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CONVENTIONAL AND NOVEL CARDIOVASCULAR RISK FACTORS IN YOUNG FINNS AND THEIR ASSOCIATIONS WITH STRUCTURAL AND FUNCTIONAL VASCULAR CHANGES OF SUBCLINICAL ATHEROSCLEROSIS

The Cardiovascular Risk in Young Finns Study

by

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For the ones I love

ABSTRACT

Juho R. H. Raiko

Conventional and novel cardiovascular risk factors in young Finns and their associations with structural and functional vascular changes of subclinical atherosclerosis. The Cardiovascular Risk in Young Finns Study. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Finland, 2011.

Background: Atherosclerosis begins in early life progressing from asymptomatic to symptomatic as we age. Although substantial progress has been made in identifying the determinants of atherosclerosis in middle to older age adults at increased cardiovascular risk, there is lack of data examining determinants and prediction of atherosclerosis in young adults.

Aims: The current study was designed to investigate levels of cardiovascular risk factors in young adults, subclinical measures of atherosclerosis, and prediction of subclinical arterial changes with conventional risk factor measures and novel metabolic profiling of serum samples.

Subjects and Methods: This thesis utilised data from the follow-ups performed in 2001 and 2007 in the Cardiovascular Risk in Young Finns study, a Finnish population-based prospective cohort study that examined 2,204 subjects who were aged 30-45 years in 2007. Subclinical atherosclerosis was studied using noninvasive ultrasound measurements of carotid intima-media thickness (IMT), carotid arterial distensibility (CDist) and brachial flow-mediated dilation (FMD). Measurements included conventional risk factors and metabolic profiling using high-throughput nuclear magnetic resonance (NMR) methods that provided data on 42 lipid markers and 16 circulating metabolites.

Results: Trends in lipids were favourable between 2001 and 2007, whereas waist circumference, fasting glucose, and blood pressure levels increased. To study the stability of noninvasive ultrasound markers, 6-year tracking (the likelihood to maintain the original fractile over time) in 6 years was examined. IMT tracked more strongly than CDist and FMD. Cardiovascular risk scores (Framingham, SCORE, Finrisk, Reynolds and PROCAM) predicted subclinical atherosclerosis equally. Lipoprotein subclass testing did not improve the prediction of subclinical atherosclerosis over and above conventional risk factors. However, circulating metabolites improved risk stratification. Tyrosine and docosahexaenoic acid were found to be novel biomarkers of high IMT.

Conclusions: Prediction of cardiovascular risk in young Finnish adults can be performed with any of the existing risk scores. The addition of metabonomics to risk stratification improves prediction of subclinical changes and enables more accurate targeting of prevention at an early stage.

Keywords: cardiovascular disease, risk factor, subclinical atherosclerosis, ultrasound, intima-media thickness, carotid artery distensibility, flow-mediated dilation, tracking, cardiovascular risk score, metabonomics, biomarker.

TIIVISTELMÄ

Juho R. H. Raiko

Perinteiset ja uudet sydän- ja verisuonitautien riskitekijät nuorilla suomalaisilla ja niiden yhteydet rakenteellisiin ja toiminnallisiin varhaisiin ateroskleroottisiin valtimomuutoksiin. Lasten Sepelvaltimotaudin Riskitekijät -projekti. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Finland, 2011.

Tausta: Ateroskleroosin kehittyminen alkaa varhain ja kehittyy ikääntymisen myötä oireettomasta sairaudesta oireelliseksi. Vaikka ateroskleroosin mittausmenetelmät ja kohonneessa sydän- ja verisuonitautiriskissä olevien keski-ikäisten ja vanhempien ihmisten tunnistaminen ovat kehittyneet huomattavasti, nuorten aikuisten sydän- ja verisuonitautiriskin määrittämistä ja varhaisen ateroskleroosin ennustamista on tutkittu vain vähän.

Tavoitteet: Tutkimuksessa selvitettiin sydän- ja verisuonitautien riskitekijöiden tasoja nuorilla aikuisilla, varhaisten ateroskleroottisten muutosten mittaamista ja varhaisten valtimomuutosten ennustamista perinteisillä riskitekijöillä ja uudella menetelmällä, seeruminäytteiden metabolisella profiloinnilla.

Tutkimushenkilöt ja -menetelmät: Tutkimuksessa käytettiin Lasten Sepelvaltimotaudin Riskitekijät –projektissa vuosina 2001 ja 2007 kerättyjä mittaustuloksia 2204 aikuiselta, jotka vuonna 2007 olivat 30-45-vuotiaita. Varhaista ateroskleroosia tutkittiin noninvasiivisesti ultraäänellä mittaamalla kaulavaltimon intima-media-paksuus (IMT) ja distensibiliteetti (CDist) ja olkavaltimon virtausvälitteinen laajeneminen (FMD). Lisäksi mitattiin perinteiset riskitekijät ja seeruminäytteiden metabolinen profiili, joka koostui 42 lipidimarkkerista ja 16 metaboliitista.

Tulokset: Vuosien 2001 ja 2007 välillä lipiditasojen kehitys oli myönteistä, mutta vyötärönympärys, paastoglukoosi ja verenpaine nousivat. IMT-mittaukset olivat stabiilimpia verrattuna CDist- ja FMD-mittauksiin. Sydän- ja verisuonitautiriskilaskurit (Framingham, SCORE, Finrisk, Reynolds ja PROCAM) ennustivat varhaista ateroskleroosia yhtä tarkasti. Lipoproteiinien alaluokkien määrittäminen ei parantanut varhaisen ateroskleroosin ennustamista perinteisiin riskitekijöihin verrattuna, mutta metaboliittien määrittäminen paransi riskin arviointia. Tyrosiini ja dokosaheksaeenihappo olivat uusia korkean IMT:n biomarkkereita.

Johtopäätökset: Varhaisen ateroskleroosin ennustamiseen nuorilla suomalaisilla voidaan käyttää kaikkia nykyisiä riskilaskureita. Metabolinen profilointi parantaa varhaisen ateroskleroosin riskin arviointia ja mahdollistaa varhaisen ja tarkan hoidon aloittamisen.

Avainsanat: sydän- ja verisuonitaudit, riskitekijä, subkliininen ateroskleroosi, ultraääni, intima-media-paksuus, kaulavaltimon distensibiliteetti, virtausvälitteinen dilataatio, urautuminen, riskilaskuri, metabonomiikka, biomarkkeri.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

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

In addition, some previously unpublished data are presented.

I Juho R.H. Raiko, Jorma S.A. Viikari, Anja Ilmanen, Nina Hutri-Kähönen, Leena Taittonen, Eero Jokinen, Matti Pietikäinen, Antti Jula, Britt-Marie Loo, Jukka Marniemi, Terho Lehtimäki, Mika Kähönen, Tapani Rönnemaa, Olli T. Raitakari, Markus Juonala, 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. 2010;267(4):370-84.

II Juho R.H. Raiko, Costan G. Magnussen, Mika Kähönen, Tomi Laitinen, Leena Taittonen, Jorma S.A. Viikari, Olli T. Raitakari, and Markus Juonala, Tracking of noninvasive ultrasound measurements of subclinical atherosclerosis in adulthood: Findings from the Cardiovascular Risk in Young Finns Study. Ultrasound in Medicine and Biology. 2010;36(8): 1237-44.

III Juho R.H. Raiko, Costan G. Magnussen, Mika Kivimäki, Leena Taittonen, Tomi Laitinen, Mika Kähönen, Nina Hutri-Kähönen, Antti Jula, Britt-Marie Loo, Russell J. Thomson, Terho Lehtimäki, Jorma S.A. Viikari, Olli T. Raitakari, and Markus Juonala, Cardiovascular risk scores in the prediction of subclinical atherosclerosis in young adults: Evidence from the Cardiovascular Risk in Young Finns Study. European Journal of Cardiovascular Prevention and Rehabilitation. 2010;17(5):549-55.

IV Peter Würtz, Juho R.H. Raiko, Costan G. Magnussen, Pasi Soininen, Antti J. Kangas, Tuulia Tynkkynen, Russell J. Thomson, Reino Laatikainen, Markku J. Savolainen, Antti Jula, Jorma S. Viikari, Mika Kähönen, Terho Lehtimäki, Markus Juonala, Mika Ala-Korpela and Olli T. Raitakari. Metabolic profiling improves prediction of subclinical atherosclerosis. Submitted.

ABBREVIATIONS

AHA the American Heart Association

ALSPAC the Avon Longitudinal Study of Parents and Children study

ApoA1 apolipoprotein-A1

ApoB apolipoprotein-B

AUC area under the curve

BMI body mass index

CARDIA the Coronary Artery Risk Development in Young Adults study

CDist carotid artery distensibility

CHD coronary heart disease

CI confidence interval

Cl confidence limit

CRP C-reactive protein

CV coefficient of variation

CVD cardiovascular disease

EGIR the European Group for the Study of Insulin Resistance

FMD flow-mediated dilation

HDL high-density lipoprotein

H-L Hosmer-Lemeshow

HOMA-IR homeostatic model assessment of insulin resistance

IDF the International Diabetes Federation

IDI integrated discrimination improvement

IDL intermediate-density lipoprotein

IMT intima-media thickness

INTERHEART the Global Case-Control Study of Risk Factors for Acute Myocardial Infarction study

LDL low-density lipoprotein

MetS metabolic syndrome

MI myocardial infarction

NCEP the National Cholesterol Education Program

NMR nuclear magnetic resonance

NO nitric oxide

NRI net reclassification improvement

OR odds ratio

PANDORA the Prevalence of peripheral Arterial disease in patients with a non-high cardiovascular disease risk, with No overt vascular Diseases nOR diAbetes mellitus

PROCAM the Prospective Cardiovascular Münster study
ROC receiver operating characteristic
SAS Statistical Analysis System
SCORE Systematic Coronary Risk Evaluation
SD standard deviation
SHAPE Screening for Heart Attack Prevention and Education
STRIP Special Turku coronary Risk factor Intervention Project
VLDL very-low-density lipoprotein
WHO World Health Organization

1. INTRODUCTION

Atherosclerosis is a systemic arterial disease with a vast a spectrum of risk factors [1-3]. The term atherosclerosis is derived from the Greek words 'athera', meaning porridge or gruel, and 'sclerosis', which means hardening. As a pathological condition atherosclerosis has been known for more than 150 years [4]. The oldest atherosclerotic lesions that have been documented to date were discovered in Egyptian mummies using computed tomography [5]. The disease is characterized by slow development of porridge-like lipid accumulates and plaques in, and stiffening of, the vessel walls of medium-sized and large arteries occurring over several decades of life [1,6]. The disease process begins during the fetal period and continues throughout childhood, adolescence and adulthood [1]. At the early stage, atherosclerotic vascular changes are predominantly asymptomatic small-scale histologic changes in the vessel wall that can affect both the structure and function of the arteries [6,7]. With advancing age, the lesions develop into more advanced stages that might manifest to clinically evident symptoms and disease such as stroke, peripheral arterial disease and coronary heart disease (CHD) [6].

Cardiovascular disease (CVD) is characterized by its increasing prevalence. At the beginning of the 20th century, CVD accounted for <10% of worldwide mortality and by 2001, the figure was 30% [8]. In the 1960s, Finland had the highest CHD mortality in the world but since this time, there has been a declining trend in the overall cardiovascular risk profile in the Finnish population [9,10]. Between 1972 and 2007, CHD mortality among middle aged Finnish men decreased 80% with improved risk factor levels explaining three-quarters of the decrease [10]. However, the number of CHD cases has increased during the past 20 years in Finland, as well as several other developed countries, and the treatment of these patients is an increasing burden to the health care system [11]. In 2009, approximately 40% of all deaths in Finland were caused by CVD [12]. In the United States (US), the estimated cost caused by atherosclerosis-related diseases was more than 500 billion US dollars in 2010 [13]. Due to the early onset of atherosclerosis, risk assessment and intervention should be targeted to young adults. Between 1986 and 2001, trends toward increased body mass index (BMI) and serum triglycerides in young Finnish adults was noted, while decrease in cholesterol level occurred at a slower rate [14]. Prevalence of obesity in adults has been increasing worldwide for decades [15,16] although recently some countries such as the US have shown signs of levelling-off [17].

Estimation of cardiovascular risk is based on determination of conventional risk factor profile (including age, sex, BMI, waist circumference, systolic and diastolic blood pressure, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides). Risk

of developing cardiovascular disease as an end-point can be calculated with risk scores such as the Framingham risk score to identify subjects with elevated risk who would benefit most from risk reduction intervention [18]. According to current guidelines, imaging studies can be used in clarifying the risk in subjects with intermediate risk based on conventional risk factors [19]. Noninvasive ultrasound measurement of carotid intima-media thickness (IMT), along with computed tomography coronary artery calcium scoring [20], is a recommended imaging method for risk clarification [21]. In order to provide additional data, imaging methods should be reproducible and they should give an estimate of the future development of the arteries. Reproducibility can be examined with tracking, the probability of maintaining the same fractile between subsequent measurements.

Conventional risk factors explain less than 50% of quantitative noninvasive measurements of atherosclerosis [22]. Therefore, there is a clear demand for further studies on novel risk factors. Metabonomics is a field of science studying the metabolic profiles of samples [23]. Combined with data on atherosclerotic end-points or cardiovascular manifestations, one could effectively search for novel cardiovascular risk markers [24]. However, widespread clinical use of novel risk assessment methods is limited by their costs since approximately 80% of global CVD mortality occurs in low- to middle-income countries [8]. Thus, new risk assessment methods must be both valid and cost-effective.

The Cardiovascular Risk in Young Finns study is an ongoing, multicenter follow-up study into cardiovascular risk from childhood to adulthood [14]. It is also one of the largest studies of its type. The main objective of this study is to examine the effects of childhood biological, psychological and lifestyle measures on cardiovascular risk in adulthood. The study began in 1980 when 3,596 Finns aged 3-18 years participated. Participation rates have been in the order of 60-80% during the follow-ups performed between 1983 and 2007.

This thesis is based on data from follow-ups in 2001 and 2007 and examines 2,204 subjects from the original cohort aged 30-45 years in 2007. Subclinical atherosclerosis was examined with carotid and brachial noninvasive vascular ultrasound studies as markers and outcome variables. Objectives of this thesis were to examine risk factor levels in 2007 and 6-year change in risk factors between 2001 and 2007, tracking of ultrasound markers of subclinical atherosclerosis, association between cardiovascular risk scores and current and future subclinical atherosclerosis and associations between high-throughput nuclear magnetic resonance (NMR)-determined lipoprotein subclasses

low-molecular weight metabolites and carotid IMT, carotid distensibility (CDist) and brachial flow-mediated dilation (FMD) with emphasis on IMT.

2. REVIEW OF THE LITERATURE

2.1. Development of atherosclerosis

Atherosclerosis is a chronic, advancing, inflammatory arterial disease with a long asymptomatic phase and an early onset in childhood [2,6]. The disease is diffuse and systemic, affecting vasculature in heart, brain and peripheral circulation [25]. Therefore, atherosclerosis can manifest as CHD, cerebrovascular complications and peripheral arterial disease. The arterial wall consists of three distinct histological layers: the innermost layer – intima; the middle layer – media; and the outermost layer – adventitia. Atherosclerosis is characterized by the development of occlusive atherosclerotic lesions in the intima layer of medium-sized muscular arteries and large elastic arteries [26]. The coronary arteries, major branches of the aortic arch and terminal abdominal aorta and its major branches are most likely to develop atherosclerotic lesions [26]. Early arterial changes reflecting the atherosclerotic process were first found in autopsy studies of young German soldiers killed in World War I [27]. These findings were later reproduced in soldiers killed in World War II [28], the Korean war [29] and the Vietnam war [30] and suggested that atherosclerosis might have a long asymptomatic phase before appearance of any clinical complications. Long term cardiovascular studies on children and young adults in the Bogalusa Heart study [31] and Pathological Determinants of Atherosclerosis in Youth study [32] have shown subclinical arteriosclerosis to be evident in early childhood. Developmental pace of atherosclerosis during childhood and early adulthood has been shown to be highly dependent on current risk factor status [31] in several studies, for instance in the Cardiovascular Risk in Young Finns study [33], the Dietary Intervention Study in Children (DISC) study [34], the Special Turku coronary Risk factor Intervention Project study (STRIP) [35], the Muscatine study [36], The Avon Longitudinal Study of Parents and Children (ALSPAC) study [37], the Coronary Artery Disease Risk Development in Young Adults (CARDIA) study [38], the Childhood Determinants of Adult Health (CDAH) study [39] and the Bogalusa Heart study [40]. Association between cardiovascular risk in childhood and atherosclerosis in adulthood has been shown in several of these studies [41-43].

Several hypotheses have been proposed to identify the triggering factors for atherosclerosis [44]. Among them is the response to injury hypothesis [6,45]. Atherosclerosis begins at sites of endothelial injury which can be caused by, for instance, increased local shear stress forces from hypertension, elevated plasma concentrations of low-density lipoprotein cholesterol (LDL-cholesterol), chemical toxins in cigarette smoke, low high-density lipoprotein cholesterol (HDL-cholesterol), insulin resistance and

glycosylated end product formation in diabetes mellitus [6,45]. These factors among others decrease endothelial cell synthesis of nitric oxide, a potent vasodilator. Moreover, endothelial cells are induced to form vasoactive molecules, cytokines and growth factors, thus disabling the normal vasodilatory, barrier and protective functions of endothelium [6,45]. Therefore, LDL-cholesterol particles can infiltrate into subendothelial space and be modified by oxidative enzymes [6]. The inflammatory response stimulates accumulation of macrophages and lymphocytes and the profileration of smooth muscle cells in intima layer which leads to thickening of the arterial wall and ultimately development of atherosclerotic lesions [6].

2.2. Classification of atherosclerotic lesions

According to the histological classification standards set by the American Heart Association (AHA), there are 6 different stages in development of atherosclerotic lesions which are displayed in Figure 1 [46,47]. The first stage of atherosclerosis (type I lesion) contains atherogenic lipoprotein which causes accumulation of macrophages in the arterial wall increasing intimal thickness. More developed lesions (type II lesions or fatty streaks) consist of subendothelial accumulations of cholesterol-engorged macrophages (foam cells) and lipid laden smooth muscle cells and they appear in the first decade of life. Currently, they are considered clinically insignificant. Intermediate lesions (type III) are typically scattered collections of extracellular lipid droplets and particles which impair the integrity of intimal smooth muscle layer. Type III lesions generally appear in the third decade of life. Atheromas (type IV) are the earliest potentially symptomatic lesions. Atheroma consists of accumulated lipd-rich necrotic debris and smooth muscle cells and they can be observed from the fourth decade onwards. The necrotic core in atheroma can be surrounded by a fibrous cap of smooth muscle cells and extracellular matrix (type V lesion or fibroatheroma). Fibroatheroma can contain calcifications, ulcerations in the intimal surface, small blood vessels from the media layer and hemorrhage from these vessels. Advanced ulcerations, hemorrhage and thrombotic deposits further increase and destabilize the integrity of the arterial change converting it to type VI lesion (complicated lesion). These lesions are most prone to cause symptoms due to acute occlusion caused by the formation of a thrombus that inflicts an ischaemic complication (e.g. stroke, myocardial or other infarctions).

Currently, it remains unknown if the mentioned lesion types are successive developmental phases in atherosclerosis. While a strong association exists between increasing age and appearance of more advanced lesion types [2,48-50], advanced lesions

tend to occur mainly at sites where early stage lesions are also situated suggesting a possible systematic progression of lesion types [2,51].

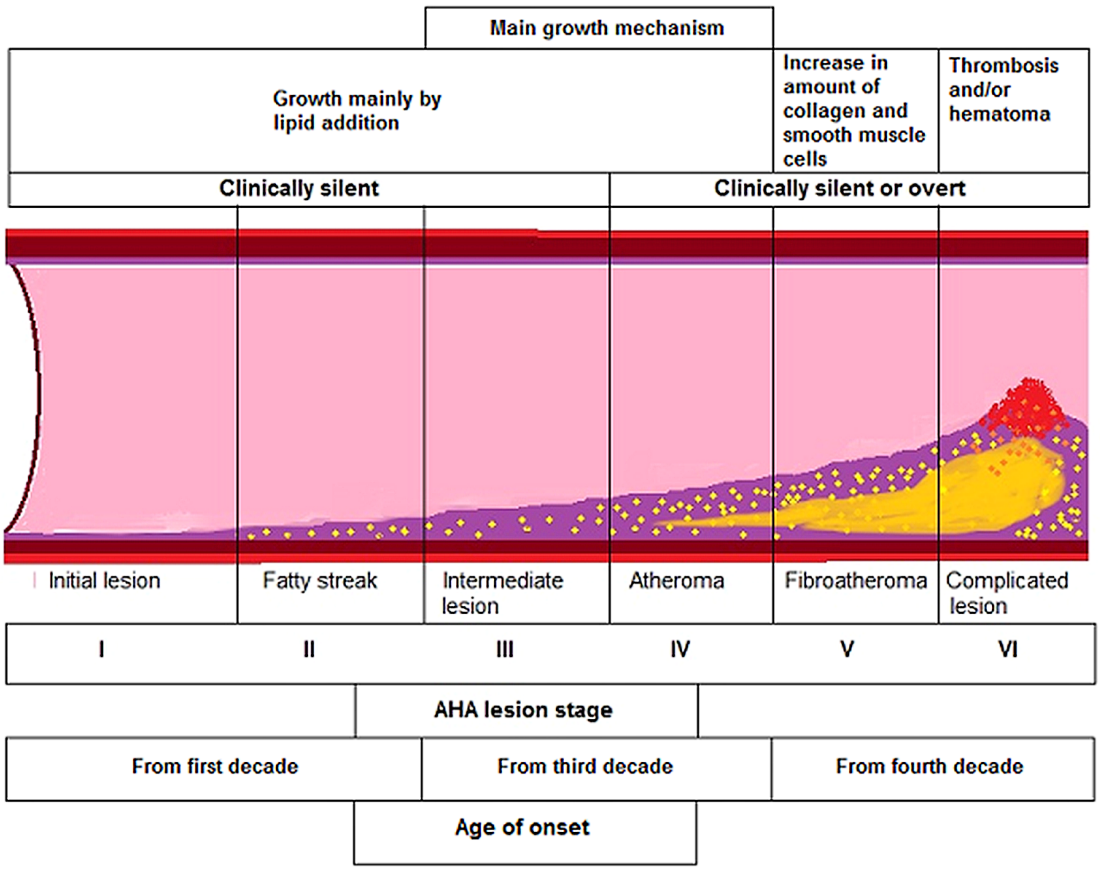


Figure 1. Development and classification of atherosclerotic lesions displayed in a simplified form. Development starts from the left as a normal arterial wall and ends at the right as an arterial thrombus.

2.3. Atherosclerotic risk factors

Prevalence and severity of atherosclerosis is related to several risk factors. Currently, there is no universal agreement on classifying atherosclerotic risk factors and markers. The AHA Prevention Conference statement in 1999 proposed a classification which divides risk factors into 3 categories: (1) major independent risk factors, (2) predisposing and (3) conditional risk factors [52].

Major independent risk factors appear to have a direct causative role in atherogenesis, whereas predisposing factors mediate some risk through causal factors and may have

independent effects. Conditional risk factors have an association with increased cardiovascular risk but their causative, independent and quantitative contributions to coronary artery disease are not well documented. Conditional risk factors may increase CVD risk in presence of causative risk factors [52,53]. Classification of risk factors is displayed in Table 1. Furthermore, there are emerging risk factors which have been extensively assessed but still require additional studies before they can be accepted for clinical utility [54]. Elevated conventional risk factor levels such as LDL-cholesterol, systolic blood pressure, smoking and BMI in childhood have been previously linked with subclinical atherosclerosis in adulthood [41,42,55]. Based on analyses from 4 longitudinal cohorts, risk factor measurements at the minimum age of 9 years are predictive of subclinical atherosclerosis in adulthood [56]. The Global Case-Control Study of Risk Factors for Acute Myocardial Infarction (INTERHEART) study has shown that dyslipidemias, smoking, hypertension, diabetes, abdominal obesity, sedentary lifestyle and no alcohol intake accounted for >90% of risk of myocardial infarction [57]. The most important conventional risk factors are reviewed in the following section.

Table 1. Classification of risk factors for atherosclerosis as presented by the AHA.

Major independent risk factor	Predisposing risk factors	Conditional risk factors
Smoking	Obesity*	Elevated serum triglycerides
Elevated serum total and LDL cholesterol	Physical inactivity*	Small LDL particles
Low serum HDL cholesterol	Abdominal obesity	Elevated serum homocysteine
Diabetes mellitus	Family history of premature CHD	Elevated serum lipoprotein(a)
Advancing age	Ethnic characteristics	Prothrombotic factors
Elevated blood pressure	Psychosocial factors	Inflammatory markers

* These risk factors are designated major risk factors by the AHA.

2.3.1. Lipid risk factors

Low-density lipoprotein cholesterol

LDL particles are the consecutive product of very-low density lipoprotein (VLDL) particles generated by the liver [58]. LDL particles transport cholesterol via the bloodstream into cells for cellular metabolism [58]. Accumulation of LDL particles in the subendothelial matrix is an important step in development of atherosclerosis and high levels of LDL-cholesterol and total cholesterol have been associated with atherosclerosis [58,59]. LDL particles undergo modifications such as oxidation, glycation and aggregation, prior to formation of foam cells and inflammation in the arterial wall [60]. LDL-cholesterol level has been shown to correlate with CVD risk and mortality and reduction in LDL-cholesterol decreases CVD events [61-62]. LDL-cholesterol levels in childhood have been shown to correlate with carotid IMT in adulthood [41-43,55]. However, high total cholesterol and LDL-cholesterol are not associated with CVD events in octogenarians without overt CHD possibly due to comorbidity and competing risk from other illnesses [63].

High-density lipoprotein cholesterol

HDL particles receive accumulated cholesterol from peripheral cells and transport it back to hepatic cells for excretion forming the reverse cholesterol transport pathway [64]. Furthermore, HDL particles have anti-inflammatory, antithrombotic and antiatherogenic capabilities [58,65] and HDL-particles stimulate nitric oxide (NO) synthesis in the endothelium [66]. HDL-cholesterol is highly protective against atherosclerosis and low levels have been associated with elevated CHD risk [67]. After adjusting for other factors of longevity, higher HDL-cholesterol levels in men at the mean age of 65 years predict survival to 85 years of age [68]. Increased CVD risk caused by low HDL-cholesterol exists at all LDL-cholesterol levels [69]. However, the main effect of HDL-particles in prevention of atherosclerosis is somewhat unclear [70]. HDL-cholesterol may provide antiatherosclerotic effects by promoting reverse cholesterol transport from macrophages which has an inverse association with carotid IMT [71]. HDL-cholesterol has been inversely associated with subclinical atherosclerosis and coronary artery calcification in children and young adults [3,43,72,73].

Triglycerides

Triglyceriders are esters consisting of glycerol and three fatty acids [74]. Dietary triglycerides, phospholipids and cholesterol are transported from the intestine first to lymphatic vessels and via the thoracic duct to bloodstream in chylomicrons [74]. Chylomicrons release glycerol and fatty acids to peripheral cells and the remnant chylomicrons are endocytosed by liver cells [74]. Liver cell release cholesterol and

triglycerides in VLDL particles for peripheral tissues [74]. Measurement of serum triglycerides displays chiefly the concentrations of triglyceride-rich lipoprotein fractions, predominantly VLDL particles [74]. High triglyceride level may be independently associated with atherogenesis or other dyslipidemias, mostly low HDL-cholesterol, may promote atherosclerosis alongside high triglyceride levels [74] and there is evidence that triglyceride-mediated pathways have a causal effect on development of CHD [75]. HDL-cholesterol and serum triglycerides affect CVD risk synergistically when LDL-cholesterol level is well treated [76]. Hypertriglyceridemia may affect coagulation and fibrinolysis [74].

Apolipoproteins

Each atherogenic lipoprotein particle (chylomicrons, IDL, VLDL, LDL and lipoprotein(a)) contains one apolipoprotein B and measurement of serum ApoB concentration displays the number of circulating atherogenic lipoproteins [77]. Apolipoprotein A-I is a structural protein in HDL particles and one HDL particle can contain a varying number of ApoA-I proteins [78]. ApoA-I is primarily responsible for the reverse cholesterol transport and if ApoA-I or other structural proteins are damaged by oxidative mechanisms, HDL particles are transformed from anti-inflammatory particles to proinflammatory particles [65]. ApoA-I concentration correlates strongly with HDL-cholesterol concentration and ApoB/ApoA-I ratio displays the ratio of atherogenic and atheroprotective particles thus giving an estimate of tendency towards development of atherosclerosis [77]. ApoB/ApoA-I ratio has been shown to be increased and ApoA levels decreased in children with family history of CHD [79,80]. However, the AHA does not recommend measurement of apolipoproteins in risk assessment in asymptomatic adults [19] though apolipoprotein ratio is a stronger predictor of CVD than conventional lipoprotein measurements [77].

2.3.2. Blood pressure

Hypertension is a major risk factor of atherosclerosis [81] and the leading risk factor for mortality globally [82]. Hypertension promotes atherosclerosis via numerous pathways [83]. Risk of CHD doubles by every 20/10 mmHg increase in blood pressure above 115/75 mmHg [84]. Elevated blood pressure in children and young adults has been linked with subclinical atherosclerosis [31,85,86]. Wide pulse pressure in childhood is associated with subclinical atherosclerosis in adulthood [87] and pulse pressure is cross-sectionally linked with endothelial dysfunction [88]. Moreover, calcified atherosclerosis correlates strongly with pulse pressure and isolated systolic hypertension [89].

2.3.3. Smoking

Smoking has been shown to increase CHD risk in several studies [90,91]. Smoking seems to act synergistically with other risk factors in promoting atherosclerosis [92,93] and cessation of smoking reduces CHD risk [94]. Pack-years of smoking but not current vs. past smoking seems to be associated with progression of atherosclerosis suggesting that some adverse effects of smoking may be cumulative and irreversible [95]. Active and passive cigarette smoking both affect all phases of atherosclerosis although the exact mechanism and the toxic components of cigarette smoke remain largely unknown [96]. Smoking has been shown to injure vascular endothelium and cause endothelial dysfunction, produce superoxide anions, reduce production and bioavailability of nitric oxide, increase production and release of endothelin and cause thrombosis [97]. Cigarette smoke increases inflammation, thrombosis and oxidation of low-density lipoproteins and recent data suggests that exposure to cigarette smoke increases oxidative stress as a potential mechanism for promoting atherosclerosis [98]. Furthermore, exposure to environmental cigarette smoke in passive smoking impairs endothelial function in children [99].

2.3.4. Obesity

Obesity has been shown to accelerate progression of CHD [100]. Overweight and obesity are associated with increased all-cause mortality in white adults while all-cause mortality is the lowest at BMI levels 20.0-24.9 [101]. However, several studies have shown that adult overweight BMI levels 25-30 have similar mortality risk as normal-weight subjects [102-105] while higher BMI at all ages is monotonously associated with worse health risk profiles [106]. Logue et al. examined BMI in a cohort of 6082 men with mean age of 55 years during a follow-up of 14.7 years and found that obesity was associated with fatal, but not non-fatal, CHD independently of known CVD risk factors and deprivation [107]. This finding suggests that obesity might have an independent role in development of CVD. Obesity in childhood and young adulthood can promote subclinical atherosclerosis [31,42,108]. Obesity is a risk factor for hypertension, dyslipidemia and insulin resistance in childhood [109,110]. Waist-to-hip ratio has been shown to provide better discrimination in prediction of coronary artery calcium score than either BMI or waist circumference [111]. Moreover, weight and BMI may not be as important in prediction of CVD in women as they are in men [112]. However, high BMI in childhood has been linked with increased risk of CHD in adulthood and the associations were stronger in boys than in girls [113]. BMI, waist circumference and waist-to-hip ratio do not singly or combined improve CVD risk prediction when data

on systolic blood pressure, history of diabetes and lipids is available indicating that type of obesity may not have a different effect on CVD risk [114]. Height can affect CVD risk alone since short stature has been linked with increased CVD risk in both sexes probably due to narrower arteries in short individuals [115].

2.3.5. Diabetes

Diabetes promotes atherosclerosis both in childhood [116] and adulthood [117]. Diabetes induces atherosclerosis via metabolic abnormalities like hyperglycemia, increased free fatty acids and insulin resistance [118]. In diabetes, hyperglycemia, altered insulin signaling, increased reactive oxygen species, inflammation and protein kinase C activation might lead to diminished NO bioavailability [119]. Diabetes can be categorized into type 1 for autoimmune induced insulin deficiency and type 2 for impaired insulin sensitivity and production, the first accounting for 5-10% of all cases of diabetes and the other for 90-95% [120]. Youth with type 1 dibetes have been shown to have elevated levels of inflammatory markers and atherogenic lipid profiles independent of good glycemic control which may contribute to accelerated athesclerosis in youth with type 1 diabetes [121]. According to the third National Health and Nutrition Examination Survey, type 1 diabetes is a common CVD risk factor in childhood with a current prevalence of 1.7 cases in 1000 adolescents [122]. Type 2 diabetes is less common in childhood than type 1 although it is an increasing problem due to increased obesity in children [123]. Obesity and increased waist circumference are the core aspects of insulin resistance in type 2 diabetes [124]. Risk of CVD in adulthood is increased by the time of exposure to insulin resistance suggesting that an early intervention is needed against childhood obesity [124,125]. Diabetes in adulthood can increase risk of cardiovascular mortality two- to fourfold [126]. In subjects without diabetes, elevated fasting blood glucose is non-linearly associated with CVD risk [127]. Independent of major risk factors, diabetic subjects die of all causes 6 years earlier than subjects without diabetes [128]. Type 2 diabetes has also been shown to equalize the risk of CHD death in subjects with no history of myocardial infarction compared to individuals with no diabetes and a history of myocardial infarction [129]. Children and young adults with diabetes have increased carotid IMT and loss of arterial elasticity [130-132].

2.3.6. Genetic factors and family history

Previously, twin studies and family history of CHD as a risk factor have indicated that genetic factors have a strong role in pathogenesis of atherosclerosis [130,133]. Family history of premature CVD is a strong genetic risk factor of atherosclerosis [134,135]. In

Young Finns study, young adults with family history of CHD have higher IMT that is partly attributed to their increased vulnerability to metabolic risk factors [136]. Several loci have been shown to affect risk of CVD individually and in aggregate with genome-wide association studies [137-143]. The 30 loci associated with CHD currently seem to explain approximately only 10% of the genetic risk of CHD [141]. Variants of 9p21 have been shown to affect CVD risk in numerous studies [144-149] while the influence is not mediated through a mechanism that affects carotid subclinical atherosclerosis and endothelial dysfunction [148]. Harismendy et al. showed that the effect of the 9p21 variant promotes atherosclerosis via increased inflammation susceptibility [150].

Despite multiple known genetic markers linked with CVD, Paynter et al. showed that a genetic risk score containing 101 single nucleotide polymorphisms does not improve prediction of CVD over and above traditional risk factors in women [151]. However, a genetic risk score improved prediction of subclinical atherosclerosis compared to conventional risk factors in Young Finns study [152]. Furthermore, a risk score containing 13 single nucleotide polymorphisms can identify the 20% of subjects of European ancestry who are at 70% elevated risk of first CHD event [153].

Recently, epigenetics, the study of transcriptional regulation of genes by chemical modification of the structure of chromatin, has been shown to influence the development of atherosclerosis [154]. For instance, dietary components may impose epigenetic marks which alter gene activation and repression resulting in an atherosclerotic cellular phenotype [154]. Gene-environment interactions seem to influence the development of CVD significantly [155].

2.3.7. Aging

Extended exposure to risk factors increases prevalence of atherosclerotic changes and risk of CVD and age can be considered as one of the strongest CVD risk factors [134,156]. For instance, the number of years lived with obesity is directly associated with all-cause and CVD mortality [157].

2.3.8. Socioeconomic factors

In Young Finns study, low socioeconomic status in childhood was linked with higher blood pressure [158] and central obesity in both sexes and low HDL-cholesterol and insulin resistance in men, independent of current socioeconomic status [159] and similar findings have been produced in other studies [160,161]. Parental socioeconomic status had modest inverse association with traditional risk factors in the offspring in young

adulthood which can contribute to future CVD risk [162]. Young adults with the lowest education have more adverse lifestyles than the more educated while parental education still affects CVD risk [163,164]. Early life socioeconomic adversity has been associated with carotid atherosclerosis in adulthood [161,165]. Including patient income in Framingham risk score minimizes the bias in CVD risk estimation caused by socioeconomic status [166]. Socioeconomic differences in health behaviour can be potentially accounted for personality-related factors [167].

2.3.9. Metabolic syndrome

CVD risk factors are largely interrelated and subjects with increased CVD risk often possess a vast spectrum of risk factors. Metabolic syndrome (MetS) is a combination of cardiovascular risk factors, hypertension, dyslipidaemia, insulin resistance and central obesity, which predicts development of CVD and type 2 diabetes [168]. The worldwide prevalence of MetS is increasing and recognition of the MetS and the treatment of its components at an early stage is important in order to prevent morbidity and mortality due to CVD [169,170]. Youth with Mets have been shown to be at risk of developing subclinical atherosclerosis, type 2 diabetes and MetS in adulthood [171]. However, recovery from childhood or adulthood MetS has been associated with decreased subclinical atherosclerosis in both anatomic and functional ultrasound markers [172,173]. MetS in adulthood can be predicted with obesity, high triglycerides, high insulin and high CRP in childhood and family history of hypertension and type 2 diabetes [174].

2.3.10. Inflammatory markers and other risk factors

During the past two decades, biomarkers have become important methods in early detection of subclinical disease in clinical practice [175]. Atherosclerosis has been associated with markers of inflammation and hemostasis [176], platelet activity and aggregation [176], homocysteine [177], infectious agents (e.g. Chlamydia pneumoniae) [178,179], sedentary lifestyle [180], no alcohol intake [181] and psychosocial status [182]. High-sensitivity C-reactive protein (CRP) in childhood and young adulthood has been related with markers of subclinical atherosclerosis [183]. High-sensitivity CRP seems to be linked with CVD but associations depend largely on other conventional risk factors and markers of inflammation and high-sensitivity CRP might not be a causative factor in development of atherosclerosis [184]. Obesity and smoking in men, and obesity and use of oral contraceptives in women were directly associated with high-sensitivity CRP level while physical activity in women decreased high-sensitivity CRP level [185]. In children with type 1 diabetes, high-sensitivity CRP has been shown to be an

independent predictor of carotid atherosclerosis suggesting that inflammation may be one of the key pathways of atherogenesis in diabetes [186]. high-sensitivity CRP in childhood has been shown to predict weakly high-sensitivity CRP in adulthood independently of conventional risk factors [187]. However, childhood high-sensitivity CRP does not predict carotid IMT in adulthood [187]. Currently, AHA recommends high-sensitivity CRP testing for subjects at intermediate risk [19].

Adipose tissue acts both as an energy storage and as the largest endocrine organ in the body [188]. Visceral and epicardial fat are strongly associated with the development of CVD while subcutaneous fat is not linked with CVD [188]. Adipose tissue secretes mediators called adipocytokines, including leptin, adiponectin, resistin and visfatin, which have multiple effects on the metabolic profile and immunological processes [189,190]. Thus, excessive fat may cause adverse metabolic and hemostatic disturbances and CVD via adipocytokines depending on the distribution of body fat [188,191,192].

Both short and excessive sleep duration act as predictors of CVD though mechanisms are not fully understood [193]. Short duration of sleep may act via reciprocal changes in levels of leptin and ghrelin [193]. These increase appetite, caloric intake, reduce energy consumption and facilitate the development of obesity and impaired glycaemic control [193]. Short duration of sleep increases secretion of cortisol, changes growth hormone metabolism and activates low-grade inflammation [193]. Effect of long duration of sleep may function via residual confounding and co-morbidities .

2.3.11. Metabonomics

Biological heterogeneity and slow development of CVD decreases the accuracy of diagnostic methods in identifying those at elevated risk [194,195]. Thus, it is of potentially great importance to examine individually the metabolic profile of every individual to assess development of pathophysiological pathways and individual risk to prevent and treat potential disease at an early stage [196]. ^{1}NMR metabonomics has been proposed as a method for metabolic profiling and advanced risk assesessment in disease prevention [196,197].

Metabonomics refers to the determination of the metabolic profile from body fluids or tissue samples [196]. The phenotype of a biological system is highly reflected by its metabolite composition [198] and metabonomics can offer a means to accurately determine pathophysiological states [199]. In examination of CVD risk factors, determination of metabolic profile can be performed with ^{1}NMR spectroscopy of serum samples which enables measurement of lipoprotein subclasses and low-molecular weight metabolites in serum samples to give a more thorough view on the

multifactorial atherogenic status [196]. NMR spectroscopy provides the spectra of all NMR-detectable compounds in the sample as molecular profiles [195]. Thus, there is no need to individually quantify each metabolite separately since spectroscopy measures all examined metabolites simultaneously [196]. NMR spectroscopy is based on the concept that each measured metabolite is identified based on the hydrogen-1 nuclei in the molecule [200]. NMR techniques provide a quick and non-destructive analysis of large amounts of metabolites in a single sample [201]. Thus, the same samples can be used again in other analyses. Moreover, NMR methods have high reproducibility enabling the use of large data sets [202]. However, NMR spectroscopy has low sensitivity with nanomolar detection of metabolites, artefacts caused by the pH and ionic composition of the sample and the overlapping spectra of the metabolites [203,204].

Metabolic profiling can be also performed with mass spectrometry [23,205] in which metabolites are identified by first ionizing the examined metabolite samples and then measuring the mass-to-charge ratio of these charged particles [206]. Mass spectrometry techniques have high sensitivity with picomolar range, high specificity and can be often used as a stand-alone method for identifying and quantifying metabolites [202]. Mass spectrometry is often combined with separation methods such as gas or liquid chromatography or capillary electrophoresis to improve resolving power [202]. Another method for determining lipoprotein subclasses is ultracentrifugation in which particles in the fluid sample are separated due to their different densities [207].

Data processing and statistical analysis are key components in metabonomics regardless of the method of measurement [202]. The Metabolomics Society [208] and the Metabolomics Standardization Initiative [209] have published data standardization initiatives to improve reproducibility in metabonomics.

Clinical utility of advanced lipoprotein testing and subfractionation has been under debate [197,210] and no clinical guidelines exist for its clinical use in estimating CVD risk. There are still methodological issues in the interpretation of NMR metabonomics data in a biochemically relevant manner [211,212] but the large array of measured metabolites is an advantage for a holistic view on metabolism [212,213]. Advanced lipoprotein testing has been used in multiple clinical trials during the past 50 years [197]. Individual patient disorders may be missed with standard lipid tests and these conditions can only be noticed with subfractionation of the lipid profile [197]. The increasing prevalence of the MetS adds to the clinical relevance of advanced lipid profiling [197]. Nevertheless, advanced lipoprotein testing is clinically useful when it adds to clinical knowledge, provides risk information that is independent of established predictors, is easy to measure and interpret and it is accurate, reproducible and internationally standardized

and it has a favourable cost-benefit ratio [214]. Metabonomics has been proposed as a viable method of studying lipotoxicity in CVD in several reviews [215,216]. However, the evidence on the clinical utility of advanced lipoprotein testing is currently based on research laboratory tests [197,210] and no clinical data is yet available. Figure 2 displays the information acquired in the NMR spectra.

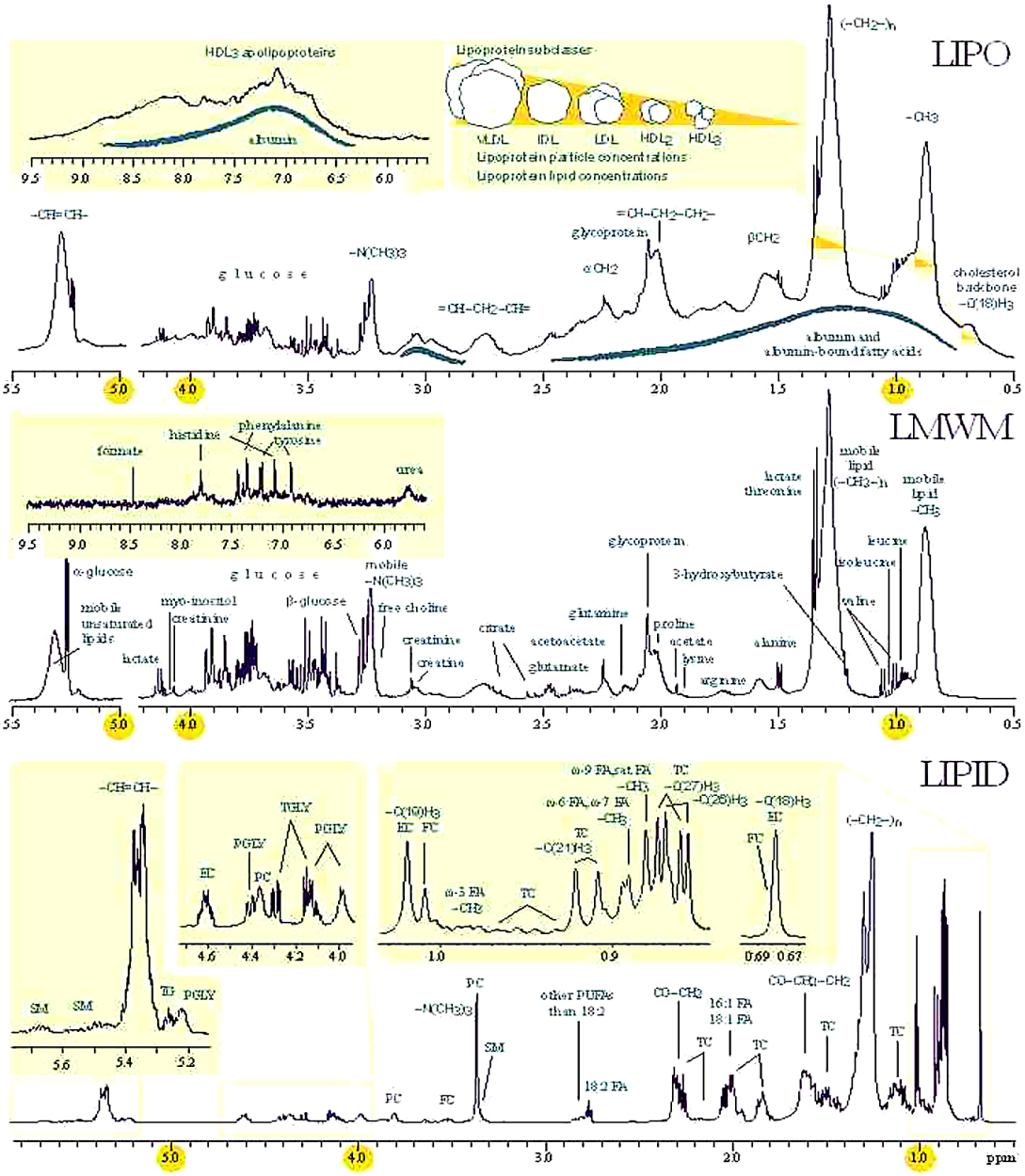


Figure 2. Information acquired in the NMR spectra in the lipo, LMWM and lipid windows. Figure modified from Soininen et al. 2009 [217].

2.4. Assessment of CVD event risk

Atherosclerotic CVD accounts worldwide for >19 million deaths annually [218]. CVD is the leading cause of death in developed countries and it is becoming more prevalent in developing countries [219]. In the US, 50% of males and 64% of females who died suddenly of CVD had no previous symptoms [220]. Approximately one third of CVD events [221] and subclinical atherosclerosis [222] cannot be attributed to traditional CVD risk factors. In the The Prevalence of peripheral Arterial disease in patients with a non-high cardiovascular disease risk, with No overt vascular Diseases nOR diAbetes mellitus (PANDORA) study, the prevalence of asymptomatic peripheral arterial disease among participants with non-high CVD risk was 17.8% [223]. These findings are often referred to as detection gap indicating that other non-conventional conditions may contribute to CVD events [224] suggesting that available screening and diagnostic methods are somewhat inadequate in detecting asymptomatic patients at risk of CVD event [225]. Thus, there is great need for early recognition of high-risk subjects in the asymptomatic phase of atherosclerosis.

2.4.1. CVD risk scores

Since CVD is typically induced by several simultaneously interacting risk factors [226], current recommendations on prevention of CHD in clinical practice emphasize the need to base intervention on assessment of total risk burden rather than on single risk factors [227-229]. Risk of CVD events is estimated with risk scores which display probability of developing CVD or CHD event in a specific time period. Estimations are based on conventional risk factors [226,230,231] and in latest models, may include family history of myocardial infarction [232,233]. According to the guidelines of the American College of Cardiology Federation and AHA imaging studies (IMT and coronary artery calcium score) should be used in patients with intermediate or higher risk and imaging studies clarify the risk in individuals at elevated risk based on their conventional risk factor status [19-21,234]. The present thesis concentrated on the following risk scores, which are detailed and contrasted in Table 2.

2.4.1.1. Framingham risk score

Framingham Heart Study is an epidemiologic study started in 1948 in an attempt to identify factors affecting CVD and eventually confirming the risk factor concept. Since then the study has developed mathematical functions for predicting CHD events [235-238]. These functions are multivariable models that include major CHD risk factors. The

model that was used in this study included sex, age, blood pressure, total cholesterol, LDL-cholesterol, HDL-cholesterol, smoking behaviour and diabetes status and reported 10-year risk of CHD event [230]. The score was developed using data on 5,573 subjects aged 30-74 years and is recommended for 30-75-year-olds [230]. Framingham score has been shown to estimate risk well in populations with similar risk levels in the US [239] and in Europe [240] but the risk score seems to overestimate absolute risk in populations with lower CHD rates [239,241,242] and in Danish [243] and German [244] populations. These findings may limit utility of the Framingham model in areas outside the US and there is little evidence supporting the use of CVD risk scores for primary prevention [245].

2.4.1.2. Reynolds risk score

Reynolds risk score is a novel sex-specific model for 10-year CVD (CHD, stroke, peripheral arterial disease) risk estimation. In addition to age, total cholesterol, HDL-cholesterol, blood pressure and smoking, function includes high-sensitivity CRP for inflammatory status, parental history of premature myocardial infarction before age 60 years and hemoglobin A_{1C} if diabetic in females [232,233]. The scores are based on studies on initially healthy 10,724 US men aged ≥50 years and 24,558 US women aged ≥45 years who were followed up for over 10 years [232,233].

2.4.1.3 SCORE

The SCORE (Systematic Coronary Risk Evaluation) project was initiated with the aim of developing a risk scoring system for clinical management of CVD risk in European clinical practice [226]. The SCORE model includes sex, smoking, systolic blood pressure, HDL-cholesterol and total cholesterol or total cholesterol/HDL-cholesterol ratio and the model predicts 10-year CHD risk [226]. Age is used to define the hazard function [226]. Separate equations were developed for high-risk and low-risk regions of Europe [226]. The score is based on 12 pooled European cohorts aged 19-80 years totalling 88,080 women and 117,098 men representing 2.7 million person years of follow-up [226].

2.4.1.4 Finrisk

Finrisk equation is based on data from a Finnish population [9] and has been shown to give similar results as Framingham in South Asian population [231]. The model includes sex, age, smoking, systolic blood pressure, total cholesterol, HDL-cholesterol and diabetes and it predicts 10-year risk of CHD and cerebrovascular event [231]. No guidelines currently exist for utility of Finrisk model. The formula is based on data on

cardiovascular morbidity and mortality in 30-64-year-old Finnish men (N=9,391) and women (N=10,056) with a 10-year follow-up period [9].

2.4.1.5 PROCAM

PROCAM risk score was developed with the cohort from the Prospective Cardiovascular Münster (PROCAM) study from the Münster and the northern Ruhr area in Germany and the score includes sex, age, smoking, systolic blood pressure, diabetes status, total cholesterol, LDL-cholesterol, triglycerides, parental history of myocardial infarction and regional adjustment factor based on geographic prevalence of CVD [246]. PROCAM score predicts 10-year risk of CVD [246]. The model is based on 18,460 men and 8,518 women aged 20-78 years at study entry with an average follow-up of 11.7 years [246].

Table 2. Description of risk scores for prediction of CVD.

	Framingham	SCORE	Finrisk	Reynolds	PROCAM
Risk factors					
Sex	✓	✓	✓	✓	✓
Age	✓	✓	✓	✓	✓
Systolic blood pressure	✓	✓	✓	✓	✓
Smoking	✓	✓	✓	✓	✓
Diabetes	✓	✓	✓	✓	✓
Total cholesterol	✓	✓	✓	✓	✓
HDL-cholesterol	✓	✓	✓	✓	
LDL-cholesterol	✓				✓
Triglycerides					✓
CRP				✓	
Family history of CVD				✓	✓
Regional adjustment of risk		✓			✓
Number of risk factors	8	8	7	9	10
Target age group	Between 30-74	Between 50-65	Middle-aged	Over 45-55	Between 45-65
Ethnicity of original cohort	US	Several European cohorts	Finnish	US	German
Predicted outcome					
CVD	✓			✓	✓
CHD		✓			
CHD and stroke			✓		
Period of prediction (years)	10	10	10	10	10

2.4.2. Novel risk assessment

In 2003, Screening for Heart Attack Prevention and Education (SHAPE) Task Force introduced a new CVD risk assessment strategy in which patients with advanced atherosclerosis are predisposed to clinical events by 3 major components: (1) vulnerable plaque, (2) vulnerable blood and (3) vulnerable myocardium [247]. However, SHAPE is a private group of CVD experts and not an official organization. Vulnerable plaques refer to all types of atherosclerotic plaques with high likelihood of rapid progression or thrombotic complications, that is, susceptible to complications [225]. However, vulnerable plaques are not the only culprit factors for acute CVD events. Tendency of thrombosis (vulnerable blood) and susceptibility to fatal arrhytmias (vulnerable myocardium) contribute extensively to development of clinically evident CVD [247] and when all these factors are considered in risk assessment they form the concept of vulnerable patient [247] indicating a subject prone to CVD events. However, few data exist for the utility of the SHAPE strategy.

2.5. Vascular ultrasound imaging methods of subclinical atherosclerosis

Noninvasive vascular ultrasound imaging methods such as carotid artery intima-media thickness (IMT), carotid artery distensibility (CDist) and brachial artery flow-mediated dilation (FMD) are currently used as markers of early atherosclerotic changes in both clinical and research settings. These methods have been shown to be reproducible and useful in identifying asymptomatic subjects at risk of CVD [248]. Recommendations for standardization of these methods for research in pediatric populations also exist [249]. However, these methods are not used, at least is Finland, in clinical settings for risk estimation.

2.5.1. Arterial wall thickness

B-mode ultrasound can be used reliably in measurement of common carotid IMT and plaque proximal to carotid bifurcation in assessment of extent and severity of structural changes in subclinical atherosclerosis [250]. In 1986, Pignoli et al. introduced measurement of IMT with ultrasound as a strong correlate of actual histological IMT using arterial samples from autopsy studies [251]. Measurement of IMT is most often performed on the carotid artery due to its convenience. Carotid IMT has been shown to correlate with CVD risk factors [252,253] and predict CHD events in asymptomatic phase [254,255]. Risk factors in childhood have been associated with increased carotid IMT in adulthood in Young Finns study [41,56], the Muscatine study [55], the Bogalusa Heart study [42], and the CDAH study [43]. In Young Finns study, the relationship between obesity in

youth and increased IMT in adulthood was explained by significant tracking of BMI from youth to adulthood [256]. The Atherosclerosis Risk in Young Adults study showed that unfavourable risk factor profile in healthy adults aged 27-30 years was associated cross-sectionally with marked increase in carotid IMT [257]. Several studies have shown that increase in carotid IMT increases risk of CVD events independent of traditional CVD risk factor levels [258-261]. However, according to Skilton et al. measures of all three layers of the carotid wall had higher correlation with cardiovascular risk factors than IMT alone [262]. For each 0.03-mm increase per year in carotid IMT, the increase in relative risk for any coronary event was 3.1-fold [263]. According to Lorenz et al., the age- and sex-adjusted overall relative risk for myocardial infarction was 1.15 and for stroke 1.18 per 0.10-mm common carotid IMT difference [264]. However, IMT regression or slowed progression by cardiovascular drug therapies is not associated with reduced risk of CVD events [265]. Slower progression of IMT has been shown to predict nonfatal myocardial infarction primarily in nonstatin trials and in subjects with low baseline carotid IMT [266].

Atherosclerosis Risk In Communities study showed that adding carotid IMT or presence of carotid plaque to traditional risk factors improved prediction of CHD [267]. IMT might also be useful in the identification of individuals who have lower CVD risk than what is based on conventional risk factors [267]. These individuals would benefit more from less intensive risk reduction thus avoiding excessive treatment. However, carotid IMT seems to be relatively modest predictor of CHD compared to carotid plaque and IMT adds little to CHD prediction by risk factors alone [268].

The utility of carotid IMT in prediction of CVD risk has been acknowledged by the AHA [269] and the Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice [270]. Measurement of carotid IMT has been proposed as screening tools for CVD risk stratification combined with risk factor assessment by the SHAPE Task Force [271] and the AHA and American College of Cardiology Federation guidelines and the Society of Atherosclerosis Imaging and Prevention statement released in 2010 recommend IMT measurement in subjects with intermediate or higher risk, metabolic syndrome, and older patients [19,21]. However, clinical use of anatomic markers of atherosclerosis has been criticised due to the lack of evidence on the independent predictive value of carotid IMT and the ability of IMT to reclassify patients into lower and higher risk categories [272-274].

2.5.2. Arterial elasticity

Arteriosclerosis is part of aging process and an independent risk factor for CVD [275]. Large arteries lose elasticity due to high ratio of collagen to elastin in the vessel wall [275,276], breaking of the elastic fibers, inflammatory reaction, fibrosis, smooth muscle necrosis in the media layer and calcification of the vessel wall [277]. In adults, CVD risk factors have been shown to promote loss of arterial elasticity in several studies [275,278-281], ageing still being the most significant factor decreasing arterial elasticity [282]. Risk factors in childhood have been shown to predict carotid elasticity in adulthood [281]. Arterial stiffness has also been shown to be an independent predictor of CVD events in subjects with elevated CVD risk [283-285].

Examination of arterial elasticity can be performed with numerous noninvasive methods [276]. Elasticity can be estimated with ultrasound by assessing the blood pressure and the change in arterial diameter during the cardiac cycle. These data can be expressed as several indices, such as distensibility, the stiffness index and Young's elastic modulus [286,287]. Distensibility describes arterial dilation during the cardiac cycle, stiffness index displays the elastic properties of the arterial wall somewhat independently of blood pressure and Young's elastic modulus measures intrinsic stiffness of the arterial wall independently of the intima-media thickness [286,288]. Arterial elasticity can be evaluated also with pulse wave velocity (PWV) and waveform analyses [276] of which PWV has been previously shown to correlate with the mentioned ultrasonographically measured indices of arterial stiffness [289]. PWV measures the difference of the pulse wave between two sites on the registered artery and the delay between points on the wave. In waveform analyses, applanation tonometry registers blood pressure waveforms during the cardiac cycle. PWV has been shown to predict CVD outcome in low-to-moderate CVD risk populations [290]. According to AHA guidelines measurement of arterial elasticity for determination of CVD risk are not recommended outside research settings [19].

2.5.3. Endothelial function

Measurement of brachial FMD is a widely used method in determining endothelium-mediated vasodilatator function [291]. Normally, endothelium maintains the local balance between vasodilation and vasocontriction and regulates thrombogenesis, fibrinolysis, platelet and leukocyte interactions and smooth muscle cell activity [292,293]. Deterioration of endothelial function (endothelial dysfunction) due to atherosclerotic progression preceeds structural atherosclerotic changes in the arterial wall [6]. Endothelial function depends on the sum of CVD risk factors, atheroprotective factors and genetics [294,295].

First measurements of endothelial function in humans were performed by measuring the response of epicardial arteries to infused acetylcholine [296]. However, such invasive measurements would not be suitable for large-scale studies in asymptomatic patients unlike the noninvasive methods like FMD [297].

Endothelial function is often measured with the technique introduced by Celermajer et al. [298]. Cuff occlusion followed by release increases blood flow in the brachial artery that increases shear stress triggering the release of NO by endothelial cells. Placement of the cuff distal to the imaged artery leads to principally NO-dependent response and proximal cuff placement causes less NO-dependent response [299]. The latter method was used in the Young Finns study.

Another noninvasive method for measuring endothelial function is examination of endothelial vasomotor function after reactive hyperemia by pulse amplitude tonometry (RH-PAT) [297,300]. RH-PAT is performed with a finger plethysmograph, a longitudinal socket which surrounds the distal index finger during measurement. The probe has a rigid outer wall and an inner membrane to provide a pressurised uniform field around the finger by applying near-diastolic pressure on the finger. This enables the probe to unload arterial wall tension and increase the signal-noise ratio [297]. Measurement of RH-PAT include quantifying arterial pulsatile volume at rest and during increased shear stress and dilation. Arterial dilation is mediated by NO released from endothelial cells in response to increased blood flow [301,302]. PAT and FMD have different relations with CVD risk factors and correlate weakly with each other [303]. FMD has been shown to correlate with CVD risk factors in childhood and adulthood [298,304,305] and there has been evidence of heritability of FMD [306,307]. Clinically, FMD is a predictor of incident CVD events in adults [308,309]. However, FMD did not predict adverse CVD outcomes in adults according to Anderson et al. [310], whereas hyperemic velocity, the stimulus for FMD, was a significant CVD risk marker [310]. Moreover, evidence on possible interrelationship between IMT and FMD is still controversial [311-313] suggesting that they might be independent markers of structural and functional arterial status. Corretti et al. [314] and Thijssen et al. [315] have introduced guidelines for measurement of FMD in studies of endothelial physiology but AHA does not recommend FMD outside research settings [19].

3. AIMS OF THE STUDY

The aims of this thesis are as follows:

1. To report cardiovascular risk factor levels in 30-45-year-old Finns in the 2007 follow-up, and the 6-year change in risk factor levels between 2001 and 2007 (I). The hypothesis was to observe a 6-year increase in obesity and blood pressure and a decrease in serum total cholesterol in young Finnish adults. Risk factors levels were expected to be elevated in study subjects in 2007.
2. To examine 6-year tracking of IMT, CDist and FMD in young adults and factors affecting tracking (II). The anatomic marker IMT was expected to display higher tracking than the functional markers CDist and FMD.
3. To examine the utility of cardiovascular risk scores in prediction of markers of subclinical atherosclerosis including increased IMT, decreased CDist and decreased FMD in young adults (III). Risk scores were expected to be more highly associated with IMT than with CDist and FMD.
4. To study the utility of metabolic profiling by serum NMR metabonomics in prediction of subclinical atherosclerosis in young adults (IV). Addition of metabonomics data to the conventional risk factor profile was expected to improve prediction of subclinical atherosclerosis.

4. SUBJECTS AND METHODS

4.1. DESCRIPTION OF THE CARDIOVASCULAR RISK IN YOUNG FINNS STUDY

This thesis is part of the Cardiovascular Risk in Young Finns Study. The Cardiovascular Risk in Young Finns Study is an on-going observational multi-centre follow-up study into cardiovascular risk factors and atherosclerosis precursors from childhood to adulthood. The study was started in 1980 and has been performed by five Finnish universities in Helsinki, Kuopio, Oulu, Tampere and Turku. In 1980, 3,596 Finns aged 3, 6, 9, 12, 15 and 18 years participated in the first cross-sectional survey. Since then, follow-ups for the whole study group have been performed in 1983, 1986, 2001 and 2007. In 2001, a total of 2,283 participants aged 24-39 years were re-examined, and in 2007, 2,204 subjects aged 30-45 years were examined. A total of 1,828 subjects participated both in 2001 and 2007. Figure 3 displays the progression of the study between 1980 and 2007 and table 3 displays dropouts and reparticipants. In 2007, among 2,217 study subjects 46 (2.1%) received statins, 152 (6.9%) received antihypertensive medication and 25 (1.1%) received treatment for diabetes according to self-report.

Vascular ultrasound studies were performed amongst 2,265 study subjects aged 24-39 years in 2001, and amongst 2,197 subjects aged 30-45 years in 2007. 1,803 subjects had vascular ultrasound available at both time-points and amongst them the use of lipid-lowering (N=7) and antihypertensive medication (N=43) was rare.

Subjects gave written informed consent in 2001 and 2007. The study complies with the Declaration of Helsinki and the research protocol was approved by local ethics committee.

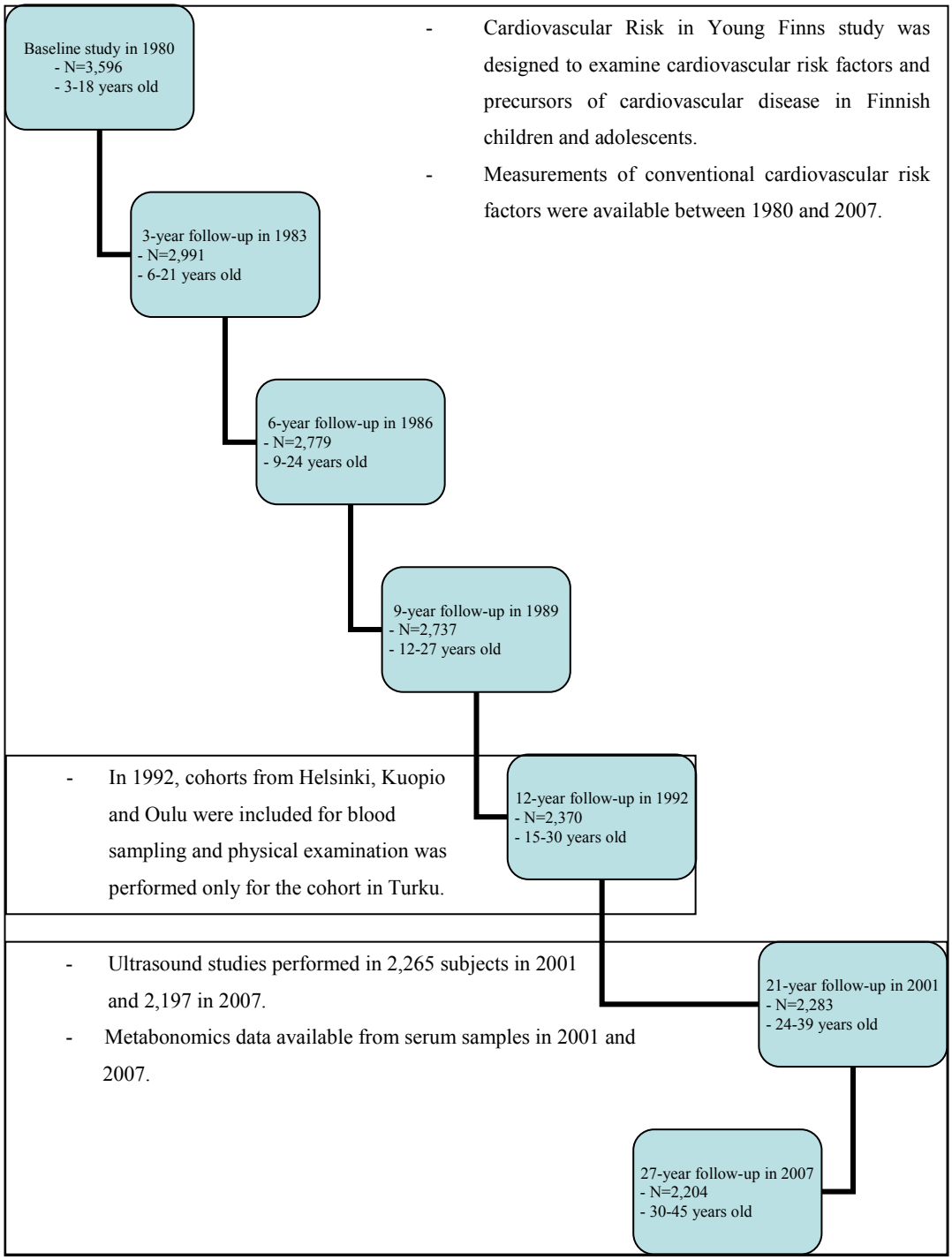


Figure 3. Progression of the study between 1980 and 2007.

Table 3. Subjects to drop out after follow-up and reparticipants from subjects who dropped out after follow-ups in mentioned years.

Year of follow-up	Participants in follow-up	Drop-outs after follow-up	Reparticipants from dropouts				
			1980	1983	1986	2001	2007
1980	3,596	728	-	-	-	-	-
1983	2,991	583	-	-	-	-	-
1986	2,779	734	189	-	-	-	-
2001	2,283	463	223	320	-	-	-
2007	2,204	-	55	60	269	-	-

4.2. BLOOD SAMPLES

In 2001 and 2007, venous blood samples were drawn primarily from the right antecubital vein after an overnight fast and serum was separated, aliquoted and stored at -70°C until analysis. If sampling from the right arm failed, the left antecubital vein was used. Serum total cholesterol levels were measured by the enzymatic cholesterol esterase – cholesterol oxidase method (Cholesterol reagent, Olympus, Ireland) [41]. The same reagent was used for estimating HDL-cholesterol levels after precipitation of LDL and VLDL with dextran sulfate-Mg^{2+} [316]. LDL-cholesterol was estimated by the Friedewald formula [317] in subjects with triglycerides levels <4.0 mmol. The serum triglycerides concentration was assayed using the enzymatic glycerol kinase - glycerol phosphate oxidase method (Triglyceride reagent, Olympus). Serum glucose concentration was determined by the enzymatic hexokinase method (Glucose reagent, Olympus). Apolipoprotein A1 (ApoA1) and B (ApoB) were analysed immunoturbidometrically (Orion Diagnostica, Espoo, Finland) [80]. The above mentioned analyses were all performed on an AU400-analyzer (Olympus, Japan). Serum insulin concentration was determined by a microparticle enzyme immunoassay (IMx insulin reagent, Abbott Diagnostics, USA) on an IMx instrument (Abbott). Serum CRP was determined turbidimetrically (2001: CRP-UL reagent, Wako, USA, 2007: CRP Latex reagent, Olympus, Ireland) on an AU400 analyzer (Olympus, Japan). All the analyses were carried out in the Laboratory for Population Research of the National Institute for Health and Welfare (Turku, Finland). The following methods of the laboratory are accreditated by the Finnish Accreditation Service according to standard ISO/IEC17025: total cholesterol, HDL-cholesterol, triglycerides, apolipoprotein A1 and B, glucose and insulin.

Due to changes in methods or reagents from 2001 to 2007, the 2007 triglycerides, glucose and insulin levels were corrected by using the following correction factor equations. The equations were determined with linear regression analysis utilizing standardized principal component adjustments.

Triglycerides = (triglycerides (2007)+0.03226)/0.9811.

Glucose = (glucose (2007)-0.0235)/0.9471.

Insulin = insulin (2007) ×1,3728-0.8795

No correction equations were needed on the 2007 total cholesterol, LDL-cholesterol and HDL- cholesterol levels, as methods had not changed.

Insulin resistance was estimated with homeostatic model assessment of insulin resistance (HOMA-IR) [124]. HOMA-IR was calculated using the following equation.

HOMA-IR = (glucose × insulin)/22.5

4.3. NMR spectroscopy and metabolite quantification

Two NMR spectra were recorded from each serum sample at 37ºC on a magnetic field strength of 500 MHz [217]. A standard proton NMR spectrum was used for lipoprotein subclass quantification [318]. The spectral information reflecting lipoprotein particle size and concentration was deconvoluted to quantify 14 lipoprotein subclasses in mmol/l by regression modeling. For detection of low-molecular-weight metabolites (LMWM) a spectrum was measured where most signals from the macromolecules are suppressed. Iterative lineshape fitting analysis was used to quantify LMWM in absolute concentration in units relative to the signal intensity.

Metabolic profiling has not been performed in children and adolescents since older serum samples from earlier follow-ups have deteriorated to a degree where reliable measurement of metabolite with NMR methods is no longer possible.

Reproducibility of measurements

The LMWM variables were not selected based on prior knowledge of biological association with atherosclerosis, but only on which metabolites could be reliably quantified in our current experimental protocol. Associations of all quantified LMMW with carotid IMT were assessed. Missing LMWM data (0.3%) was imputed using the nonlinear iterative partial least squares algorithm based on all other covariates [319].

4.4. PHYSICAL EXAMINATION AND QUESTIONNAIRES

The physical examination consisted of measurement of height, weight, systolic and diastolic blood pressure, and waist and hip circumferences [41]. Height was measured with a Seca anthropometer to the nearest centimeter and weight with Seca weighing scales. BMI was calculated by dividing the weight in kilograms by the square of the height in meters. Waist circumference was assessed midway between the iliac crest and the lowest rib and hip circumference at the level of the greater trochanters as the

average of two measurements with an accuracy of 0.1 cm. Blood pressure was measured in the right arm at least three times with a random zero sphygmomanometer in sitting position after a 5 minute rest. Blood pressure was estimated as the average of the three readings of systolic and diastolic blood pressure. The use of antihypertensive medication was regarded as an indication of hypertension. Lifestyle risk factors such as smoking, alcohol consumption and physical activity were examined with questionnaires. The subjects smoking daily were regarded as smokers. Physical activity index was calculated using a metabolic equivalents (MET) index [320]. Study subjects reported the frequency, intensity and duration of both leisure-time and working-time physical activity. One MET unit equals the energy consumption of one kilocalorie per one kilogram of body weight per one hour.

Parental history of myocardial infarction was reported <55 years in either parent in 2001 and <55 years in males and <65 years in females in 2007. History of stroke was reported at any age.

4.5. ULTRASOUND STUDIES

Ultrasound studies were performed on the carotid and brachial arteries with Acuson Sequoia512 ultrasound mainframes (Acuson, Mountain View, California) with 13.0 MHz linear array transducer by the same single measurer in both follow-ups.

Common carotid IMT was measured on the posterior wall of the left common carotid artery approximately 10mm proximal to the carotid bifurcation. A minimum of 4 measurements were performed to calculate mean carotid IMT. The left common carotid artery and carotid bulb area were also scanned for atherosclerotic plaques, defined as distinct areas of the far and near vessel walls protruding into the lumen >50% of the adjacent intima-media layer[321]. Carotid IMT is displayed in Figure 4.

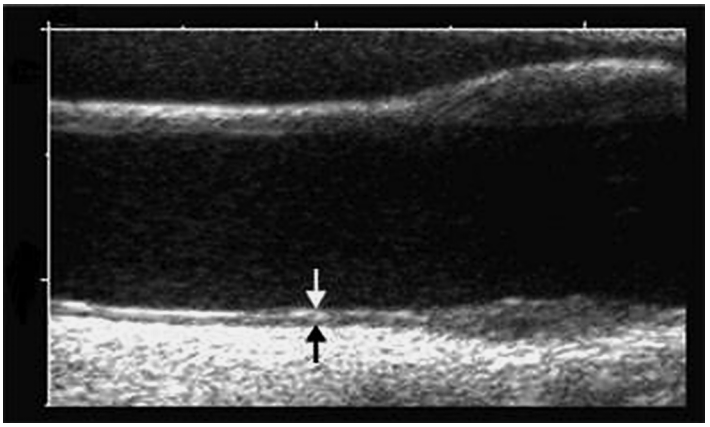


Figure 4. Carotid IMT was measured in the common carotid artery proximal to the carotid bifurcation. IMT is displayed in the figure as the distance between the arrows.

CDist was assessed by measuring the common carotid artery diameter in end-diastole and end-systole. The proportional change between systolic and diastolic values was calculated and distensibility was expressed as the ratio between change in diameter and pulse pressure derived from concomitant brachial blood pressure[321]. CDist=[(systolic diameter–diastolic diameter)/diastolic diameter]/pulse pressure. Carotid diameter is displayed in Figure 5.

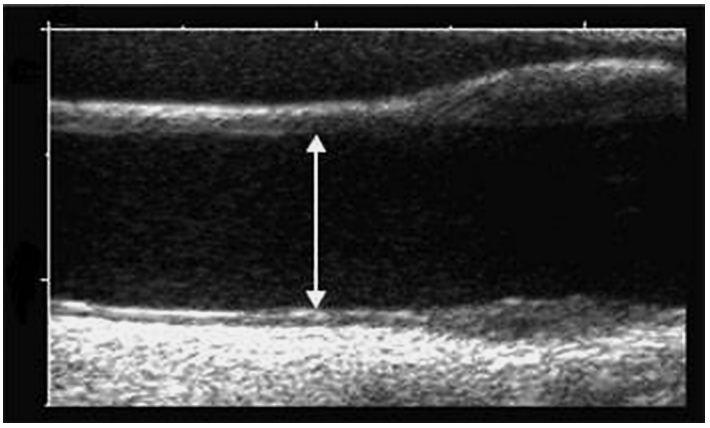


Figure 5. Carotid distensibility was measured as the difference between the carotid diameter in end-systole and end-diastole divided by pulse pressure. Carotid diameter is displayed with the arrow.

Brachial FMD was examined by measuring the left brachial artery diameter both at rest and during reactive hyperemia. The increased blood flow was induced by inflating a

pneumatic tourniquet placed around the forearm to a pressure of 250mmHg for 4.5 minutes and then deflating the tourniquet[321]. Measurement of arterial diameter was performed at end-diastole at fixed distance from an anatomic marker at rest and 40, 60 and 80 seconds after cuff release[321]. The maximum vessel diameter during dilation was expressed as the percentage relative to resting scan. Brachial artery diameter at rest and and during reactive hyperemia is displayed in Figures 6a-b.

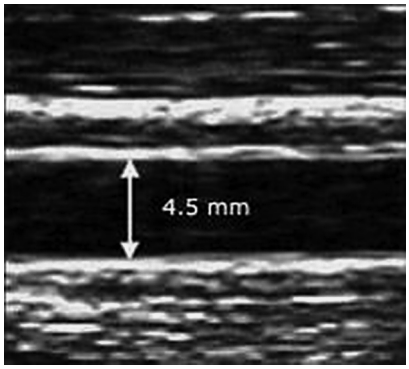


Figure 6a. Brachial artery diameter at rest is displayed with the arrow.

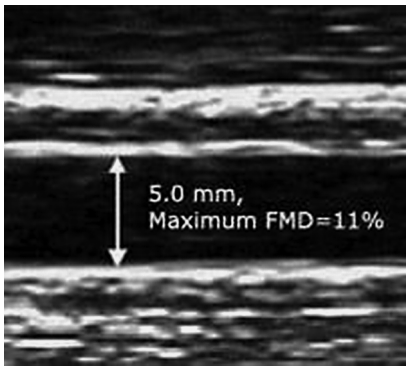


Figure 6b. The diameter of the same brachial artery is displayed during reactive hyperemia and expressed as percentage relative to diameter at rest.

In 2001, 57 subjects were re-examined 3 months after their original scan to assess variability in measurements. The between-visit coefficients of variation (CV) for IMT, CDist and FMD were 6.4%, 16.3% and 26.0% respectively[321].

4.6. STUDY DESIGN OF STUDIES I-IV

In study I, cardiovascular risk factor levels in 2007 and their 6-year change between 2001 and 2007 were studied. Risk factor levels were examined in 2,204 subjects aged 30-45 years in 2007. 6-year changes were assessed as changes in mean values of risk

factor levels related to sex in the follow-up studies in 2001 and 2007. In order to analyse the results, only subjects of the same age were selected for the analyses. Therefore, the age cohort of 30-39-year-old participants in 2001 was compared with its counterpart in the 2007 follow-up. Because some of the subjects belonged to both groups (i.e. study years 2001 and 2007), all statistical analyses concerning 6-year change were performed separately for subjects aged 30-33-years and 36-39 years to avoid analyses using data from the same subjects in both study years.

In study II, 6-year tracking of IMT, Cdist and FMD and factors affecting tracking were studied in 1,809 subjects.

In study III, the prediction of subclinical atherosclerosis with cardiovascular risk scores was examined. Subclinical atherosclerosis was measured by IMT, CDist and FMD in 1,809 subjects.

In study IV, associations between lipoprotein subclasses and low-molecular-weight metabolites determined with NMR-metabonomics and subclinical atherosclerosis were studied. Subclinical atherosclerosis was measured with IMT, CDist and FMD. A total of 1,587 subjects had all metabonomics variables and IMT, CDist and FMD measurements available were subsequently included for this analysis.

4.7. STATISTICAL ANALYSES

The results are stated as mean ± SD, unless stated otherwise. Group comparisons were performed using *t*-test for continuous variables and χ^2-test for categorical variables. Values for serum triglycerides and insulin were $\log_{10}$-transformed prior to analyses due to skewed distributions. The statistical analyses were performed using Statistical Analysis System (SAS, version 9.2), STATA (version 10.0) and MatLab (version 7.5) softwares. Statistical significance was inferred at a 2-tailed P-value <0.05. Scatter plots were examined to determine the distribution of measurements and detect possible outliers. No clear outliers were detected in the data.

Retrospective power analyses were not performed since confidence intervals have been shown to display inadequate sample size to readers better than retrospective power analyses [322].

Study I:

Cardiovascular risk factor levels in 2007 are expressed as mean ± SD and the effect of age on risk factors was studied using linear regression analysis. The similarities of the associations between age and risk factors between men and women were studied with multivariate linear regression models, which included the risk factor as the dependent variable and age, sex and age × sex interaction term as independent variables. The

differences in risk factor level means between men and women in all age groups were determined by applying *t*-test for continuous variables and χ^2-test for categorical variables.

6-year changes were assessed as changes in mean values of risk factor levels related to sex in the follow-up studies in 2001 and 2007. In addition, for easier presentation of results, the mean values for variables are calculated for the combined group. The statistical analysis for secular changes was performed using an unpaired *t*-test. There was no difference in age in either gender between the above mentioned groups in 2001 and 2007 (P always > 0.2), and therefore no adjustments for age were performed.

The prevalences of MetS and its components are expressed as proportions. The effects of age and time (secular trend) on the prevalence of MetS and its components in 2001 and 2007 were studied with logistic regression analysis.

Study II:

Ultrasound measurements and risk factor levels are expressed as mean ± SD unless stated otherwise. Comparisons of mean ultrasound measurement levels were performed using the Mann Whitney test due to skewed distributions of IMT, CDist and FMD. Trends by age were examined with linear regression analyses.

To give reliable long-term estimation on the development of atherosclerotic changes ultrasound measurements should maintain in the same fractiles over time. Tracking was studied to assess whether the examined ultrasound methods fulfilled this requirement.

Two approaches were used to examine tracking of vascular ultrasound measurements. First, IMT, CDist and FMD were divided into age- and sex-specific quintiles at both time-points and the probability of remaining in quintiles expressed. Second, the correlation between measurements in 2001 and 2007 was examined with Spearman's partial correlation analysis. The partial correlation analyses were standardised for sex and age unless analyses were stratified by these variables. Tracking of conventional risk factors was examined with Spearman's correlation. All studies were standardized by age except analyses on Framingham risk score which includes age as a CVD risk factor. The degree of tracking was estimated for correlation coefficients as follows: <0.30 for low, 0.30-0.60 for moderate, 0.60-0.90 for moderately high and >0.90 for high [323].

Spearman's partial correlation models between ultrasound measurements in 2001 and 2007 were standardised by age and stratified by BMI, blood pressure and Framingham risk score groups in analyses concerning the effect of categorical risk factors on

tracking. Comparisons between tracking correlation coefficients were examined using Fisher r-to-z transformation [324].

Effect of cardiovascular risk on stability was studied by measuring tracking in groups below and above sex-specific median of 10-year CVD risk according to Framingham [325] and SCORE [226] risk scores based on risk factors in 2001. Median for Framingham risk score was 0.66% in females and 1.70% in males and median for SCORE was 0.010% in females and 0.14% in males.

Short-term reproducibility of ultrasound measurements was examined using Spearman's partial correlations between ultrasound measurements in 2001 and the re-examination 3 months later and the measurements in 2007. Correlation analyses were also performed between mean values of the measurements in 2001 and the re-examinations and measurements 6 years later. Analyses were standardised by sex and age.

Study III:

Cross-sectional (2001 and 2007) and longitudinal associations between risk scores and ultrasound measurements were examined using Spearman's correlations.

The utility of baseline risk scores to predict 6-year subclinical atherosclerosis was examined. These analyses were based on separate logistic models that included a single binary subclinical outcome with a single risk score as the predictor variable. For comparisons, the Framingham risk score was used as the reference risk score, with subsequent comparisons made between Framingham with Finrisk, SCORE, PROCAM, or Reynolds risk scores. A number of criteria, put forward by the AHA [326], were used to compare performance between risk scores.

Calibration of each model within groups (tenths) was assessed using the Hosmer-Lemshow (H-L) chi-square statistic[327]. Values >20 ($P<0.01$) suggest a lack of adequate calibration [328].

Discrimination was estimated using area under the receiver operating characteristic curve (AUC) determined for each logistic regression model. Differences in AUC between Framingham and Finrisk, SCORE, PROCAM, or Reynolds risk score models was estimated using the DeLong algorithm [329].

Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were calculated to determine the extent to which Finrisk, SCORE, PROCAM or Reynolds risk scores compared with the Framingham score reassigned participants to a risk level or category that better reflected their final outcome status (case or control) [330,331]. For NRI, participants were assigned to four categories reflecting their 6-year risk of the subclinical outcome. Risk categories for high IMT/plaque were <14%, 14-16%,

16-20%, ≥20%. Categories for low CDist and low FMD were <9%, 9-11%, 11-15%, ≥15%.

IDI is the continuous version of NRI where, instead of assigning categories of risk, differences between risk probabilities for the two models are averaged and differenced for cases and controls. A P≤0.01 for IDI comparisons suggests improved model performance [330].

Study IV:

A dichotomous score representing increased subclinical atherosclerosis was defined as incidence of carotid IMT≥90th percentile and/or presence of carotid plaque at 6-year follow-up. Individuals with high carotid IMT or plaque at baseline were excluded. Baseline characteristics were compared using two-tailed χ^2-test, t-test and Kolmogorov-Smirnov test, adjusted for age, sex, and BMI, for categorical, normally distributed and skewed variables, respectively. The relations of systemic metabolites to subclinical atherosclerosis were investigated according to criteria suggested by the AHA [332]. Logistic regression models for lipids and lipoprotein subclasses were adjusted for sex, age, systolic and diastolic blood pressure, BMI, and family history of CVD. Models for low molecular weight and lipid metabolites were further adjusted for conventionally assayed LDL-cholesterol, HDL-cholesterol, and triglycerides. For validation of novel amino acid biomarkers, cross-sectional associations with carotid IMT were assessed using linear regression for 1028 individuals from the Health 2000 study [148]. To assess association between CDist and FMD and metabolic profiling, logistic regression analyses for low CDist (CDist≤20th percentile) and FMD (FMD≤20th percentile) prevalence at 6-year follow-up were adjusted for sex, baseline age, body mass index and systolic blood pressure. The low-molecular-weight and serum extract metabolite associations were further adjusted for baseline conventional serum total cholesterol and triglycerides. Association between high progression of IMT (IMT progression in 6 years ≥80th percentile) and metabolic profiling was examined with logistic regression analyses adjusted for sex, baseline age, body mass index, systolic blood pressure and baseline IMT. The low-molecular-weight and serum extract metabolite associations were further adjusted for baseline conventional serum total cholesterol and triglycerides.

Derivation of prediction models

The incremental value of adding circulating metabolic biomarkers to established risk factors for prediction of high IMT was examined based on multivariate logistic regression models. The prediction models were derived according to the following steps: non-laboratory risk factors were selected based on the lowest deviance (-2·log-

likelihood), indicating the best model fit. All combinations of the following variables were tested: age, sex, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, smoking status, and family history of CVD. Subsequently, conventional lipids were tested with the selected non-laboratory risk factors forced into the models. The model with lowest deviance was selected from all possible combinations of total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, apoB, apoA1, total cholesterol/HDL-cholesterol, and apoB/apoA1 -ratio, glucose, and C-reactive protein. This model (age, sex, BMI, systolic and diastolic blood pressure, family history of CVD, LDL-cholesterol, and HDL-cholesterol) constitutes the reference (Model A). The reference model was compared to prediction models where NMR-based metabolites were allowed to complement conventional lipid risk factors. In order to derive the most parsimonious prediction models only metabolites with nominally significant odds ratios (OR) ($P<0.05$) were included in the model selection [333]. Backwards stepwise selection (threshold $P<0.05$) was used with non-laboratory risk factors forced into the models. Three comparison models were derived: NMR-based lipoprotein lipid and subclass measures were allowed to complement or replace conventional lipids (Model B), low-molecular-weight metabolites were additionally included in the model selection (Model C), and finally, lipid metabolites were allowed to enter the model (Model D). For derivation of prediction models missing metabolite data (1.0%) were imputed using the nonlinear iterative partial least squares algorithm based on all other covariates in the models [319]. Individuals (n=17) with missing data in the selected biomarkers, tyrosine and docosahexaenoic acid, were subsequently excluded for evaluation of prediction model performance.

Model selection for prediction of low CDist and FMD was performed in the same way. For low CDist, reference model (model A) included age, systolic and diastolic blood pressure, waist circumference, LDL-cholesterol, apoB, glucose and CRP. Reclassification analyses were performed between the derived models. No models were derived for prediction of low FMD since all associations between low FMD and metabonomics variables were nonsignificant.

Evaluation of prediction models

The ability to discriminate risk was estimated using AUC. Comparison of AUC between the models were estimated using the DeLong algorithm [334]. Log-likelihood ratio (χ^2 for each model as compared to a model with nonlaboratory risk factors only) and Aikake Information Criterion (AIC) were used to provide estimates of global fit. Calibration of each model within risk deciles was assessed using HL goodness-of-fit [335]. NRI and IDI were calculated to determine the extent to which addition of

metabolite data reassigned participants to risk levels that better reflected their outcome status [336,337]. For NRI, participants were assigned to one of four categories (<5%, 5%-10%, 10%-20%, and >20%) that reflected their 6-year risk of incident high IMT or plaque based on each model. The proportions of participants correctly reclassified to either higher- or lower-risk categories using models B, C, or D were compared with model A. All risk prediction models for incident high IMT were evaluated using 10-fold cross-validation so that prediction of an individual's risk is not influenced by his or her own outcome status in order to avoid overfitting. The median of discrimination, reclassification, global fit and calibration metrics for 50 cross-validation repeats are presented. Because there is no clinical consensus on what signifies high IMT, low CDist and FMD, the predictive performance of the models using alternate cut-points to define high IMT, low CDist and FMD, were examined with similar results obtained. Statistical significance was inferred for $P<0.05$.

5. RESULTS

5.1. Study I:
Follow-ups of the Cardiovascular Risk in Young Finns Study in 2001 and 2007: Levels and 6-year changes in risk factors

5.1.1. Attrition analyses

Risk factor levels in 2001 in participants and non-participants in the follow-up in 2007 were examined for potential differences in baseline risk factor levels and measurements of subclinical atherosclerosis between the groups (Table 4a-b). The objective was to determine if non-participation at follow-up of subjects with high or low risk factors was responsible for any of the observed changes in risk factor levels. In men systolic blood pressure and prevalence of smoking were higher in non-participants than in participants. In women, non-participants were younger and had higher waist circumference, systolic blood pressure and prevalence of smoking than participants. Thus, increases in systolic pressure and waist circumference might have been higher than observed. Moreover, prevalence of smoking could have been higher in both sexes in 2007.

There were no significant differences in mean levels of subclinical atherosclerosis between participants and non-participants. Therefore, the mean state of atherosclerosis was not affected by selection bias.

Table 4a. Differences in risk factor levels (in 2001) between participants (participated both in 2001 and 2007) and non-participants (participated in 2001, but not in 2007) in the follow-up in 2007.

	Men		
	Participants	Non-Participants	P-value for difference
N	803	223	
Age in 2001	31.6	31.0	0.12
BMI (kg/m^2)	25.7	26.0	0.27
Waist (cm)	89.8	90.0	0.78
Systolic BP (mmHg)	121.1	123.3	0.02
Diastolic BP (mmHg)	72.9	74.2	0.12
Total cholesterol (mmol/l)	5.24	5.32	0.28
LDL cholesterol (mmol/l)	3.43	3.40	0.67
HDL cholesterol (mmol/l)	1.15	1.18	0.18
ApoA1 (g/l)	1.40	1.42	0.14
ApoB (g/l)	1.13	1.14	0.57
Triglycerides (mmol/l)	1.51	1.61	0.081
Insulin (mU/l)	7.6	8.2	0.19
Glucose (mmol/l)	5.2	5.3	0.41
Smoking (%)	27.0	42.1	<0.0001
Alcohol consumption (daily doses)	1.22	1.40	0.15

	Women		
	Participants	Non-Participants	P-value for difference
N	1025	232	
Age in 2001	31.7	30.8	0.0072
BMI (kg/m^2)	24.4	25.0	0.094
Waist (cm)	79.0	80.9	0.025
Systolic BP (mmHg)	112.3	114.5	0.015
Diastolic BP (mmHg)	68.6	69.4	0.34
Total cholesterol (mmol/l)	5.09	5.08	0.95
LDL cholesterol (mmol/l)	3.16	3.16	0.90
HDL cholesterol (mmol/l)	1.40	1.38	0.32
ApoA1 (g/l)	1.57	1.56	0.74
ApoB (g/l)	1.00	1.02	0.39
Triglycerides (mmol/l)	1.17	1.22	0.31
Insulin (mU/l)	7.8	8.3	0.25
Glucose (mmol/l)	4.91	4.93	0.66
Smoking (%)	17.6	26.6	0.0022
Alcohol consumption (daily doses)	0.49	0.55	0.45

Comparison between participants and non-participants performed using *t*-test.

To examine the effect of age difference on differences in risk factors between participants and non-participants, analyses were performed stratified by sex and age. The results were similar suggesting that observed differences were not driven by age difference.

Table 4b. Differences in measurements of subclinical atherosclerosis (in 2001) between participants (participated both in 2001 and 2007) and non-participants (participated in 2001, but not in 2007) in the follow-up in 2007.

	Men Participants	Non-Participants	P-value for difference
N	794	224	
IMT (mm)	0.59	0.60	0.26
CDist (%/mmHg)	2.00	2.01	0.88
FMD (%)	6.84	7.32	0.13
	Women Participants	Non-Participants	P-value for difference
N	1015	232	
IMT (mm)	0.57	0.57	0.63
CDist (%/mmHg)	2.33	2.25	0.14
FMD (%)	8.78	9.03	0.46

Comparison between participants and non-participants performed using *t*-test.

To examine the effect of age difference on differences in measurements between participants and non-participants, analyses were performed stratified by sex and age. The results were similar suggesting that observed differences were not driven by age difference.

5.1.2. Cardiovascular risk factors in 2007

Mean risk factor profile and mean levels of subclinical atherosclerosis in 2007 in young adults are displayed in Tables 5 and 6a-b. In 30-45-year-old subjects, the mean serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride concentrations in 30-45-year-old adults were 5.05, 3.09, 1.34 and 1.40 mmol/l, respectively. Men had higher total cholesterol, LDL-cholesterol, total cholesterol to HDL-cholesterol ratio, triglycerides, ApoB, systolic and diastolic blood pressure, BMI, waist circumference, alcohol consumption and IMT than women in all age groups. Women had higher HDL-cholesterol and ApoA1 levels, ApoA1 to ApoB ratio, CDist and FMD. In conclusion, men had higher mean CVD risk than women.

Table 5. Lipid risk factors in Finnish women and men aged 30-45 years in 2007 (mean ± SD).

Age (years)	N	Total cholesterol (mmol/l)	LDL-cholesterol (mmol/l)	HDL-cholesterol (mmol/l)	Total cholesterol/ HDL-cholesterol ratio	Triglycerides (mmol/l)	ApoA1 (g/l)	ApoB (g/l)	ApoB/ ApoA1 ratio
Women									
30	170	4.79±0.88	2.71±0.70	1.52±0.40	3.30±0.85	1.27±0.71	1.74±0.34	0.91±0.23	2.03±0.62
33	206	4.74±0.81	2.81±0.64	1.42±0.31	3.44±0.75	1.12±0.64	1.64±0.26	0.90±0.23	1.92±0.52
36	195	4.95±0.82	2.95±0.69	1.44±0.33	3.59±0.97	1.22±0.64	1.68±0.27	0.95±0.23	1.86±0.52
39	234	4.93±0.88	2.96±0.74	1.43±0.30	3.59±0.96	1.20±0.71	1.65±0.25	0.96±0.25	1.83±0.50
42	218	5.11±0.84	3.13±0.75	1.44±0.32	3.70±0.96	1.18±0.66	1.66±0.24	0.99±0.24	1.78±0.53
45	190	5.05±0.87	3.09±0.75	1.44±0.30	3.65±0.91	1.17±0.59	1.65±0.23	0.98±0.23	1.79±0.48
Men									
30	162	4.84±0.88	3.02±0.78	1.19±0.25	4.27±1.28	1.50±1.02	1.47±0.19	1.01±0.26	1.55±0.46
33	146	5.03±0.93	3.19±0.83	1.16±0.27	4.53±1.33	1.58±0.99	1.46±0.21	1.08±0.26	1.44±0.46
36	176	5.07±0.91	3.19±0.76	1.21±0.28	4.45±1.39	1.52±1.00	1.50±0.21	1.08±0.26	1.48±0.44
39	173	5.33±0.92	3.40±0.85	1.21±0.28	4.65±1.43	1.71±1.11	1.53±0.20	1.15±0.27	1.43±0.51
42	182	5.47±0.96	3.48±0.82	1.21±0.29	4.75±1.31	1.83±1.37	1.53±0.21	1.20±0.27	1.35±0.40
45	157	5.38±0.95	3.36±0.81	1.27±0.32	4.41±1.13	1.74±1.17	1.58±0.23	1.16±0.26	1.44±0.41
All women	1210	4.93±0.86	2.95±0.73	1.44±0.33	3.55±0.91	1.19±0.66	1.67±0.27	0.95±0.24	1.86±0.53
All men	994	5.19±0.95	3.28±0.82	1.21±0.28	4.52±1.33	1.65±1.13	1.51±0.21	1.11±0.27	1.45±0.45
P-value*		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
All	2204	5.05±0.91	3.09±0.79	1.34±0.33	3.99±1.22	1.40±0.93	1.60±0.26	1.02±0.27	1.67±0.54
Effect of age									
β (women) †		0.023	0.027	-0.0035	0.024	-0.00294	-0.0038	0.0060	-0.016
P-value (women)		<0.0001	<0.0001	0.0688	<0.0001	0.4418	0.0149	<0.0001	<0.0001
β (men) †		0.041	0.027	0.0052	0.016	0.021	0.0070	0.011	-0.0090
P-value (men)		<0.0001	<0.0001	0.0036	0.053	0.0028	<0.0001	<0.0001	0.0016
P-value for interaction ‡		0.02	0.92	0.001	0.42	0.002	<0.0001	0.02	0.54

*t-test applied between men and women.

† Values are regression coefficients (expressed in mmol/l, g/l, mU/l, mmHg, kg/m^2 or cm) for a 1 unit change in age.

‡ Significant interaction means that the association between a risk factor and age in men is different from that of women.

Table 6a. Non-lipid risk factors in Finnish women and men aged 30-45 years in 2007 (mean ± SD).

Age (years)	N	Insulin (mU/l)	Glucose (mmol/l)	Impaired fasting glucose (%) ¶	blood pressure (mmHg)	blood pressure (mmHg)	BMI (kg/m^2)	Waist (cm)	Smoking (%)	Alcohol consumption (daily doses)
Women										
30	170	8.5±6.3	4.98±0.50	2.9	115±14	71±12	24.3±4.6	80.5±11.6	20.4	0.51±0.79
33	206	8.3±7.8	5.03±0.39	1.0	113±12	71±10	24.4±4.1	80.8±10.5	13.3	0.47±0.68
36	195	9.2±7.8	5.28±1.29	4.2	115±13	73±11	25.6±5.7	84.4±14.5	16.4	0.51±0.66
39	234	8.2±6.0	5.19±0.64	4.3	117±15	74±12	26.4±5.6	85.8±13.5	15.1	0.56±0.71
42	218	8.3±6.3	5.28±1.04	5.1	118±14	75±11	25.3±4.1	84.3±12.0	11.8	0.61±0.79
45	190	8.8±6.9	5.33±0.60	8.4	120±16	76±11	26.0±5.7	85.8±13.3	14.0	0.63±0.67
Men										
30	162	9.1±6.7	5.41±1.48	5.6	123±12	76±11	25.9±4.4	91.1±12.4	26.5	1.25±1.28
33	146	8.7±6.6	5.36±0.63	4.8	124±12	76±10	26.3±4.1	93.0±11.5	26.9	1.52±1.97
36	176	7.8±5.8	5.42±0.48	8.0	125±12	78±10	26.4±4.5	92.2±11.7	17.7	1.35±2.55
39	173	9.6±8.3	5.60±0.98	12.1	127±12	81±10	27.2±4.1	96.2±11.3	26.7	1.54±1.73
42	182	10.5±8.4	5.57±0.65	15.6	128±15	82±11	27.2±3.6	96.2±10.0	21.2	1.26±1.40
45	157	10.3±10.0	5.75±1.26	19.1	128±14	81±12	27.5±4.6	97.2±13.0	18.9	1.54±1.86
All women	1210	8.7±8.3	5.19±0.82	4.3	117±14	73±11	25.4±5.1	83.7±12.8	15.0	0.55±0.72
All men	994	9.6±9.7	5.52±0.98	11.0	126±13	79±11	26.7±4.2	94.4±11.9	22.9	1.40±1.84
P-value*		0.013	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
All	2204	9.1±9.0	5.34±0.91	7.3	121±14	76±11	26.0±4.8	88.6±13.5	18.5	0.93±1.41
Effect of age										
β (women) †		0.0036	0.023		0.40	0.34	0.11	0.36		0.011
P-value (women)		0.93	<0.0001	0.0004	<0.0001	<0.0001	0.0002	<0.0001	0.0963	0.0108
β (men) †		0.13	0.024		0.37	0.43	0.11	0.43		0.0076
P-value (men)		0.01	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1043	0.5143
P-value for interaction ‡		0.05	0.90		0.82	0.30	0.99	0.49		0.79

*t-Test applied between men and women.

† Values are regression coefficients (expressed in mmol/l, g/l, mU/l, mmHg, kg/m^2 or cm) for a 1 unit change in age.

‡ Significant interaction means that the association between a risk factor and age in men is different from that of women.

¶ Fasting glucose>6.0 mmol.

Table 6b. Ultrasound measurements of subclinical atherosclerosis in Finnish women and men aged 30-45 years in 2007 (mean ± SD).

Age (years)	N	IMT (mm)	Cdist (%/mmHg)	FMD (%)
Women				
30	170	0.57±0.07	2.35±0.72	10.62±4.95
33	207	0.58±0.07	2.23±0.68	9.48±4.92
36	192	0.60±0.08	2.10±0.78	10.18±5.24
39	233	0.63±0.08	1.97±0.70	9.73±4.92
42	216	0.63±0.09	1.85±0.66	10.08±4.52
45	191	0.65±0.09	1.70±0.65	9.38±4.61
Men				
30	162	0.59±0.09	1.94±0.68	8.22±4.14
33	146	0.62±0.08	1.94±0.64	7.68±3.71
36	174	0.63±0.10	1.80±0.55	7.25±3.86
39	171	0.65±0.10	1.68±0.59	7.72±3.71
42	181	0.68±0.10	1.59±0.53	7.52±3.38
45	156	0.69±0.12	1.52±0.57	7.31±3.65
All women	1209	0.61±0.09	2.02±0.73	9.89±4.87
All men	988	0.64±0.11	1.74±0.61	7.61±3.74
P-value*		<0.0001	<0.0001	<0.0001
All	2197	0.62±0.10	1.90±0.69	8.86±4.54
Effect of age				
β (women) †		0.00576	-0.04334	-0.04119
P-value (women)		<0.0001	<0.0001	0.15
β (men) †		0.00728	-0.03139	-0.04283
P-value (men)		<0.0001	<0.0001	0.07
P-value for interaction ‡		0.047	0.033	0.97

**t*-Test applied between men and women.

† Values are regression coefficients (expressed in mmol/l, g/l, mU/l, mmHg, kg/m^2 or cm) for a 1 unit change in age.

‡ Significant interaction means that the association between a risk factor and age in men is different from that of women.

5.1.3. Changes in risk factor levels between 2001 and 2007

Between 1986 and 2001, there was an increase in BMI and triglycerides levels in 24-year-olds while total cholesterol levels decreased only 5% [14]. Changes in risk factor levels and subclinical atherosclerosis between 2001 and 2007 were examined among 30-33-year-olds and 36-39-year-olds (Tables 7, 8a-b and 9 and Figures 7-10). A significant decline in total cholesterol among both sexes was observed. A significant decrease was also observed in LDL-cholesterol. There was a significant increase in HDL-cholesterol in 30-33-year-old women but no significant change was observed in other age groups. The ratio of total cholesterol and HDL-cholesterol decreased significantly among both sexes in all age groups. ApoA1 level increased and ApoB level decreased among both sexes. The trend of the ratio of ApoB to ApoA1 was favourable among both sexes. There was a significant increase in glucose levels in all age groups except 30-33-year-old men. Waist circumference and systolic blood pressure increased among 36-39-year-old men and women, whereas diastolic blood pressure increased in all age groups. Body weight and BMI increased significantly only among 36-39-year-old women. There was a minor increase in hip circumference in 30-33-year-old women. Between 2001 and 2007 the prevalence of impaired fasting glucose (fasting glucose> 6.0 mmol) increased significantly in men but not in women. The trends in triglycerides, serum insulin and alcohol consumption were nonsignificant. The MET index indicated nonsignificant change in mean physical activity. There was no significant change in either mean age or sex structure of the cohort between 2001 and 2007. Secular trends in hip circumference are displayed in Figures 1-2 in the Appendix.

In women, IMT increased in 36-39-year-olds between 2001 and 2007. FMD increased in 30-33-year old and 36-39-year old women and in 30-33-year old men. Levels of ultrasound measurements in 2001 and 2007 are displayed in Figures 3-7 in the Appendix.

In 2001, according to the questionnaires among 1,779 subjects aged 30-39 years, 58 (3.3%) were using antihypertensive medication, 7 (0.4%) were using medication for hypercholesterolaemia and 7 (0.4%) subjects received any treatment for diabetes. In 2007, among 1,459 subjects aged 30-39 years, 65 subjects (4.5%) were using antihypertensive medication, 18 subjects (1.2%) received medication for hypercholesterolaemia, 5 (0.3%) were given orally administrative medication for diabetes and 11 (0.8%) were using insulin.

While examining only those study subjects who participated both in 2001 and 2007, similar results were found as when examining those subjects who participated in at

least one of the two follow-ups. Total cholesterol, LDL-cholesterol and ApoB levels had decreased significantly in both sexes. However, the change in diastolic blood pressure was nonsignificant in 30-33-year-old men (73.6 mmHg vs. 75.2 mmHg, P=0.08). Otherwise, there were no differences between the analyses.

To assess the effects of antihypertensive medication and medication for hypercholesterolaemia on the risk factor changes trends in lipid levels and blood pressure were examined in study subjects excluding those with the mentioned medication. The trends in total cholesterol, LDL-cholesterol, ApoA1 and ApoB in both sexes without lipid medication were significant (P always <0.001) and essentially similar with the trends in the total cohort. The trends in triglyceride levels in both sexes and in HDL-cholesterol in men was nonsignificant whereas in 30-33-year-old women it was significant and favourable. The trends in systolic and diastolic blood pressure in subjects without antihypertensive medication were significant (P always <0.01) and essentially similar with the trends in the total cohort.

Table 7. Changes in laboratory cardiovascular risk factors during 2001-2007 in 30-39-year-old Finnish adults.

	WOMEN				MEN			
Risk factor	**2001**	**2007**	**%**	**P-value**	**2001**	**2007**	**%**	**P-value**
Total cholesterol (mmol/l)								
Age 30-33 years	5.10	4.77	-6.6	<0.0001	5.27	4.93	-6.5	<0.0001
Age 36-39 years	5.21	4.94	-5.3	<0.0001	5.60	5.20	-7.2	<0.0001
Age 30-39 years	5.16	4.86	-5.8	<0.0001	5.43	5.07	-6.6	<0.0001
LDL-cholesterol (mmol/l)								
Age 30-33 years	3.18	2.77	-13.0	<0.0001	3.43	3.10	-9.6	<0.0001
Age 36-39 years	3.31	2.96	-10.7	<0.0001	3.71	3.30	-11.1	<0.0001
Age 30-39 years	3.24	2.87	-11.6	<0.0001	3.57	3.20	-10.2	<0.0001
HDL-cholesterol (mmol/l)								
Age 30-33 years	1.39	1.47	5.8	0.002	1.15	1.18	2.2	0.24
Age 36-39 years	1.39	1.43	2.7	0.08	1.19	1.21	1.7	0.36
Age 30-39 years	1.39	1.45	3.9	0.0006	1.17	1.19	2.1	0.10
Triglycerides (mmol/l)								
Age 30-33 years	1.19	1.19	0.2	0.91	1.55	1.54	-1.0	0.58
Age 36-39 years	1.14	1.21	5.9	0.18	1.65	1.62	1.9	0.73
Age 30-39 years	1.17	1.20	3.0	0.30	1.60	1.58	-1.2	0.71
ApoA1 (g/l)								
Age 30-33 years	1.57	1.69	7.5	<0.0001	1.40	1.47	5.0	<0.0001
Age 36-39 years	1.55	1.66	7.2	<0.0001	1.45	1.51	4.6	<0.0001
Age 30-39 years	1.56	1.67	7.3	<0.0001	1.42	1.49	4.9	<0.0001
ApoB (g/l)								
Age 30-33 years	1.01	0.90	-10.9	<0.0001	1.14	1.05	-8.3	<0.0001
Age 36-39 years	1.02	0.96	-6.4	<0.0001	1.22	1.11	-8.8	<0.0001
Age 30-39 years	1.02	0.93	-8.4	<0.0001	1.18	1.08	-8.3	<0.0001
Insulin (mU/l)								
Age 30-33 years	7.75	8.92	15.1	0.67	7.60	8.71	14.7	0.76
Age 36-39 years	7.48	8.92	19.3	0.37	7.80	8.71	11.6	0.65
Age 30-39 years	7.62	8.68	13.9	0.46	7.70	9.01	17.0	0.06
Glucose (mmol/l)								
Age 30-33 years	4.89	5.01	2.4	0.004	5.26	5.38	2.4	0.16
Age 36-39 years	5.01	5.23	4.5	0.0002	5.32	5.51	3.6	0.0014
Age 30-39 years	4.95	5.13	3.7	<0.0001	5.29	5.45	3.1	0.002

Table 8a. Changes in non-laboratory cardiovascular risk factors during 2001-2007 in 30-39-year-old Finnish adults.

	WOMEN				MEN			
Risk factor	**2001**	**2007**	**%**	**P-value**	**2001**	**2007**	**%**	**P-value**
Body mass index (kg/m^2)								
Age 30-33 years	24.9	24.4	-2.0	0.13	26.0	26.1	0.4	0.83
Age 36-39 years	24.8	26.0	5.0	0.0008	26.5	26.8	1.1	0.39
Age 30-39 years	24.8	25.3	1.7	0.08	26.2	26.5	0.9	0.33
Weight (kg)								
Age 30-33 years	68.5	67.4	-1.6	0.24	83.9	85.4	1.8	0.20
Age 36-39 years	68.2	71.7	5.1	0.0007	84.6	86.4	2.1	0.11
Age 30-39 years	68.3	69.7	2.0	0.0002	84.2	85.9	2.0	0.04
Waist circumference (cm)								
Age 30-33 years	80.3	80.7	0.5	0.61	90.5	92.0	1.7	0.07
Age 36-39 years	80.7	85.1	5.5	<0.0001	92.5	94.2	1.8	0.048
Age 30-39 years	80.5	83.1	3.2	<0.0001	91.5	93.2	1.9	0.006
Hip circumference (cm)								
Age 30-33 years	100.4	100.8	0.4	0.015	100.4	99.9	-0.4	0.48
Age 36-39 years	99.7	100.8	1.1	0.13	100.1	99.6	-0.5	0.41
Age 30-39 years	100.1	99.9	-0.2	0.68	100.2	99.8	-0.4	0.22
Systolic blood pressure (mmHg)								
Age 30-33 years	112.8	116.2	3.0	0.14	121.8	123.5	1.5	0.06
Age 36-39 years	113.5	116.2	2.3	0.0035	123.2	126.0	2.3	0.002
Age 30-39 years	113.2	115.2	1.8	0.001	122.4	124.8	1.9	0.0004
Diastolic blood pressure (mmHg)								
Age 30-33 years	68.6	73.2	6.6	0.0007	74.0	75.9	2.6	0.02
Age 36-39 years	70.5	73.2	3.8	0.0002	76.2	79.3	4.0	0.0002
Age 30-39 years	69.5	72.2	3.9	<0.0001	75.1	77.7	3.5	<0.0001
Alcohol consumption (daily doses)								
Age 30-33 years	0.47	0.49	4.3	0.18	1.21	1.38	14.4	0.28
Age 36-39 years	0.51	0.54	4.7	0.38	1.28	1.44	12.9	0.29
Age 30-39 years	0.49	0.51	4.3	0.56	1.24	1.41	13.9	0.06
Smoking (%)								
Age 30-33 years	21.6	16.5	-23.9	0.14	30.2	26.6	-11.7	0.40
Age 36-39 years	18.0	15.7	-12.7	0.36	29.8	22.2	-25.6	0.02
Age 30-39 years	19.2	16.1	-16.6	0.08	29.7	24.3	-18.0	0.02

Table 8b. Changes in ultrasound measurements of subclinical atherosclerosis during 2001-2007 in 30-39-year-old Finnish adults.

	WOMEN				MEN			
Risk factor	**2001**	**2007**	**%**	**P-value**	**2001**	**2007**	**%**	**P-value**
IMT (mm)								
Age 30-33 years	0.57	0.58	1.8	0.15	0.59	0.60	1.7	0.30
Age 36-39 years	0.60	0.62	3.3	0.02	0.63	0.64	1.6	0.09
Age 30-39 years	0.59	0.60	1.7	0.002	0.61	0.62	1.6	0.03
CDist (%/mmHg)								
Age 30-33 years	2.32	2.29	-1.3	0.45	1.98	1.94	-2.0	0.51
Age 36-39 years	2.07	2.03	-1.9	0.36	1.78	1.74	-2.2	0.44
Age 30-39 years	2.20	2.15	-2.3	0.14	1.88	1.84	-2.1	0.20
FMD (%)								
Age 30-33 years	9.31	9.99	7.3	0.05	6.91	7.96	15.2	0.001
Age 36-39 years	8.77	9.94	13.3	0.0005	7.01	7.49	6.8	0.12
Age 30-39 years	9.05	9.96	10.0	0.0002	6.96	7.71	10.8	0.0006

Table 9. Changes in non-laboratory cardiovascular risk factors during 2001-2007 in 30-39-year-old Finnish adults. Analyses were nonstratified by sex.

Risk factor	2001	2007	%	P-value	Risk factor	2001	2007	%	P-value
Total cholesterol (mmol/l)					**Body mass index (kg/m^2)**				
Age 30-33 years	5.14	4.84	-5.8	<0.0001	Age 30-33 years	25.2	25.0	-0.8	0.47
Age 36-39 years	5.36	5.06	-5.6	<0.0001	Age 36-39 years	25.4	26.4	3.9	0.0004
LDL-cholesterol (mmol/l)					**Weight (kg)**				
Age 30-33 years	3.28	2.92	-11.0	<0.0001	Age 30-33 years	75.0	75.2	0.3	0.83
Age 36-39 years	3.47	3.11	-10.4	<0.0001	Age 36-39 years	75.1	78.5	4.5	0.0006
HDL-cholesterol (mmol/l)					**Waist circumference (cm)**				
Age 30-33 years	1.28	1.35	5.5	0.002	Age 30-33 years	84.4	85.5	1.3	0.15
Age 36-39 years	1.30	1.33	2.3	0.12	Age 36-39 years	85.8	89.4	4.2	<0.0001
Triglycerides (mmol/l)					**Hip circumference (cm)**				
Age 30-33 years	1.31	1.31	0.1	0.93	Age 30-33 years	100.1	99.3	-0.8	0.11
Age 36-39 years	1.30	1.38	6.2	0.10	Age 36-39 years	99.9	100.3	0.4	0.45
ApoA1 (g/l)					**Systolic blood pressure (mmHg)**				
Age 30-33 years	1.49	1.60	7.4	<0.0001	Age 30-33 years	116.3	117.4	0.9	0.18
Age 36-39 years	1.50	1.59	6.0	<0.0001	Age 36-39 years	117.1	120.4	2.8	<0.0001
ApoB (g/l)					**Diastolic blood pressure (mmHg)**				
Age 30-33 years	1.06	0.96	-9.4	<0.0001	Age 30-33 years	70.8	73.1	3.2	0.0007
Age 36-39 years	1.10	1.03	-6.4	<0.0001	Age 36-39 years	72.6	75.8	4.4	<0.0001
Insulin (mU/l)					**Alcohol consumption (daily doses)**				
Age 30-33 years	7.32	8.84	20.1	0.14	Age 30-33 years	0.80	0.86	7.5	0.18
Age 36-39 years	7.66	8.72	13.8	0.38	Age 36-39 years	0.79	0.97	22.8	0.38
Glucose (mmol/l)					**Smoking (%)**				
Age 30-33 years	5.05	5.17	2.4	0.04	Age 30-33 years	23.0	18.6	-19.1	0.08
Age 36-39 years	5.13	5.34	4.1	<0.0001	Age 36-39 years	19.3	18.6	-3.7	0.75

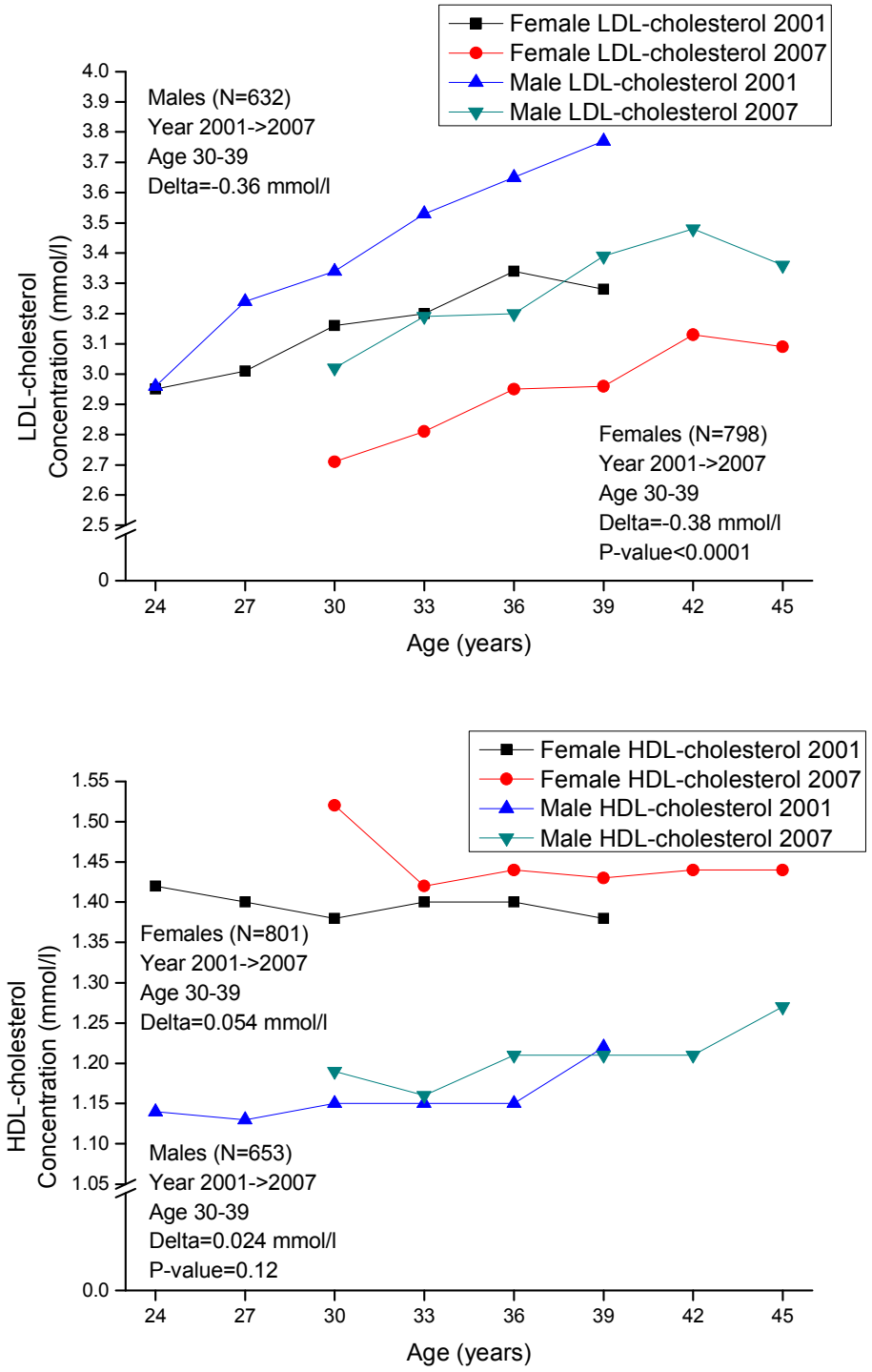


Figure 7. Secular trends in LDL-cholesterol and HDL-cholesterol in men and women between 2001 and 2007.

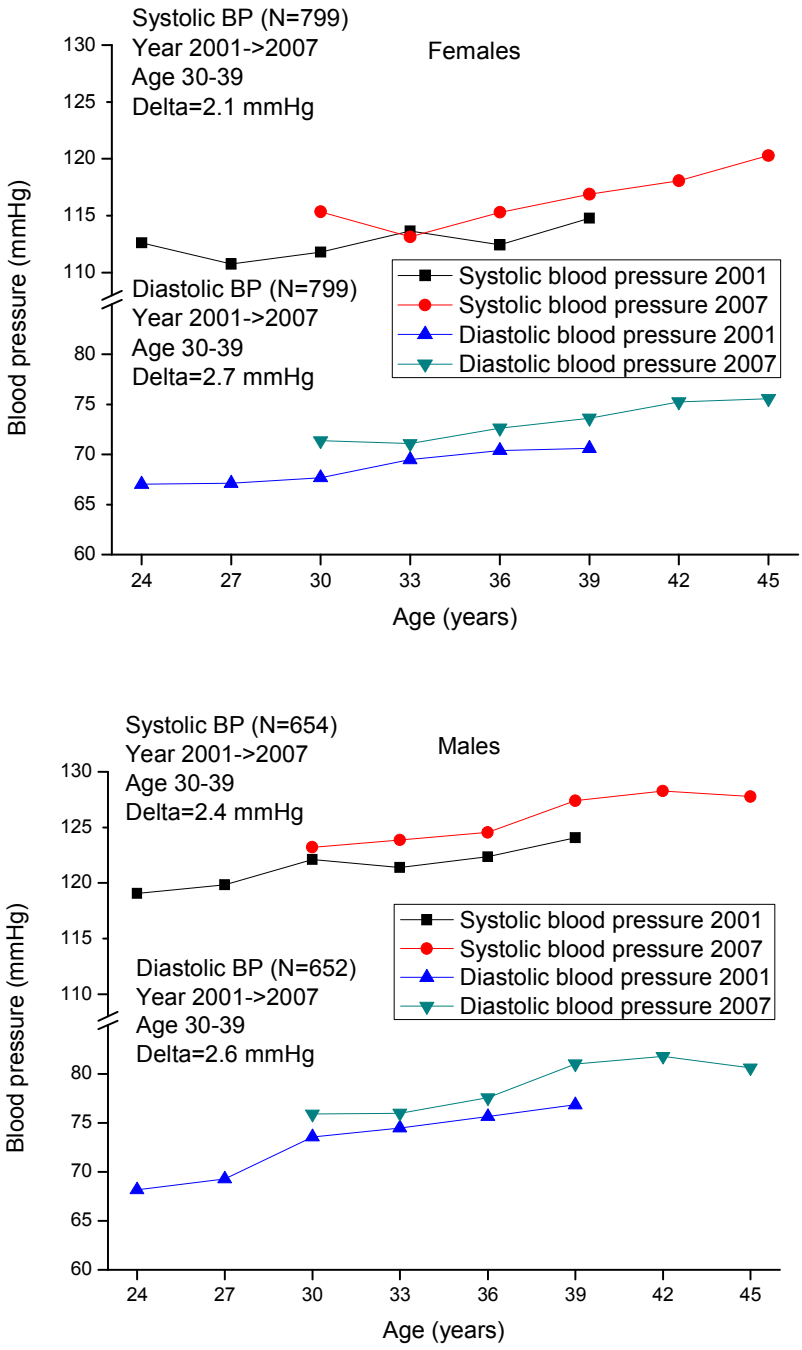


Figure 8. Secular trends in blood pressure in men and women between 2001 and 2007.

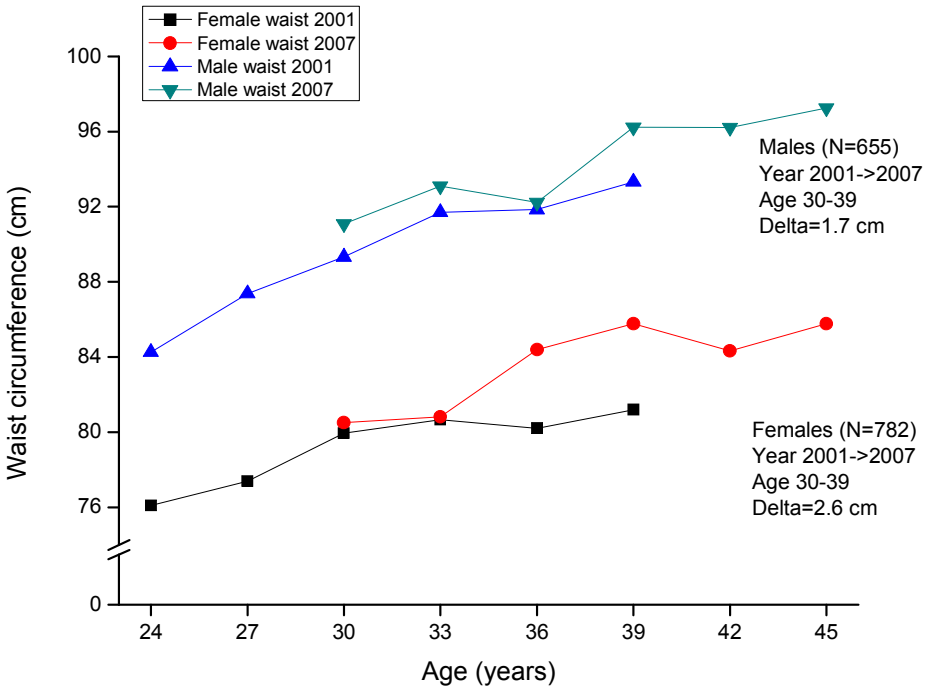


Figure 9. Secular trends in waist circumference in men and women between 2001 and 2007.

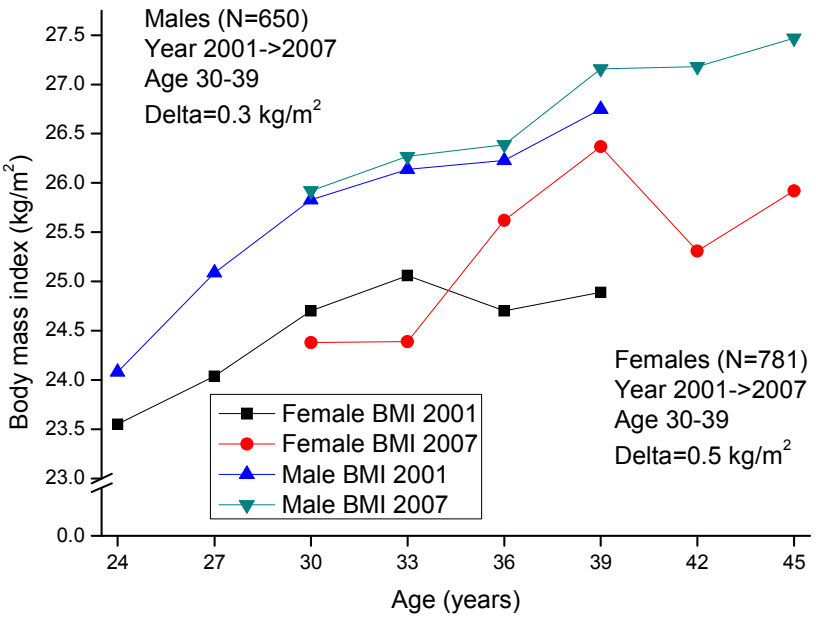


Figure 10. Secular trends in BMI in men and women between 2001 and 2007.

5.1.4. Prevalence of metabolic syndrome

Significant increase in the prevalence of MetS by age was found in both sexes in 2007 according to all classifications ($P<0.05$). According to the NCEP classification the prevalence of MetS was significantly greater among men than among women in the age groups of 33, 39, 42 and 45 ($P<0.05$). Tables 10a and 10b describe the secular trends of MetS in the age groups of 30-39-year-olds between 2001 and 2007 according to the updated NCEP, EGIR and IDF classifications. Between 2001 and 2007 MetS increased significantly both in men and in women in the age group of 30-39-year-olds according to the EGIR classification, whereas the change was nonsignificant in both genders according to the updated NCEP and IDF classifications. Prevalence and secular trends of MetS in 2001 and 2007 has been displayed in Figures 8-13 in the Appendix.

Examination of the components of the updated NCEP classification in the age groups of 30-39-year-olds indicated significant increases between 2001 (N=1516) and 2007 (N=1384) in the prevalences of obesity in women (21.6% vs. 29.2%, P=0.0005), hypertension in men (32.8% vs. 39.3%, P=0.01) and high glucose in both sexes (women: 8.3% vs. 12.5%, P=0.006, men: 20.0% vs. 26.5%, P=0.005). The prevalence of low HDL-cholesterol had decreased between 2001 and 2007 in women (41.1% vs. 35.5%, P=0.009).

Table 10a. Prevalence of the metabolic syndrome and secular trends between 2001 and 2007 in women.

	NCEP			EGIR			IDF		
Age (years)	**2001**	**2007**	P-value	**2001**	**2007**	P-value	**2001**	**2007**	P-value
24	6.6			8.4			10.2		
27	5.1			5.6			6.1		
30	8.4	11.1		7.3	13.1		12.0	14.4	
33	12.7	3.7		9.1	7.8		14.0	7.3	
36	9.7	16.9		7.3	17.4		13.5	17.9	
39	14.2	18.8		6.3	15.6		16.8	22.8	
42		17.9			10.4			20.8	
45		20.3			16.1			25.7	
Prevalence in total cohort	9.6	15.0		7.3	13.4		12.2	18.4	
Prevalence among 30-33-year-olds	10.7	7.0	0.08	8.3	10.1	0.37	13.1	10.4	0.26
Prevalence among 36-39-year-olds	11.8	17.9	0.02	6.8	16.4	<0.0001	15.1	20.6	0.04
Prevalence among 30-39-year-olds	11.3	12.9		7.5	13.6		14.1	15.9	

Table 10b. Prevalence of the metabolic syndrome and secular trends between 2001 and 2007 in men.

	NCEP			EGIR			IDF		
Age (years)	**2001**	**2007**	P-value	**2001**	**2007**	P-value	**2001**	**2007**	P-value
24	8.5			6.6			4.6		
27	12.8			10.8			11.4		
30	18.2	18.0		10.2	19.9		17.1	21.8	
33	18.5	18.4		11.6	21.4		22.5	20.6	
36	22.1	18.9		19.2	17.8		24.7	21.9	
39	21.0	30.3		17.2	20.6		26.4	31.5	
42		33.5			33.0			39.8	
45		29.6			27.0			32.2	
Prevalence in total cohort	17.1	25.0		12.8	23.4		18.1	28.3	
Prevalence among 30-33-year-olds	18.3	18.2	0.96	10.8	20.6	0.0006	19.7	21.2	0.64
Prevalence among 36-39-year-olds	21.6	24.6	0.36	18.4	19.2	0.81	25.5	26.7	0.74
Prevalence among 30-39-year-olds	19.9	21.6		14.5	19.8		22.6	24.1	

5.2. Study II:

Tracking of noninvasive ultrasound measurements of subclinical atherosclerosis in adulthood

Tracking of ultrasound markers of atherosclerosis and factors affecting tracking were examined to determine if noninvasive ultrasound measurements were reproducible and reliable enough to be used in clinical practice.

5.2.1. Tracking of ultrasound measurements

Table 11 displays the effect of age on IMT, CDist and FMD. IMT increases and CDist decreases by age. FMD shows no age-related change. Table 12 displays tracking of ultrasound measurements between 2001 and 2007 stratified by age and sex. Correlations in IMT (all $P<0.0001$) and CDist (all $P<0.001$) were significant in all subgroups. With the exception of nonsignificant correlations in 24-year-old females and 33-year-old males, FMD in 2001 was associated with FMD 6 years later (P always <0.05). In analyses combining data on all age groups, Spearman's nonpartial correlations were statistically significant in males and females for IMT ($r=0.61$, $P<0.0001$; $r=0.52$, $P<0.0001$), CDist ($r=0.41$, $P<0.0001$; $r=0.41$, $P<0.0001$) and FMD ($r=0.23$, $P<0.0001$; $r=0.20$, $P<0.0001$). Similarly, correlations standardised by age were significant in both males and females for IMT ($r=0.56$, $P<0.0001$; $r=0.46$, $P<0.0001$), CDist ($r=0.35$, $P<0.0001$; $r=0.36$, $P<0.0001$) and FMD ($r=0.23$, $P<0.0001$; $r=0.20$, $P<0.0001$).

Figure 11 displays the probability of subjects to remain in their original fractile between follow-ups. Probabilities tended to be the highest in the lower and upper quintiles in both sexes. Males with IMT, CDist and FMD in the highest quintile in 2001 were most likely to maintain the same quintile in 2007 (57.8%, 38.5%, 31.9% respectively). Females in the fifth quintile for IMT and CDist (45.6%, 38.2% respectively) and the first quintile for FMD (33.7%) were most likely to maintain their status in 2007.

Table 11. The effect of age on ultrasound measures in 2001 and 2007.

The β-values are parameter estimates (95% CI) from regression analyses for 1-year increase in age.

* = P<0.0001

	IMT in 2001	IMT in 2007	Cdist in 2001
Men	0.0063 (0.0051-0.0074)***	0.0073 (0.0061-0.0085)***	-0.0414 (-0.0491--0.0337)***
Women	0.0052 (0.0043-0.0061)***	0.0058 (0.0048-0.0067)***	-0.0416 (-0.0500--0.0332)***
	Cdist in 2007	FMD in 2001	FMD in 2007
Men	-0.0314 (-0.0388--0.0240)***	0.0088 (-0.0426-0.0603)	-0.0428 (-0.0891-0.0035)
Women	-0.0433 (-0.0513--0.0354)***	0.0383 (-0.0141-0.0907)	-0.0412 (-0.0968-0.0144)

* P<0.05, ** P<0.01, *** P<0.001

Table 12. Spearman's correlation coefficients between ultrasound measurements in 2001 and 2007 stratified by sex and age.

Men

IMT				**CDist**		**FMD**	
Age	n	r	P-value	r	P-value	r	P-value
24	115	0.45 (0.29-0.58)	<0.0001	0.31 (0.13-0.47)	0.0007	0.24 (0.05-0.41)	0.011
27	111	0.39 (0.22-0.54)	<0.0001	0.46 (0.30-0.60)	<0.0001	0.19 (-0.01-0.37)	0.057
30	142	0.61 (0.50-0.70)	<0.0001	0.30 (0.14-0.44)	0.0003	0.33 (0.17-0.47)	0.0001
33	147	0.49 (0.36-0.60)	<0.0001	0.23 (0.07-0-38)	0.0056	0.10 (-0.07-0.26)	0.24
36	148	0.62 (0.51-0.71)	<0.0001	0.43 (0.29-0.55)	<0.0001	0.25 (0.08-0.41)	0.0055
39	131	0.69 (0.59-0.77)	<0.0001	0.40 (0.25-0-54)	<0.0001	0.21 (0.03-0.38)	0.022

Women

IMT				**CDist**		**FMD**	
Age	n	r	P-value	r	P-value	r	P-value
24	132	0.52 (0.38-0.63)	<0.0001	0.31 (0.15-0.46)	0.0003	0.03 (-0.15-0.20)	0.73
27	170	0.44 (0.31-0.55)	<0.0001	0.28 (0.14-0.41)	0.0002	0.20 (0.05-0.34)	0.013
30	159	0.33 (0.18-0.46)	<0.0001	0.35 (0.21-0.48)	<0.0001	0.32 (0.17-0.46)	<0.0001
33	199	0.45 (0.33-0.55)	<0.0001	0.34 (0.21-0.46)	<0.0001	0.18 (0.04-0.32)	0.012
36	190	0.48 (0.36-0.58)	<0.0001	0.41 (0.29-0.52)	<0.0001	0.22 (0.08-0.36)	0.0027
39	165	0.59 (0.48-0.68)	<0.0001	0.48 (0.35-0.59)	<0.0001	0.24 (0.09-0.38)	0.0029

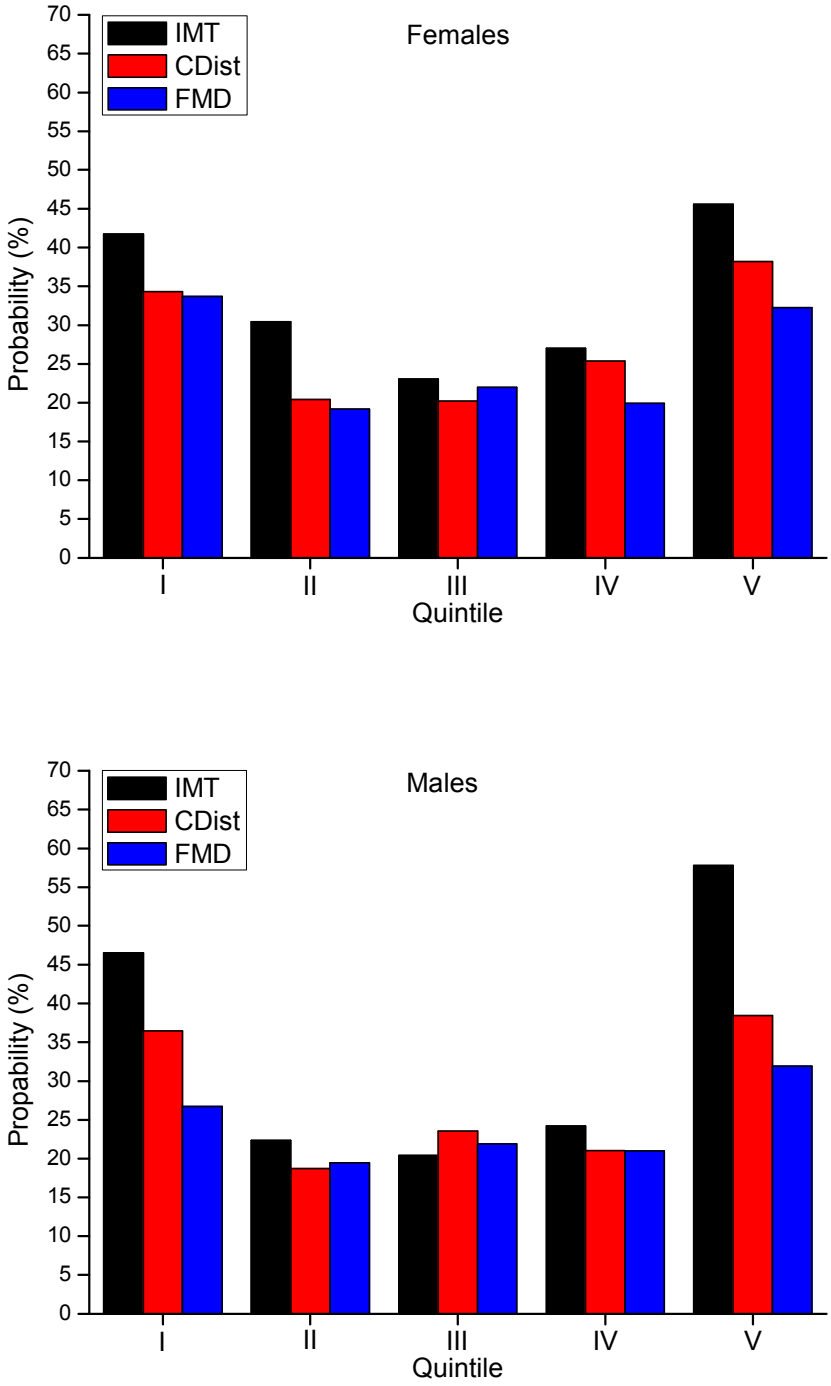


Figure 11. Probability of ultrasound variables to remain in the same fractile from 2001 to 2007.

5.2.2. Factors affecting tracking of ultrasound measurements

In men, 6-year tracking of IMT was better in those aged 33-39 years at baseline compared with those aged 24-30 years (r=0.60 vs. r=0.50, P=0.03). An apparent sex difference in tracking of IMT was observed, in men tracking more strongly compared with women (r=0.56 vs. 0.46, P=0.0059).

To examine how risk factor levels influence tracking, tracking of ultrasound measurements in groups with different risk factor levels was compared. Figures 12a-c display tracking of ultrasound measurements between 2001 and 2007 among BMI, SCORE risk score groups and normotensive and hypertensive in 2001. Tracking of CDist tended to be decreased in subjects with baseline BMI≥30kg/m^2 (r=0.36 vs. r=0.19, P=0.01). Tracking of IMT (r=0.44 vs. R=0.57, P=0.0001) and CDist (r=0.32 vs. r=0.40, P=0.03) were significantly higher in subjects with 10-year CVD risk above median according to SCORE risk score at baseline. Tracking of IMT displayed tendency towards increasing in subjects with 10-year CVD risk above median according to Framingham risk score (r=0.47 vs. r=0.53, P=0.06), whereas no difference was observed in tracking of CDist (r=0.32 vs. r=0.38, P=0.14) and FMD (r=0.21 vs. r=0.22, P=0.83). The results are displayed in Figure 14 in the Appendix. Tracking in normotensive and hypertensive subjects was equally strong (IMT: r=0.49 vs. 0.55, P=0.14; CDist: r=0.33 vs. r=0.27, P=0.25; FMD: r=0.21 vs. r=0.23, P=0.65; P for all correlation coefficients <0.0001). Figure 12c displays tracking stratified by sex. In men, tracking of CDist was higher in normotensive (r=0.35 vs. r=0.19 ,P=0.04).

Influence of age and sex on the effect of risk factors was examined by performing the analyses stratified by sex and comparing mean age with two-tailed T test. No difference was observed between low and high BMI in tracking of IMT in men (r=0.54 vs. r=0.45, P=0.16) and women (r=0.45 vs. r=0.50, P=0.50), in tracking of FMD in men (r=0.22 vs. r=0.25, P=0.78) and women (r=0.20 vs. r=0.06, P=0.17) and in tracking of CDist in men (r=0.36 vs. r=0.24, P=0.21). Tracking of CDist in women was higher in subjects with low BMI (r=0.38 vs. r=0.16, P=0.02). Men with BMI≥30kg/m^2 were older than men with lower BMI (34.0 years vs. 31.6 years, P<0.0001). No difference was observed in women (32.2 years vs. 31.9 years, P=0.64). Older men had higher tracking of IMT than younger men, which may have been responsible for the observed nonsignificant change between low and high BMI groups in tracking of IMT.

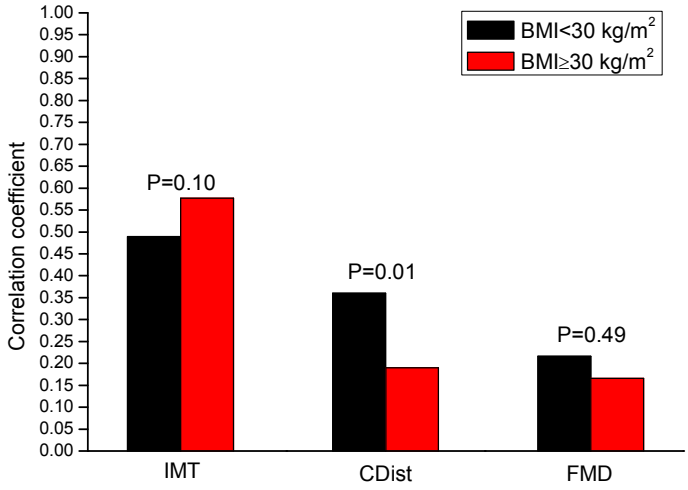


Figure 12a. Correlation between ultrasound measurements in 2001 and 2007 in BMI groups (BMI<30 kg/m^2 and BMI≥30 kg/m^2) based on BMI values in 2001. Significant P-values indicate a difference in correlation between BMI groups.

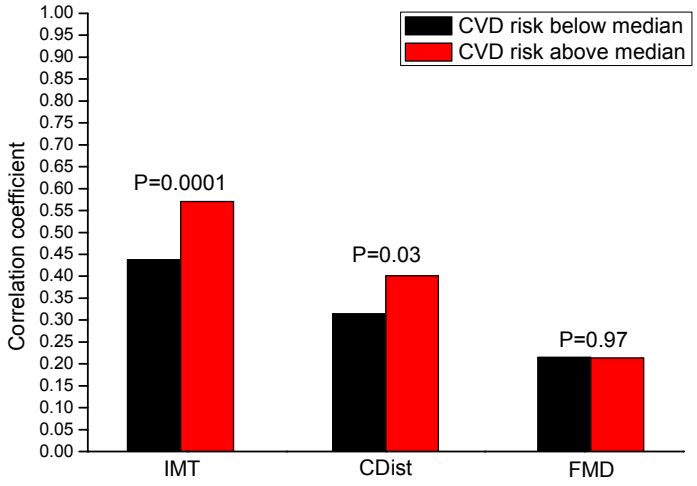
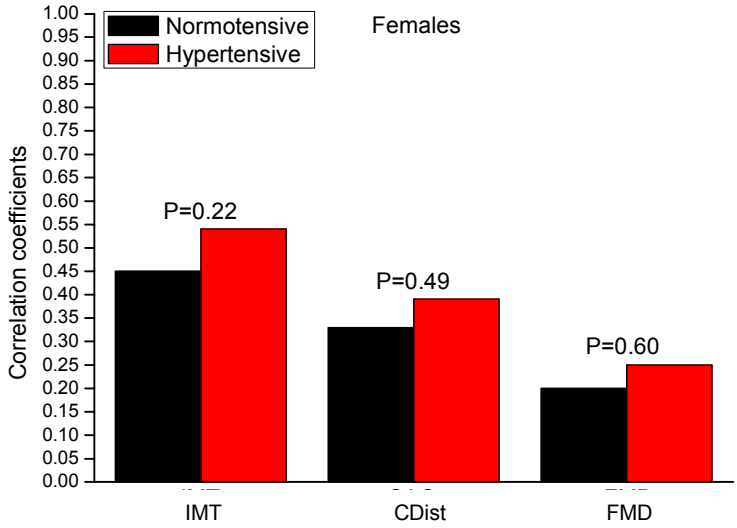


Figure 12b. Correlation between ultrasound measurements in 2001 and 2007 in groups below and above median of estimated 10-year risk of CVD event according to the SCORE risk score based on data in 2001. Significant P-values indicate a difference in correlation between CVD risk score groups.



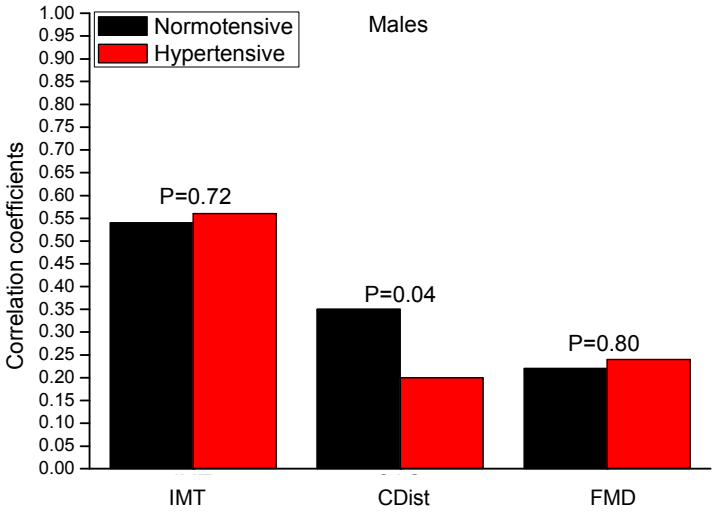


Figure 12c. Correlation between ultrasound measurements in 2001 and 2007 in normotensive and hypertensive men and women.

5.2.3. Comparison of 3-month and 6-year tracking of ultrasound measurements

Figure 13 demonstrates Spearman's correlations between the original measurements in 2001 and the re-examinations conducted in 57 subjects 3 months later and the measurements among the same subjects in the follow-up in 2007. In IMT, there was little short-term variability but long-term tracking between 2001 and 2007 was only moderate (r=0.64 vs. r=0.49, P<0.0003 in both). CDist demonstrated poorer reproducibility and tracking (r=0.51 vs. r=0.48, P<0.0005 in both). Significant short-term variability and moderate tracking in FMD (r=0.39 vs. r=0.36, P<0.02 in both) was observed. The difference between 3-month and 6-year tracking was nonsignificant in all methods.

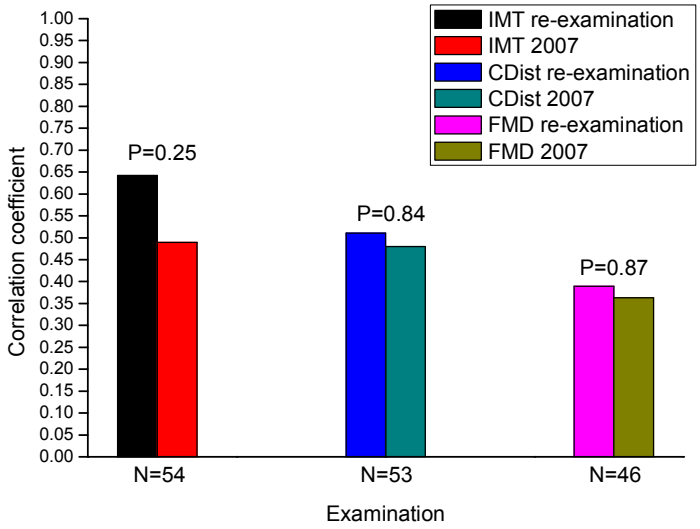


Figure 13. Correlation between original ultrasound measurements in 2001 and re-examinations in 2001 (3-month tracking) and measurements in 2007 (6-year tracking). P-values indicate whether there is significant difference between 3-month tracking and 6-year tracking.

5.3. Study III:

Cardiovascular risk scores in the prediction of subclinical atherosclerosis in young adults

Prediction of ultrasound markers of atherosclerosis were studied to examine if risk scores with clinical outcomes could also predict subclinical asymptomatic changes.

5.3.1. Association between risk scores and ultrasound measurements

Mean and median values for risk scores are displayed in Tables 13 and 14. Table 15 displays Spearman's correlation between 10-year risk scores and ultrasound measurements. For IMT and CDist, all correlations were significant (P always <0.001). Correlations for FMD were not consistent and either nonsignificant or low ($-0.07 \leq r \leq 0.09$).

Table 13. Descriptive data in 2001 and 2007.

Year 2001 Women						
Age (years)	24	27	30	33	36	39
N	132	170	159	199	190	165
Framingham (%)	0.28±0.32	0.38±0.35	0.71±0.68	1.05±0.94	1.43±0.98	1.95±1.27
SCORE (%)	<0.01±<0.01	<0.01±0.01	0.01±<0.01	0.01±0.01	0.03±0.01	0.06±0.03
Finrisk (%)	0.23±0.10	0.26±0.12	0.35±0.18	0.42±0.20	0.52±0.24	0.66±0.28
PROCAM (%)	0.05±0.06	0.06±0.04	0.09±0.10	0.12±0.10	0.18±0.18	0.21±0.17
Reynolds (%)	0.12±0.12	0.11±0.07	0.16±0.13	0.22±0.20	0.27±0.19	0.31±0.20
Men						
Age (years)	24	27	30	33	36	39
N	115	111	142	147	148	131
Framingham (%)	0.41±0.46	0.91±0.80	1.66±1.75	2.75±2.30	3.49±2.19	5.31±3.39
SCORE (%)	0.02±0.01	0.04±0.02	0.10±0.05	0.19±0.11	0.32±0.17	0.58±0.33
Finrisk (%)	0.28±0.15	0.45±0.23	0.63±0.38	0.98±0.77	1.21±0.74	1.75±1.02
PROCAM (%)	0.41±0.31	0.74±0.56	1.07±1.05	2.00±2.90	2.23±2.54	3.31±2.93
Reynolds (%)	0.12±0.06	0.23±0.11	0.41±0.25	0.67±0.41	0.91±0.44	1.45±0.79
Year 2007 Women						
Age (years)	30	33	36	39	42	45
N	132	170	159	199	190	165
Framingham (%)	0.71±0.75	0.86±0.74	1.62±1.70	1.98±1.46	2.69±1.72	3.45±2.07
SCORE (%)	0.01±<0.01	0.01±0.01	0.03±0.01	0.06±0.02	0.11±0.04	0.20±0.09
Finrisk (%)	0.32±0.18	0.39±0.18	0.56±0.40	0.65±0.33	0.83±0.41	1.01±0.42
PROCAM (%)	0.07±0.08	0.09±0.07	0.18±0.26	0.19±0.18	0.28±0.22	0.38±0.30
Reynolds (%)	0.18±0.16	0.17±0.12	0.30±0.27	0.34±0.28	0.45±0.30	0.60±0.43
Men						
Age (years)	30	33	36	39	42	45
N	115	111	142	147	148	131
Framingham (%)	1.41±1.37	2.19±1.30	3.30±2.54	5.44±3.97	6.78±3.67	8.32±4.37
SCORE (%)	0.09±0.05	0.17±0.08	0.30±0.15	0.56±0.30	0.87±0.43	1.27±0.62
Finrisk (%)	0.54±0.31	0.77±0.35	1.07±0.65	1.71±1.24	2.17±1.04	2.78±1.44
PROCAM (%)	0.78±0.64	1.26±0.97	1.74±1.80	3.14±3.89	3.69±2.82	4.41±3.58
Reynolds (%)	0.36±0.21	0.58±0.25	0.90±0.50	1.50±0.87	1.99±0.91	2.67±1.29

Data are mean±SD.

Table 14. Median values of risk estimates. Minimum and maximum values are expressed inside the brackets.

Year 2001 Women						
Age (years)	24	27	30	33	36	39
Framingham (%)	0.18 (0.03-2.81)	0.27 (0.03-2.66)	0.49 (0.11-3.94)	0.71 (0.08-5.52)	1.12 (0.25-5.82)	1.70 (0.40-6.96)
SCORE (%)	<0.01 (<0.01-0.01)	<0.01 (<0.01-0.01)	0.01 (<0.01-0.02)	0.01 (0.01-0.05)	0.03 (0.01-0.07)	0.05 (0.03-0.17)
Finrisk (%)	0.21 (0.08-0.82)	0.23 (0.09-1.01)	0.30 (0.11-1.41)	0.36 (0.15-1.16)	0.47 (0.14-1.75)	0.61 (0.21-2.03)
PROCAM (%)	0.03 (0.01-0.67)	0.04 (0.01-0.31)	0.06 (0.01-0.86)	0.08 (0.02-0.61)	0.12 (0.02-1.39)	0.17 (0.05-0.98)
Reynolds (%)	0.08 (0.02-1.07)	0.09 (0.02-0.56)	0.12 (0.04-0.78)	0.15 (0.04-1.22)	0.21 (0.06-1.60)	0.26 (0.08-1.35)
Men						
Framingham (%)	0.23 (0.05-2.81)	0.67 (0.08-4.92)	1.09 (0.21-15.06)	1.91 (0.41-11.24)	2.81 (0.55-11.93)	4.24 (1.60-16.97)
SCORE (%)	0.01 (0.01-0.06)	0.04 (0.02-0.11)	0.08 (0.04-0.27)	0.15 (0.08-0.71)	0.28 (0.12-1.29)	0.47 (0.22-2.13)
Finrisk (%)	0.23 (0.13-0.91)	0.39 (0.14-1.38)	0.50 (0.19-2.79)	0.74 (0.27-5.78)	0.98 (0.33-6.08)	1.47 (0.63-7.35)
PROCAM (%)	0.31 (0.09-1.60)	0.57 (0.12-2.64)	0.72 (0.15-8.68)	1.08 (0.26-20.21)	1.47 (0.29-23.33)	2.53 (0.53-22.03)
Reynolds (%)	0.10 (0.05-0.41)	0.21 (0.08-0.59)	0.34 (0.11-1.89)	0.55 (0.23-2.35)	0.79 (0.28-2.44)	1.23 (0.52-4.46)
Year 2007 Women						
Age (years)	30	33	36	39	42	45
Framingham (%)	0.44 (0.06-4.03)	0.62 (0.10-4.42)	1.13 (0.19-17.01)	1.52 (0.22-7.63)	2.16 (0.53-12.20)	3.09 (0.70-12.66)
SCORE (%)	0.01 (<0.01-0.04)	0.01 (0.01-0.04)	0.03 (0.01-0.09)	0.05 (0.02-0.14)	0.10 (0.05-0.28)	0.16 (0.08-0.66)
Finrisk (%)	0.28 (0.09-1.08)	0.35 (0.11-1.20)	0.46 (0.17-4.09)	0.55 (0.19-2.56)	0.71 (0.15-3.30)	0.92 (0.35-2.23)
PROCAM (%)	0.05 (0.01-0.53)	0.13 (0.03-0.77)	0.11 (0.02-2.67)	0.14 (0.03-1.66)	0.22 (0.04-1.20)	0.30 (0.06-1.97)
Reynolds (%)	0.12 (0.03-1.01)	0.06 (0.01-0.47)	0.20 (0.05-1.76)	0.25 (0.04-2.12)	0.37 (0.08-1.86)	0.47 (0.10-2.26)
Men						
Framingham (%)	0.88 (0.08-8.16)	1.85 (0.40-6.42)	2.56 (0.56-20.38)	4.19 (0.58-23.99)	5.95 (1.54-22.90)	7.08 (2.43-23.34)
SCORE (%)	0.07 (0.03-0.33)	0.15 (0.07-0.47)	0.26 (0.12-0.82)	0.47 (0.19-1.93)	0.74 (0.32-2.84)	1.09 (0.54-3.90)
Finrisk (%)	0.42 (0.16-1.73)	0.69 (0.23-1.79)	0.90 (0.30-4.90)	1.36 (0.39-9.94)	1.95 (0.72-6.35)	2.33 (0.92-9.04)
PROCAM (%)	0.49 (0.10-3.22)	0.98 (0.14-4.80)	1.21 (0.31-14.85)	2.02 (0.30-29.01)	1.84 (0.56-5.46)	2.36 (0.83-6.14)
Reynolds (%)	0.31 (0.08-1.30)	0.54 (0.20-1.38)	0.76 (0.27-3.96)	1.27 (0.48-5.60)	2.88 (0.52-19.04)	3.54 (0.67-25.06)

Table 15. Spearman's correlation between 10-year CVD risk scores and ultrasound measurements.

	IMT			CDist			FMD		
Men	2001-2001	2007-2007	2001-2007	2001-2001	2007-2007	2001-2007	2001-2001	2007-2007	2001-2007
Framingham	0.31***	0.37***	0.40***	-0.35***	-0.25***	-0.25***	0.004	-0.03	-0.02
Finrisk	0.30***	0.37***	0.39***	-0.33***	-0.25***	-0.25***	0.02	-0.02	-0.008
SCORE	0.33***	0.38***	0.40***	-0.34***	-0.26***	-0.26***	0.01	-0.05	-0.02
Reynolds	0.35***	0.40***	0.42***	-0.39***	-0.28***	-0.28***	0.01	-0.03	-0.02
PROCAM	0.27***	0.33***	0.38***	-0.31***	-0.22***	-0.23***	0.02	-0.01	0.006
Women	2001-2001	2007-2007	2001-2007	2001-2001	2007-2007	2001-2007	2001-2001	2007-2007	2001-2007
Framingham	0.29***	0.39***	0.35***	-0.33***	-0.31***	-0.30***	0.07*	0.002	-0.04
Finrisk	0.28***	0.38***	0.32***	-0.24***	-0.25***	-0.24***	0.04	-0.01	-0.04
SCORE	0.32***	0.37***	0.35***	-0.31***	-0.33***	-0.32***	0.05	-0.03	-0.07*
Reynolds	0.26***	0.38***	0.32***	-0.34***	-0.32***	-0.27***	0.09**	0.03	-0.002
PROCAM	0.28***	0.39***	0.32***	-0.27***	-0.27***	-0.24***	0.08**	0.02	-0.02

(*P<0.05; **P<0.01; ***P<0.001)

2001-2001 = correlation between CVD risk score in 2001 and ultrasound measurement in 2001.

2007-2007 = correlation between CVD risk score in 2001 and ultrasound measurement in 2007.

2007-2007 = correlation between CVD risk score in 2007 and ultrasound measurement in 2007.

5.3.2. Comparison of baseline risk scores to predict 6-year subclinical atherosclerosis

Table 16 displays model fit, discrimination, calibration, and reclassification indices. Figures 14a-c display the ROC curves for risk scores in prediction of 6-year atherosclerosis. For the outcome of high carotid IMT or plaque, Finnrisk, SCORE, and Reynolds risk scores tended to perform equally well as Framingham, but calibration was best for the Reynolds model (lack of fit, as indicated by the H-L statistic, remained substantial however. PROCAM demonstrated reduced AUC, NRI, and IDI in comparison with Framingham, but only IDI was statistically significant. Finrisk performed equally with Framingham in predicting 6-year low CDist, whereas reclassification was less accurate for SCORE (IDI) and PROCAM (NRI). Although discrimination was similar between Framingham and Reynolds risk scores, NRI and IDI were more accurate when the model with Reynolds was used. For the prediction of 6-year low FMD, Finrisk and Reynolds risk scores tended to perform equally well. SCORE improved discrimination over Framingham risk score (AUC 0.596 vs. 0.568), but calibration was poorer (18.7 vs. 4.4). Reclassification was less accurate when PROCAM was used in place of Framingham to predict low FMD.

Table 16. Model fit, discrimination, calibration, and reclassification indices for prediction of 6-year subclinical outcomes from year 2001 risk scores.

	Framingham	Finrisk		SCORE		PROCAM		Reynolds	
Outcome	Statistic	Statistic	P-value*	Statistic	P-value*	Statistic	P-value*	Statistic	P-value*
High carotid IMT (>90th percentile) or plaque									
OR (95%CI)†	1.7 (1.5-2.0)	1.7 (1.5-2.0)	***	1.7 (1.5-1.9)	***	1.5 (1.3-1.7)	***	1.7 (1.5-1.9)	***
AUC (95%CI)	0.728 (0.698-0.758)	0.733 (0.702-0.763)	0.41	0.726 (0.695-0.757)	0.83	0.712 (0.681-0.744)	0.15	0.729 (0.698-0.759)	0.95
H-L	51.3	60.6	***	54.7	***	57.2	***	46.3	***
NRI	-	2.2%	0.25	3.7%	0.18	-4.9%	0.12	3.9%	0.16
IDI	-	-0.17%	0.29	-0.90%	0.02	-2.76%	<0.01	-0.10%	0.41
Low CDist (<10th percentile)									
OR (95%CI)†	1.4 (1.2-1.5)	1.3 (1.2-1.5)	***	1.3 (1.1-1.4)	***	1.2 (1.1-1.4)	***	1.4 (1.3-1.6)	***
AUC (95%CI)	0.652 (0.612-0.692)	0.652 (0.611-0.693)	0.97	0.642 (0.603-0.681)	0.45	0.639 (0.598-0.680)	0.41	0.658 (0.618-0.697)	0.54
H-L	25.8	29.9	***	27.8	***	27.2	***	25.1	***
NRI	-	1.9%	0.32	-3.8%	0.22	-27.4%	<0.01	6.9%	0.04
IDI	-	-0.05%	0.42	-0.50%	0.005	-0.75%	0.01	0.78%	0.003
Low brachial FMD (<10th percentile)									
OR (95%CI)†	1.2 (1.1-1.4)	1.2 (1.1-1.4)	***	1.2 (1.1-1.4)	***	1.1 (1.0-1.3)	***	1.2 (1.1-1.4)	***
AUC (95%CI)	0.568 (0.521-0.615)	0.578 (0.531-0.624)	0.13	0.596 (0.550-0.642)	<0.05	0.594 (0.548-0.639)	0.08	0.582 (0.535-0.629)	0.09
H-L	4.4	8.0	***	18.7	***	19.1	***	8.8	***
NRI	-	-1.5%	0.35	-7.1%	0.10	-13.6%	<0.01	5.1%	0.08
IDI	-	-0.29%	0.01	-0.22%	0.10	-0.50%	<0.01	-0.08%	0.27

*P-values for comparisons between Framingham (reference risk score) vs. each of Finrisk, SCORE, PROCAM, or Reynolds risk scores.

†Odd ratios are expressed for a 1 standard deviation increase in Framingham, Finrisk, SCORE, PROCAM, or Reynolds risk scores.

Analyses for each outcome were limited to subjects that had sufficient risk variables to calculate all risk scores (IMT, N = 1761; CDist, N = 1754; FMD, N = 1751).

Abbreviations. AUC = area under receiver-operating characteristic curve; CDist = carotid artery distensibility; CI = confidence interval; FMD = flow-mediated dilatation; H-L = Hosmer-Lemeshow chi-square statistic; IMT = intima-media thickness; NRI = Net reclassification improvement; OR = odds ratio; IDI = integrated discrimination improvement.

Note.

Population mean for high IMT/plaque = 15.5%. Risk categories, of <14%, 14-16%, 16-20%, ≥20% used for calculation of NRI

Population mean for low CDist = 10.2%. Risk categories, of <9%, 9-11%, 11-15%, ≥15% used for calculation of NRI

Population mean for low FMD = 10.3%. Risk categories, of <9%, 9-11%, 11-15%, ≥15% used for calculation of NRI

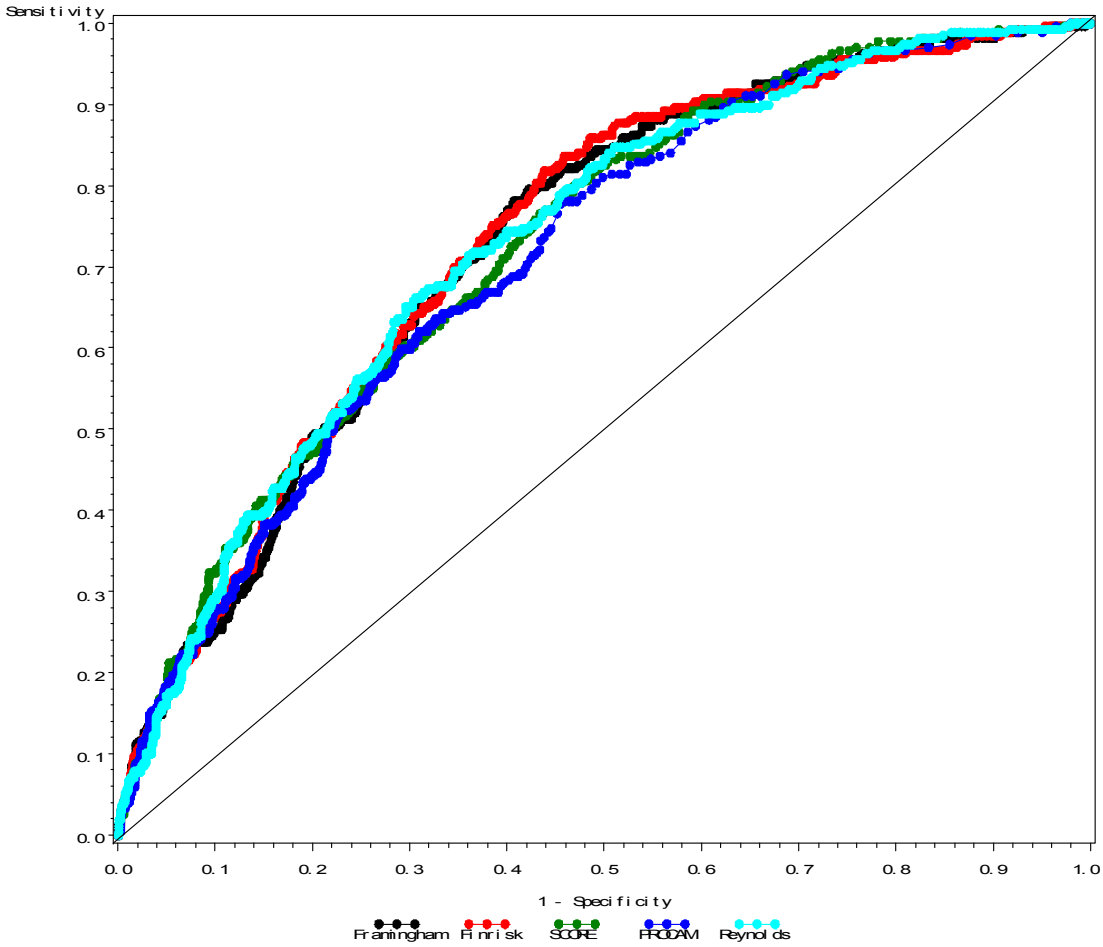


Figure 14a. ROC curve for use of Framingham, Finrisk, SCORE, PROCAM and Reynolds risk scores in 2001 in prediction of high IMT (highest decile) or carotid plaque in 2007 in both sexes

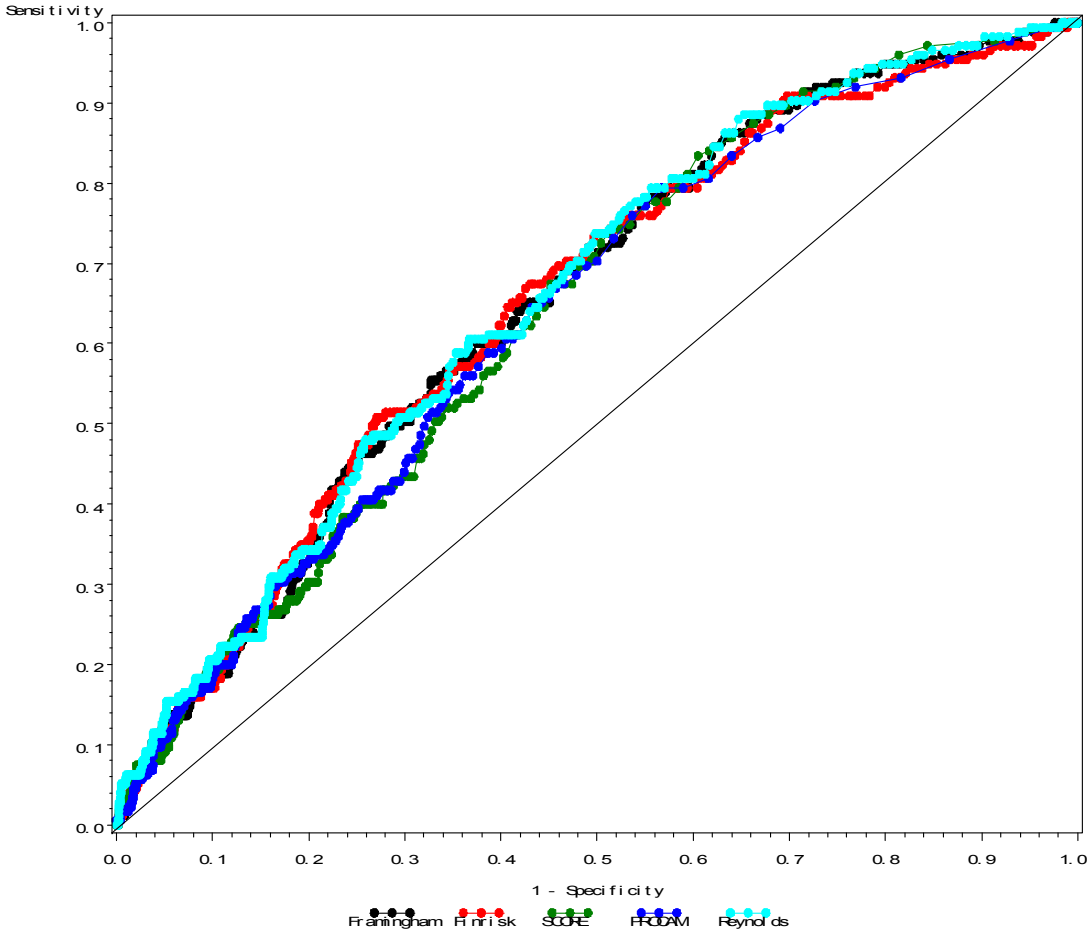


Figure 14b. ROC curve for use of Framingham, Finrisk, SCORE, PROCAM and Reynolds risk scores in 2001 in prediction of low CDist (lowest decile in 2007 in both sexes.

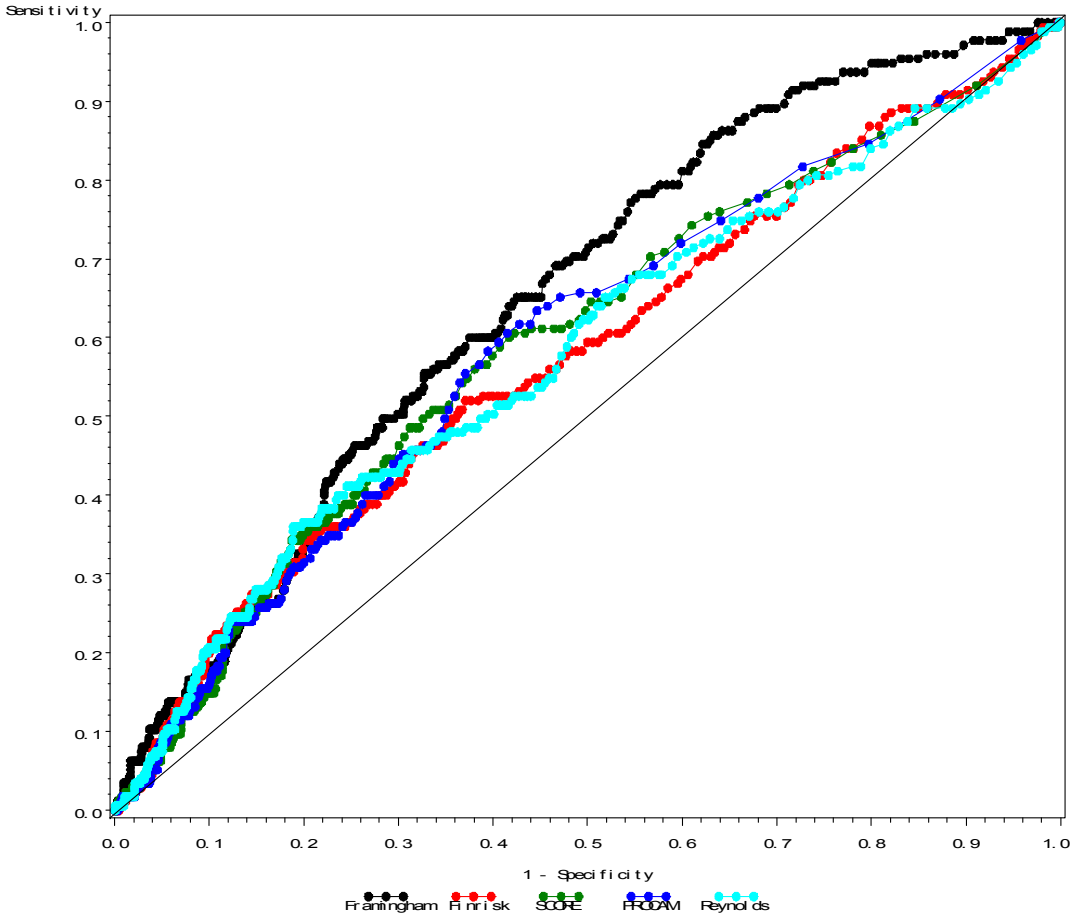


Figure 14c. ROC curve for use of Framingham, Finrisk, SCORE, PROCAM and Reynolds risk scores in 2001 in prediction of low FMD (lowest decile) in 2007 in both sexes.

5.4. Study IV:

Metabolic profiling in prediction of subclinical atherosclerosis

Due to the multifactorial character of atherosclerosis accurate risk estimation needs to be based on a large array of risk factors [338]. Measurement of vast numbers of metabolites in serum samples enables the identification of atherogenic phenotypes and thus allows an early intervention with the aim of improving the prognosis [339]. To examine link between metabonomics and early atherosclerosis associations between NMR-determined metabolites and subclinical atherosclerosis were analysed.

5.4.1. Prediction of incident high carotid IMT or plaque

1,587 subjects had complete ultrasound and lipoprotein lipid data available and 147 subjects developed IMT≥90th percentile and/or plaque between 2001 and 2007. Baseline characteristics are displayed in Table 17. OR for incident high IMT are shown in Table 18. Several lipoprotein fractions determined by NMR had higher OR compared the corresponding conventional lipoprotein measurements. In HDL subclasses, large HDL had the lowest OR which was lower than that of HDL-cholesterol. Tyrosine and glutamine had similar OR compared to conventional LDL-cholesterol. The associations remained significant when the models were adjusted for HOMA-IR in 2001 to examine if the associations were independent of insulin resistance. The association of tyrosine and incident high IMT was attenuated but remained significant (OR=1.28, P=0.01) and the association of glutamine was largely unaltered (OR=1.38, P=0.002). The associations of tyrosine and glutamine were verified cross-sectionally in an independent population of the Health 2000 study [148] since the biomarkers have not been linked with the development of atherosclerosis in previous studies. Results are shown in Table 19. The association remained significant for glutamine in the Health 2000 cohort while the association for tyrosine was nonsignificant. Both amino acids were significantly associated with incident IMT when the Health 2000 and Young Finns cohorts were combined. Esterified cholesterol and polyunsaturated fatty acid levels were associated with incident high IMT. Linoleic acid was directly associated and docosahexaenoic acid was inversely associated with incident high IMT.

Table 17. Baseline characteristics.

	IMT<90th percentile (*n*=1440)	IMT≥90th percentile or plaque (*n*=147)	P
Male sex [%]	41.3	62.6	<0.001†
Age [y]	31.5 (4.9)	34.3 (4.2)	<0.001†
Body mass index [kg/m^2]	24.6 (4.1)	26.7 (4.9)	<0.001†
Diastolic blood pressure [mm Hg]	70 (10)	73 (11)	0.78
Systolic blood pressure [mm Hg]	115 (13)	121 (13)	0.03
Current smoker [%]	21.9	21.9	0.71
Family history of cardiovascular disease [%]	13.0	19.0	0.07
LDL-cholesterol [mmol/l]	3.2 (0.81)	3.7 (0.92)	<0.001
HDL-cholesterol [mmol/l]	1.3 (0.31)	1.2 (0.27)	0.09
Triglycerides [mmol/l]	1.1 [0.80-1.5]	1.2 [0.90-1.8]	0.39
Glucose [mmol/l]	5.0 [4.7-5.2]	5.1 [4.9-5.4]	0.63
C-reactive protein [mmol/l]	0.71 [0.31-1.7]	0.83 [0.35-1.8]	0.77
Carotid intima-media thickness [mm]	0.56 [0.51-0.62]	0.65 [0.58-0.70]	<0.001

Baseline characteristics in subjects with incident carotid IMT≥90th percentile or plaque at 6-year follow-up. Values are mean (SD) for normally distributed variables, prevalence for dichomotous variables and median [25th-75th percentile] for skewed distributions. P-values for comparison are adjusted for age, sex and BMI.

†: unadjusted P-values.

Table 18. Odds ratios for 6-year incident high IMT.

	OR	95% CI	P
Low-molecular-weight metabolites			
Alanine	1.24	1.03-1.49	0.03
Glutamine	1.37	1.12-1.68	0.002
Histidine	1.21	1.00-1.45	0.05
Isoleucine	1.16	0.90-1.49	0.25
Leucine	1.14	0.93-1.38	0.21
Phenylalanine	1.03	0.84-1.26	0.79
Tyrosine	1.34	1.11-1.62	0.002
Valine	1.06	0.85-1.31	0.62
Glucose	1.11	0.96-1.29	0.16
Lactate	1.09	0.93-1.28	0.30
Pyruvate	1.15	0.97-1.37	0.12
Citrate	0.91	0.75-1.10	0.33
Glycoproteins	1.16	0.92-1.45	0.21
3-hydroxybutyrate	1.06	0.87-1.28	0.59
Acetate	0.96	0.79-1.16	0.64
Acetoacetate	1.08	0.91-1.30	0.38
Creatinine	0.92	0.74-1.14	0.42
Urea	0.90	0.75-1.08	0.25
Serum extract metabolites			
Total fatty acids	1.40	1.00-1.98	0.05
Esterified cholesterol	1.47	1.11-1.97	0.008
Free cholesterol	1.14	0.86-1.51	0.38
Total phosphoglycerides	1.15	0.86-1.54	0.33
Phosphatidylcholine	1.12	0.83-1.50	0.46
Sphingomyelins	1.15	0.91-1.46	0.24
Total cholines	1.15	0.86-1.54	0.33
omega-3 fatty acids	0.90	0.74-1.10	0.30
omega-6 and omega-7 fatty acids	1.44	1.10-1.89	0.009
omega-9 and saturated fatty acids	1.36	0.94-1.97	0.11
omega-3/(omega-6 and omega-7)	0.82	0.68-0.99	0.04
omega-3/omega-9	0.83	0.69-1.01	0.07
Linoleic acid	1.43	1.14-1.80	0.002
Docosahexaenoic acid	0.77	0.62-0.96	0.02
Average methylene groups in fatty acid chain	1.07	0.91-1.26	0.43
Average methylene groups per double bond	1.21	0.95-1.55	0.12
Average double bonds in fatty acid chain	0.82	0.63-1.07	0.14
Ratio of bisallylic groups to double bonds	0.82	0.66-1.02	0.07
Ratio of bisallylic groups to total fatty acids	0.80	0.62-1.02	0.07

OR and 95% CI for incident carotid IMT≥90th percentile or plaque at follow-up according to metabolite measures at baseline. Odds ratios are adjusted for sex, baseline age, body mass index, diastolic and systolic blood pressure, and family history of CVD. The low-molecular-weight and serum extract metabolite associations were further adjusted for baseline conventional LDL-cholesterol, HDL-cholesterol, and triglycerides. Values are expressed for a 1-SD increase in the predictor variable.

Table 19. Linear regression of carotid IMT adjusted for sex, age, body mass index, systolic and diastolic blood pressure, LDL-cholesterol, HDL-cholesterol, and triglycerides.

	Health 2000			Cardiovascular Risk in Young Finns†			Combined
Metabolite	β (*SE*) [mm]	P	*n*	β (*SE*) [mm]	P	*n*	P
Tyrosine	7.6 (4.7)	0.08	1018	5.8 (3.2)	0.03	823	0.004
Glutamine	9.7 (4.6)	0.03	1013	7.3 (3.3)	0.01	779	0.001

†: Individuals excluded from prospective analyses due to missing data at either time-point.

5.4.1.1. Model derivation

The models derived for prediction of incident high IMT are shown in Table 20. The best combination of non-laboratory risk factors were age, sex, BMI, systolic and diastolic blood pressure, and family history of CVD, which were subsequently forced into all models. For the conventional lipid measures, LDL-cholesterol and HDL-cholesterol gave rise to the best fit in this study. All comparison models included LDL-cholesterol$_{NMR}$ and IDL$_{NMR}$, whereas conventional lipids measures were never selected. Interestingly, tyrosine was the only amino acid remaining in model C, but inclusion in the selection procedure prompted medium HDL and IDL-cholesterol to remain in the models. Finally, when lipid metabolites were included in model selection, model C was extended to include docosahexaenoic acid.

5.4.1.2. Evaluation of prediction models

In prediction of incident high IMT, the predictive ability of the reference model versus the three comparison models was evaluated in terms of discrimination, reclassification, model fit, and calibration in Table 20. Figure 15 displays ROC curves for models A, C and D predicting 6-year incidence of high IMT. All models displayed good calibration. Replacement of conventional lipids by LDL-cholesterol$_{NMR}$ and IDL in model B did not significantly improve discrimination between individuals with high and low IMT, however, it was accompanied by an improvement in reclassification of 9.4% (P=0.02). When the model selection was extended with low-molecular-weight metabolites (model C) and lipid metabolites (model D) also a significant improvement in AUC was achieved (P=0.03 for both models). Models C and D performed similarly in terms of improved discrimination, and reclassified about 15% of the individuals towards more

correct risk categories. Results are presented here for 6-year incident high IMT or plaque. Essentially similar results were obtained for 6-year prevalence of high IMT or plaque (data not shown).

Table 20. Model fit, discrimination, calibration, and reclassification indices for prediction of 6-year incidence of high IMT with models including conventional risk factors and metabonomics data.

Model	AUC	95% CI	P_{AUC}†	NRI [%]	P_{NRI}†	IDI [%]	P_{IDI}†	χ^2‡	$P_{\chi 2}$	AIC	HL	P_{HL}
A: age, sex, BMI, diastolic blood pressure, systolic blood pressure, family history of CVD, LDL-cholesterol, HDL-cholesterol (reference model)	0.741	0.700-0.781	–	–	–	–	–	10.3	0.006	887	7.1	0.53
B: non-laboratory risk factors*, $LDL\text{-}cholesterol_{NMR}$, IDL	0.748	0.707-0.789	0.49	9.4	0.02	1.73	<0.001	24.4	<0.001	873	5.2	0.73
C: non-laboratory risk factors*, $LDL\text{-}cholesterol_{NMR}$, IDL, IDL-cholesterol, medium HDL, tyrosine	0.763	0.723-0.802	0.03	14.4	0.003	2.43	<0.001	33.2	<0.001	870	5.4	0.71
D: non-laboratory risk factors*, $LDL\text{-}cholesterol_{NMR}$, IDL, IDL-cholesterol, medium HDL, tyrosine, docosahexaenoic acid	0.768	0.728-0.807	0.03	15.4	0.004	3.05	<0.001	37.8	<0.001	867	8.5	0.39

†: P-values for comparison of the reference model A with model B, model C, and model D.

‡: Log-likelihood ratio χ^2 as compared to a model with non-laboratory risk factors only.

* Non-laboratory risk factors: age, sex, body mass index, diastolic and systolic blood pressure, and family history of CVD.

For NRI, participants were assigned to four categories (<5%, 5-10%, 10%-20%, and ≥20%) that reflected their 6-year risk of incident high IMT based on each model. Median values of 10-fold cross-validation with 50 repeats are shown.

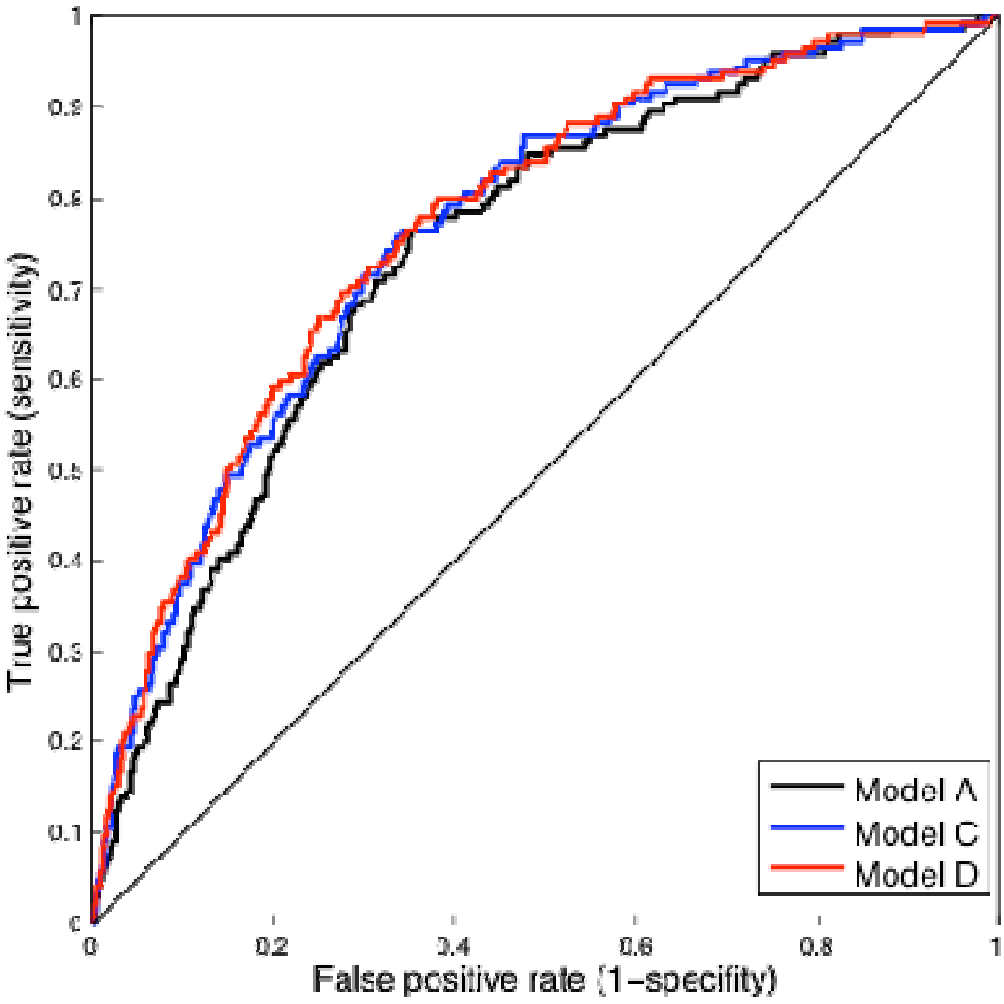


Figure 15. ROC curves for 6-year incidence of carotid IMT≥90th percentile or plaque for the reference model A with conventional lipid risk-factors, model C with NMR-based lipoprotein measures and tyrosine, and model D further including docosahexaenoic acid.

5.4.2. Prediction of 6-year prevalence of low CDist and FMD

Odds ratios for low CDist and low FMD prevalences (values $\leq 20^{th}$ percentile) are displayed in Tables 21 and 22. CDist in 6 years was decreased by medium, small and very small VLDL, total and IDL triglycerides, omega-9 and saturated fatty acids, free cholesterol, lactate, pyruvate and average methylene groups per double bond. Associations between glycoprotein (OR=1.17, P=0.05) and VLDL triglycerides (OR=1.14, P=0.05) with low CDist were borderline significant. Average double bonds in fatty acid chains and ratio of bisallylic groups to total fatty acids protected from low 6-year CDist. All metabolites with significant associations were added to a logistic regression model predicting low CDist prevalence in 6 years with age, sex, BMI, systolic blood pressure, total cholesterol and triglycerides as covariates. Average double bonds in fatty acid chains was the only metabolite with significant association in this model (OR=0.55, [0.33-0.91], P=0.02). Free cholesterol (OR=1.18, P=0.06) was nonsignificantly related to prevalence of low FMD at 6-years.

In predicting prevalence of low CDist, reference model and three comparison models are compared in Table 23. Figure 16 displays the ROC curves of the prediction models. All models had good calibration. However, metabonomics did not improve discrimination compared to reference model according to AUC and NRI. Model D had improved discrimination according to IDI (IDI=1.3%, P=0.0005).

Table 21a. Odds ratios for low 6-year CDist prevalence.

Lipoprotein subclasses	OR (95% CI)	P-value
Extremely large VLDL	1.07 (0.95-1.22)	0.25
Very large VLDL	1.08 (0.95-1.23)	0.22
Large VLDL	1.10 (0.97-1.25)	0.13
Medium VLDL	1.14 (1.01-1.30)	0.047
Small VLDL	1.18 (1.03-1.35)	0.02
Very small VLDL	1.14 (1.01-1.30)	0.04
IDL	1.12 (0.99-1.27)	0.08
Large LDL	1.12 (0.99-1.28)	0.08
Medium LDL	1.12 (0.99-1.28)	0.08
Small LDL	1.10 (0.96-1.25)	0.17
Very large HDL	0.99 (0.85-1.16)	0.94
Large HDL	0.97 (0.83-1.14)	0.72
Medium HDL	0.97 (0.84-1.11)	0.66
Small HDL	1.05 (0.92-1.20)	0.47
NMR Lipids		
Total cholesterol	1.12 (0.99-1.27)	0.08
IDL cholesterol	1.12 (0.98-1.27)	0.09
LDL cholesterol	1.12 (0.98-1.27)	0.10
HDL cholesterol	0.98 (0.84-1.14)	0.78
Total triglycerides	1.16 (1.02-1.32)	0.03
VLDL triglycerides	1.14 (1.00-1.30)	0.05
IDL triglycerides	1.16 (1.02-1.32)	0.02
Conventional lipoproteins		
Total cholesterol	1.09 (0.96-1.24)	0.20
LDL-cholesterol	1.06 (0.93-1.20)	0.40
HDL-cholesterol	1.03 (0.89-1.19)	0.66
Triglycerides	1.07 (0.94-1.20)	0.32
Non-HDL-cholesterol	1.08 (0.95-1.24)	0.24
Total cholesterol/HDL cholesterol ratio	1.06 (0.93-1.21)	0.37
ApoA1	1.11 (0.97-1.28)	0.12
ApoB	1.16 (1.01-1.33)	0.03
ApoB/ApoA1 ratio	1.09 (0.95-1.25)	0.22

OR and 95% CI for CDist≤20th percentile at follow-up according to metabolite measures at baseline. Odds ratios are adjusted for sex, baseline age, body mass index and systolic blood pressure. The low-molecular-weight and serum extract metabolite associations were further adjusted for baseline conventional total cholesterol and triglycerides. Values are expressed for a 1-SD increase in the predictor variable.

Table 21b. Odds ratios for low 6-year CDist prevalence.

NMR-based Low-Molecular-Weight Metabolites (LMWM)	OR (95% CI)	P-value
3-hydroxybutyrate	1.03 (0.90-1.18)	0.63
Acetoacetate	1.01 (0.89-1.16)	0.84
Alanine	1.05 (0.92-1.20)	0.48
Creatinine	1.07 (0.94-1.22)	0.31
Glucose	1.01 (0.90-1.15)	0.82
Glutamine	0.99 (0.86-1.15)	0.93
Glycoproteins	1.17 (0.99-1.37)	0.05
Histidine	0.98 (0.86-1.11)	0.76
Isoleucine	0.95 (0.78-1.15)	0.59
Leucine	0.94 (0.82-1.07)	0.34
Urea	0.94 (0.83-1.07)	0.37
Tyrosine	1.03 (0.90-1.18)	0.68
Valine	0.90 (0.77-1.06)	0.21
Lactate	1.21 (1.07-1.37)	0.002
Pyruvate	1.30 (1.14-1.47)	<0.0001
Citrate	1.04 (0.91-1.19)	0.60
Lipid particle components		
Total fatty acids	1.23 (0.98-1.56)	0.08
Esterified cholesterol	1.07 (0.85-1.35)	0.57
Free cholesterol	1.30 (1.06-1.59)	0.01
Total phosphoglycerides	1.07 (0.91-1.27)	0.42
Phosphatidylcholine	1.08 (0.91-1.27)	0.38
Sphingomyelins	1.05 (0.89-1.24)	0.58
Total cholines	1.06 (0.89-1.26)	0.53
omega-3 fatty acids	0.97 (0.84-1.12)	0.69
omega-6 and omega-7 fatty acids	1.13 (0.92-1.37)	0.24
omega-9 and saturated fatty acids	1.29 (1.01-1.65)	0.04
Omega-3/omega-6&7	0.94 (0.83-1.07)	0.38
Omega-3/omega-9	0.91 (0.80-1.04)	0.18
Linoleic acid (18:2)	1.13 (0.94-1.34)	0.19
Docosahexaenoic acid (22:6)	1.01 (0.87-1.16)	0.93
Other polyunsaturated fatty acids than 18:2	0.97 (0.83-1.15)	0.74
Average methylene groups in fatty acid chain	1.11 (0.98-1.25)	0.10
Average methylene groups per double bond	1.32 (1.11-1.57)	0.001
Average double bonds in fatty acid chain	0.78 (0.65-0.93)	0.006
Ratio of bisallylic groups to double bonds	0.89 (0.77-1.04)	0.13
Ratio of bisallylic groups to total fatty acids	0.82 (0.69-0.97)	0.02
Average fatty acid chain length	0.97 (0.85-1.11)	0.67

Table 22a. Odds ratios for low 6-year FMD prevalence.

Lipoprotein subclasses	OR (95% CI)	P-value
Extremely large VLDL	0.99 (0.88-1.12)	0.90
Very large VLDL	0.99 (0.88-1.12)	0.89
Large VLDL	0.98 (0.87-1.11)	0.79
Medium VLDL	0.99 (0.87-1.12)	0.82
Small VLDL	1.02 (0.90-1.15)	0.81
Very small VLDL	0.98 (0.87-1.10)	0.69
IDL	0.97 (0.86-1.09)	0.57
Large LDL	0.98 (0.87-1.10)	0.67
Medium LDL	0.98 (0.87-1.10)	0.75
Small LDL	0.97 (0.86-1.09)	0.59
Very large HDL	0.92 (0.81-1.04)	0.18
Large HDL	0.99 (0.87-1.14)	0.92
Medium HDL	1.03 (0.91-1.15)	0.67
Small HDL	1.04 (0.93-1.16)	0.48
NMR Lipids		
Total cholesterol	0.99 (0.88-1.11)	0.82
IDL cholesterol	0.98 (0.87-1.09)	0.67
LDL cholesterol	0.98 (0.87-1.10)	0.74
HDL cholesterol	1.00 (0.88-1.13)	0.97
Total triglycerides	0.99 (0.87-1.12)	0.82
VLDL triglycerides	0.99 (0.87-1.12)	0.86
IDL triglycerides	0.98 (0.87-1.10)	0.68
Conventional lipoproteins		
Total cholesterol	0.93 (0.83-1.05)	0.24
LDL-cholesterol	0.95 (0.84-1.06)	0.36
HDL-cholesterol	1.01 (0.90-1.15)	0.83
Triglycerides	0.91 (0.80-1.03)	0.13
Non-HDL-cholesterol	0.92 (0.82-1.04)	0.20
Total cholesterol/HDL cholesterol ratio	0.94 (0.82-1.07)	0.34
ApoA1	1.02 (0.90-1.15)	0.77
ApoB	0.94 (0.83-1.06)	0.32
ApoB/ApoA1 ratio	0.93 (0.82-1.06)	0.28

OR and 95% CI for FMD$\leq 20^{th}$ percentile at follow-up according to metabolite measures at baseline. Odds ratios are adjusted for sex, baseline age, body mass index and systolic blood pressure. The low-molecular-weight and serum extract metabolite associations were further adjusted for baseline conventional total cholesterol and triglycerides. Values are expressed for a 1-SD increase in the predictor variable.

Table 22b. Odds ratios for low 6-year FMD prevalence.

NMR-based Low-Molecular-Weight Metabolites (LMWM)	OR (95% CI)	P-value
3-hydroxybutyrate	0.95 (0.84-1.06)	0.35
Acetoacetate	0.99 (0.89-1.11)	0.88
Alanine	1.02 (0.91-1.14)	0.74
Creatinine	1.01 (0.90-1.14)	0.84
Glucose	1.06 (0.96-1.18)	0.25
Glutamine	1.06 (0.94-1.20)	0.35
Glycoproteins	1.12 (0.97-1.28)	0.12
Histidine	0.97 (0.87-1.08)	0.54
Isoleucine	1.13 (0.94-1.36)	0.18
Leucine	1.03 (0.92-1.16)	0.62
Urea	1.00 (0.90-1.12)	0.95
Tyrosine	1.00 (0.88-1.13)	0.97
Valine	1.06 (0.92-1.21)	0.43
Lactate	0.94 (0.84-1.06)	0.33
Pyruvate	0.94 (0.83-1.06)	0.29
Citrate	0.97 (0.87-1.09)	0.59
Lipid particle components		
Total fatty acids	1.13 (0.91-1.41)	0.25
Esterified cholesterol	1.09 (0.90-1.33)	0.37
Free cholesterol	1.18 (0.99-1.41)	0.06
Total phosphoglycerides	1.13 (0.98-1.30)	0.09
Phosphatidylcholine	1.12 (0.97-1.29)	0.13
Sphingomyelins	0.93 (0.80-1.08)	0.33
Total cholines	1.05 (0.91-1.22)	0.49
omega-3 fatty acids	1.09 (0.96-1.24)	0.18
omega-6 and omega-7 fatty acids	1.06 (0.89-1.26)	0.53
omega-9 and saturated fatty acids	1.15 (0.91-1.45)	0.24
Omega-3/omega-6&7	1.07 (0.96-1.19)	0.23
Omega-3/omega-9	1.07 (0.96-1.19)	0.24
Linoleic acid (18:2)	1.10 (0.94-1.28)	0.24
Docosahexaenoic acid (22:6)	1.07 (0.95-1.21)	0.28
Other polyunsaturated fatty acids than 18:2	1.04 (0.90-1.21)	0.61
Average methylene groups in fatty acid chain	1.00 (0.89-1.12)	0.96
Average methylene groups per double bond	1.01 (0.87-1.18)	0.89
Average double bonds in fatty acid chain	1.00 (0.86-1.17)	0.99
Ratio of bisallylic groups to double bonds	0.99 (0.87-1.13)	0.91
Ratio of bisallylic groups to total fatty acids	1.00 (0.86-1.16)	0.98
Average fatty acid chain length	0.99 (0.88-1.12)	0.87

Table 23. Model fit, discrimination, calibration, and reclassification indices for prediction of 6-year prevalence of low CDist with models including conventional risk factors and metabonomics data.

Model	AUC	95% CI	P_{AUC}†	NRI [%]	P_{NRI}†	IDI [%]	P_{IDI}†	χ^2‡	Pχ2	AIC	HL	P_{HL}
A: age, systolic blood pressure, diastolic blood pressure, waist circumference, LDL-cholesterol, apoB, glucose, CRP (reference model)	0.753	0.724-0.782	–	–	–	–	-	2.9	0.09	887	3.8	0.87
B: non-laboratory risk factors*, small VLDL	0.751	0.721-0.780	0.55	0.5	0.66	-0.2	0.26	0.4	0.55	1484	8.0	0.43
C: non-laboratory risk factors*, small VLDL, lactate	0.756	0.726-0.784	0.36	0.6	0.76	0.8	0.02	2.2	0.14	1474	5.7	0.68
D: non-laboratory risk factors*, small VLDL, lactate, omega-9 and saturated fatty acids, average number of double bonds in a fatty acid chain	0.757	0.727-0.786	0.29	1.3	0.60	1.3	0.0005	2.1	0.15	1472	7.4	0.50

†: P-values for comparison of the reference model A with model B, model C, and model D.

‡: Log-likelihood ratio χ^2 as compared to a model with non-laboratory risk factors only.

* Non-laboratory risk factors: age, systolic and diastolic blood pressure, and waist circumference.

For NRI, participants were assigned to four categories (<5%, 5-10%, 10%-20%, and ≥20%) that reflected their 6-year risk of prevalence of low CDist based on each model.

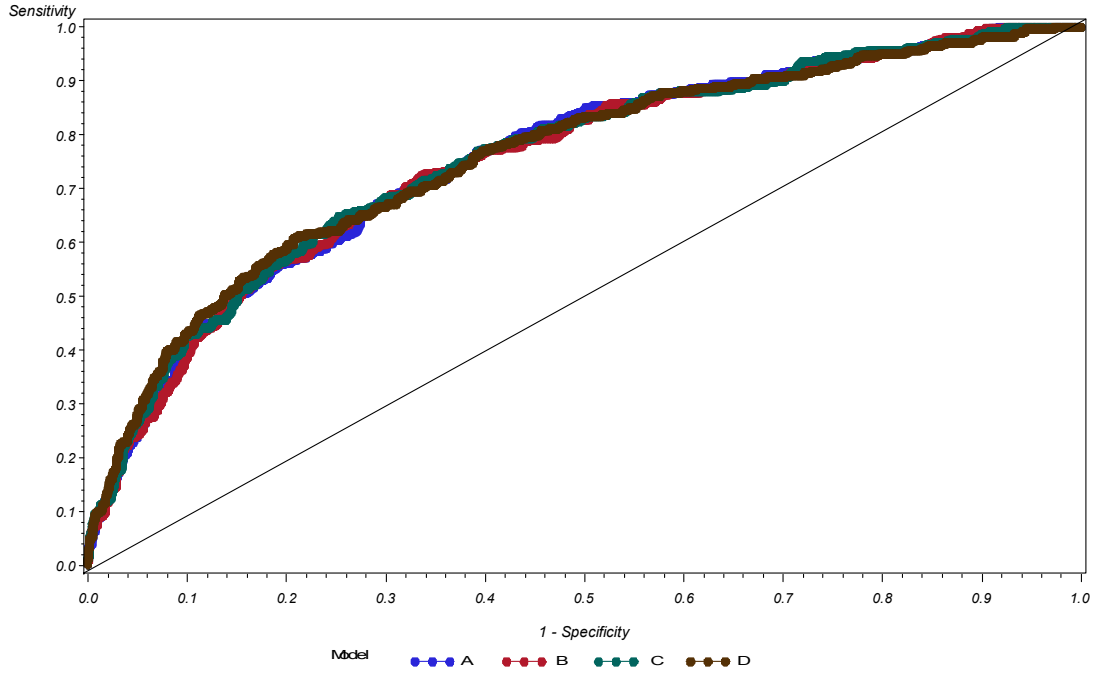


Figure 16. ROC curves for 6-year prevalence of CDist≤20th percentile for the reference model A with conventional lipid risk-factors, model B with NMR-based lipoprotein measures, model C with NMR-based lipoprotein measures and lactate, and model D with omega-9 and saturated fatty acids and average double bonds in a fatty acid chain.

5.4.3. Prediction of high 6-year IMT progression

Tables 24a-b display OR for high IMT proression (values ≥80th percentile). High IMT progression was predicted by small VLDL, large, medium and small LDL, medium and small HDL, LDL-cholesterol, HDL-cholesterol, urea, tyrosine, omega-3 fatty acids, ratio of bisallylic to double bonds and ratio to total fatty acids. The inverse association between urea and IMT progression was probably caused by significant direct association between urea and IMT in 2001 and nonsignificant association in 2007. Therefore, urea might not be protective from IMT progression.

Table 25 displays the reclassification analyses in prediction of high IMT progression. Compared to the reference model, addition of lipoprotein subclasses to conventional risk factors did not improve discrimination (AUC=0.718 vs. AUC=0.725, P=0.15). Addition of LMWM data increased discrimination significantly (AUC=0.718 vs. AUC=0.733, P=0.04). Figure 17 displays the ROC curves for the models.

Table 24a. Odds ratios for high 6-year IMT progression.

Lipoprotein subclasses	OR for progression(95% CI)	P-value
Extremely large VLDL	0.99 (0.87-1.13)	0.93
Very large VLDL	1.04 (0.92-1.19)	0.52
Large VLDL	1.04 (0.92-1.19)	0.53
Medium VLDL	1.07 (0.94-1.23)	0.32
Small VLDL	1.24 (1.08-1.42)	0.002
Very small VLDL	1.08 (0.95-1.23)	0.24
IDL	1.09 (0.96-1.24)	0.19
Large LDL	1.15 (1.01-1.31)	0.03
Medium LDL	1.17 (1.03-1.34)	0.02
Small LDL	1.15 (1.00-1.31)	0.04
Very large HDL	0.90 (0.78-1.05)	0.17
Large HDL	0.80 (0.68-0.94)	0.007
Medium HDL	0.82 (0.71-0.95)	0.007
Small HDL	0.97 (0.85-1.10)	0.64
NMR Lipids		
Total cholesterol	1.09 (0.96-1.24)	0.16
IDL cholesterol	1.12 (0.98-1.27)	0.09
LDL cholesterol	1.17 (1.03-1.34)	0.01
HDL cholesterol	0.83 (0.71-0.96)	0.01
Total triglycerides	1.08 (0.95-1.24)	0.25
VLDL triglycerides	1.07 (0.93-1.22)	0.35
IDL triglycerides	1.07 (0.94-1.22)	0.32
Conventional lipoproteins		
Total cholesterol	1.02 (0.85-1.21)	0.87
LDL-cholesterol	1.06 (0.89-1.27)	0.49
HDL-cholesterol	0.77 (0.63-0.94)	0.01
Triglycerides	1.11 (0.94-1.32)	0.23
Non-HDL-cholesterol	1.10 (0.92-1.31)	0.32
Total cholesterol/HDL cholesterol ratio	1.21 (1.03-1.42)	0.02
ApoA1	0.90 (0.75-1.08)	0.27
ApoB	1.19 (0.99-1.43)	0.07
ApoB/ApoA1 ratio	1.22 (1.02-1.45)	0.03

Table 24b. Odds ratios for high 6-year IMT progression.

NMR-based Low-Molecular-Weight Metabolites (LMWM)	OR for progression(95% CI)	P-value
3-hydroxybutyrate	1.02 (0.90-1.16)	0.72
Acetoacetate	0.98 (0.85-1.12)	0.73
Alanine	1.05 (0.92-1.20)	0.45
Creatinine	0.89 (0.76-1.03)	0.11
Glucose	1.00 (0.88-1.13)	0.96
Glutamine	1.08 (0.94-1.24)	0.30
Glycoproteins	1.04 (0.89-1.22)	0.60
Histidine	1.03 (0.91-1.17)	0.63
Isoleucine	0.99 (0.82-1.21)	0.94
Leucine	0.94 (0.81-1.08)	0.35
Urea	0.80 (0.70-0.91)	0.0006
Tyrosine	1.17 (1.02-1.34)	0.02
Valine	0.99 (0.85-1.15)	0.89
Lactate	1.08 (0.96-1.21)	0.22
Pyruvate	1.06 (0.93-1.20)	0.41
Citrate	0.93 (0.82-1.07)	0.31
Lipid particle components		
Total fatty acids	1.00 (0.80-1.25)	0.97
Esterified cholesterol	1.17 (0.95-1.46)	0.15
Free cholesterol	1.02 (0.83-1.24)	0.88
Total phosphoglycerides	0.87 (0.73-1.04)	0.12
Phosphatidylcholine	0.87 (0.73-1.03)	0.11
Sphingomyelins	1.03 (0.87-1.21)	0.77
Total cholines	0.89 (0.75-1.06)	0.20
omega-3 fatty acids	0.86 (0.74-1.00)	0.05
omega-6 and omega-7 fatty acids	1.08 (0.90-1.31)	0.41
omega-9 and saturated fatty acids	0.98 (0.78-1.23)	0.83
Omega-3/omega-6&7	0.88 (0.73-1.05)	0.15
Omega-3/omega-9	0.88 (0.73-1.05)	0.16
Linoleic acid (18:2)	1.17 (0.94-1.45)	0.17
Docosahexaenoic acid (22:6)	0.89 (0.73-1.08)	0.22
Other polyunsaturated fatty acids than 18:2	0.93 (0.74-1.18)	0.55
Average methylene groups in fatty acid chain	0.99 (0.84-1.18)	0.95
Average methylene groups per double bond	1.10 (0.87-1.39)	0.43
Average double bonds in fatty acid chain	0.86 (0.67-1.10)	0.22
Ratio of bisallylic groups to double bonds	0.77 (0.62-0.94)	0.01
Ratio of bisallylic groups to total fatty acids	0.77 (0.60-0.97)	0.03
Average fatty acid chain length	0.94 (0.78-1.14)	0.55

Table 25. Model fit, discrimination, calibration, and reclassification indices for prediction of high 6-year IMT progression with models including conventional risk factors and metabonomics data.

Model	AUC	95% CI	P_{AUC}†	NRI [%]	$P\text{-}_{NRI}$†	IDI [%]	P_{IDI}†	χ^2‡	$P\chi^2$	AIC	HL	P_{HL}
A: age, waist circumference, systolic BP, smoking status, baseline IMT, HDL/total cholesterol-ratio (reference model)	0.718	0.689-0.749	–	–	–	–	–	130.8	<0.0001	1673	9.2	0.32
B: non-laboratory risk factors*, small LDL, medium LDL, large LDL, HDL-cholesterol	0.725	0.696-0.755	0.15	4.5	0.05	0.8	0.005	135.9	<0.001	1499	17.1	0.03
C: non-laboratory risk factors*, small LDL, medium LDL, large LDL, HDL-cholesterol, urea, tyrosine	0.733	0.704-0.762	0.04	8.3	0.005	2.2	<0.0001	150.9	<0.001	1481	7.9	0.45

†: P-values for comparison of the reference model A with model B and model C.

‡: Log-likelihood ratio χ^2 as compared to a model with non-laboratory risk factors only.

* Non-laboratory risk factors: age, sex, waist circumference, systolic blood pressure, smoking status, and baseline IMT.

For NRI, participants were assigned to four categories (<5%, 5-10%, 10%-20%, and ≥20%) that reflected their 6-year risk of high IMT progression based on each model.

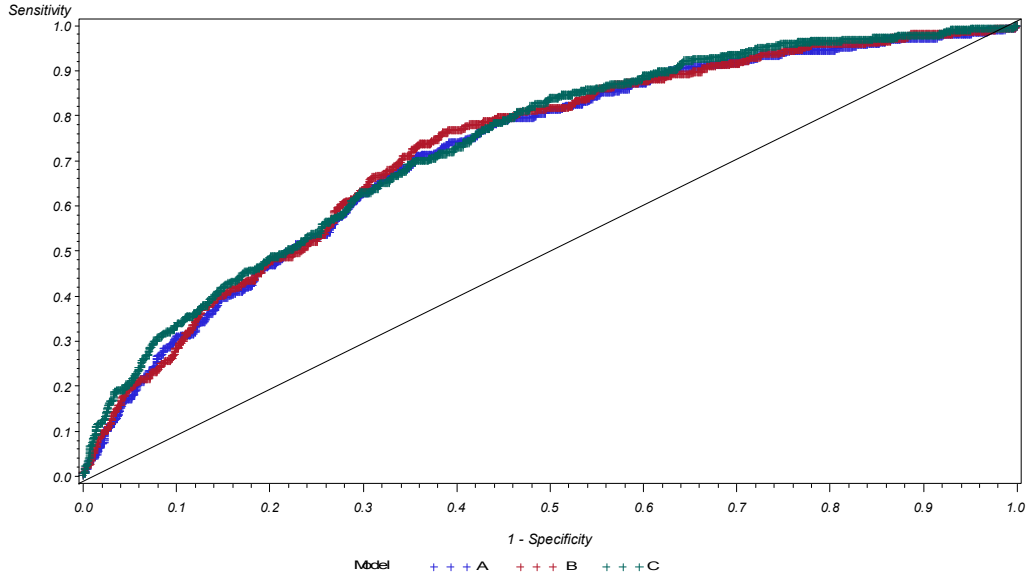


Figure 17. ROC curves for high 6-year progression of IMT (IMT progression ≥80th percentile) for the reference model A with conventional lipid risk-factors, model B with NMR-based lipoprotein measures, model C with NMR-based lipoprotein measures and lactate, and model D with omega-9 and saturated fatty acids and average double bonds in a fatty acid chain.

6. DISCUSSION

6.1. Study cohort

The aim in the Cardiovascular Risk in Young Finns study is to examine CVD risk factors in childhood, adolescence and young adulthood and their effects in development of CVD [33,41]. The cohort was selected to closely represent Finnish children and adolescents in the 1980s. The participants were chosen from both urban and rural areas and eastern and western parts of Finland.

Five cities with medical faculties and 12 rural municipalities in the surrounding areas participated in forming the cohort. Of 4,320 subjects who were invited to the first follow-up in 1980, 3,596 subjects aged 3, 6, 9, 12, 15 and 18 years participated in the study. Subsequent follow-ups were performed in 1983, 1986, 1989, 2001 and 2007. In 1989, follow-up was performed only in Turku. In 2001, a total of 2,283 participants aged 24-39 years were re-examined, and in 2007, 2,204 subjects aged 30-45 years took part in the follow-up. Subjects were predominantly clinically healthy in 2001 and 2007. In 2007, among 2,217 study subjects 46 (2.1%) received statins, 152 (6.9%) received antihypertensive medication and 25 (1.1%) received treatment for diabetes. Therefore, results are principally unaffected by medication and our cohort represents healthy or at least mostly unmedicated young Finns.

Loss to follow-up was examined between 2001 and 2007. 223 men and 232 women dropped out between 2001 and 2007. Moreover, male non-participants had higher systolic blood pressure and prevalence of smoking in 2001 than participants and female non-participants were younger and had higher systolic blood pressure, waist circumference and prevalence of smoking. According to these findings the observed 6-year changes in the mean levels of the mentioned variables might be different due to the observed selection bias in the cohort. Mean levels of subclinical atherosclerosis were similar in participants and non-participants. Despite higher risk factor levels, subclinical atherosclerosis had not developed more rapidly in non-participants probably due to their young age and the small differences in the risk factor levels compared to participants.

6.2. RESULTS

6.2.1. Risk factor levels in the follow-up in 2007 and changes in risk factor levels between 2001 and 2007

According to this study, significant decreases were observed in total cholesterol and LDL-cholesterol in young adults between 2001 and 2007. HDL-cholesterol appeared to increase in women simultaneously. Nevertheless, systolic and diastolic blood pressure, fasting glucose levels and waist circumference increased. Furthermore, prevalence of the MetS increased significantly according to the EGIR classification and both the updated NCEP and IDF classification displayed nonsignificant increase. The changes were mostly caused by the significant increases in serum glucose levels, blood pressure and waist circumference. Increase in prevalence of abdominal obesity between 2001 and 2007 continues the adverse trend in the development of body shape in the Finnish population from the 1980s to the early 2000s [340,341]. Although BMI has steadily increased in the Finnish population between 1980s and 2000s [342], our study found significant increase in BMI only in 36-39-year-old women. This indicates that adipose tissue seems to be more concentrated in the abdomen in 2007 than in 2001.

The 2007 follow-up in FINRISK study confirmed that total cholesterol level had started to decrease after a levelling-off period between 1997 and 2002 [10]. FINRISK discovered that blood pressure had ceased to decrease between 2002 and 2007 after the favourable trend between 1972 and 2002 [10]. Young Finns study found that blood pressure increased in both sexes. Globally, age-standardised mean systolic blood pressure was 128.1 mmHg in men and 124.4 mmHg in women with high regional variation [343] while in 2007 in Young Finns the levels were 126 mmHg and 117 mmHg.. Between 1980 and 2008, systolic blood pressure decreased by 0.8 mmHg in men and 1.0 mmHg in women per decade [343]. In Young Finns, systolic blood pressure increased 2.4 mmHg in men and 2.0 mmHg between 2001 and 2007. In global comparison, Finnish systolic blood pressure may be at an acceptable level but there is an increasing trend contrary to the worldwide development. In the FINDIET 2007 study [344,345], the changes in total cholesterol and blood pressure were suggested to be caused by changes in consumption of dietary fats, salt and alcohol. Between 2002 and 2007 the portion of saturated fats decreased and the amount of polyunsaturated fats increased in the Finnish diet [345]. Consumption of alcohol increased but the effect on blood pressure was compensated by the decrease in use of salt [345]. Our study found nonsignificant increase in alcohol use.

In order to protect both physical and financial welfare, more efforts should be made to increase awareness of the association between CVD risk factors and cardiovascular disease, to decrease CVD risk in currently healthy young adults and thus prevent development of clinical cardiovascular disease.

In 2007, the mean cholesterol concentration was 5.05 mmol/l in the Young Finns study. In 2008, the age-standardised global mean level of total cholesterol was 4.64 mmol/l for men and 4.76 mmol/l for women [346]. Mean levels of total cholesterol in Young Finns in 2007 (5.19 mmol/l for men and 4.93 mmol/l for women) were higher than the global levels. Among 30-45-year-old study subjects, 46.6% had their serum cholesterol above 5.0 mmol/l and 50.0% had their LDL-cholesterol above 3.0 mmol/l. 15.8% had their total cholesterol level below the ideal population mean concentration of 4.14 mmol/l set by WHO committee [347]. Between 1986 and 2001, serum triglycerides increased in 24-year-olds in Young Finns study, total cholesterol decreased only 5%, HDL-cholesterol increased. Globally, total cholesterol decreased 0.1 mmol/l per decade between 1980 and 2008 [346]. Therefore, trends in lipoprotein have rendered the mean lipid profile less atherogenic in 2007 than in 2001. Lifestyle changes may have had higher effect on the development in lipids rather than lipid medication since use of statins was still low in Young Finns cohort in 2007 although national use was almost 10% of the population in 2006 [348].

Clustering of risk factors in subjects with central obesity is well established [349]. Therefore, increased prevalence of central obesity might partially account for the observed increase in blood pressure and impaired glucose tolerance in young adults. Moreover, prevalence of MetS showed nonsignificant increase according to the NCEP and IDF criteria and significant increase according to the EGIR criterion. Most of the improvement in CVD occurred in risk factors that are not part of the MetS classifications though they are associated with MetS [350]. Apart from HDL-cholesterol, no components of MetS showed any improvement in 6 years suggesting that cardiometabolic profile in young Finns might have deteriorated between 2001 and 2007. This could be later observed as increased prevalence of MetS and CVD morbidity during the following the decades. Rokholm et al. found increases in obesity in adults aged 16-84 years since 1999 in European countries like Sweden and Denmark and Asian countries while stability was found in the US [17]. Between 1980 and 2000, obesity increased in Finnish adults in all age groups aged 30 years or older [342]. The increase was highest in individuals with the lowest education and women [342]. However, also well-educated subjects showed increase [342]. In Young Finns, increase in BMI was observed between 1986 and 2001 in 24-year-old subjects [14]. Worldwide prevalence of

obesity has increased for decades [16,351-353]. Between 1980 and 2008, global mean BMI increased 0.4kg/m^2 per decade [354]. Between 2001 and 2007, increase in BMI was 0.5 in women and 0.3 in men aged 30-39 years in Young Finns. Thus, increase in obesity might be slightly faster in Finland than in other nations. The trends in BMI vary substantially between nations [354]. It has even been estimated that majority of the world population will be overweight or obese by 2030 if the current development continues [15]. In the US, direct medical costs of CVD is currently 273 billion US dollars and the cost is expected to triple by 2030 and prevalence of CVD will increase by 9.9% [355]. However, numerous studies have shown a levelling-off in prevalence of obesity in children during the 2000s [17,356-359]. Therefore, increasing obesity and future comorbidity might be an issue only in Finns currently aged 30-39 years whereas future generation of young adults might not have a similar prevalence of obesity.

In 2010, AHA issued 2020 Impact Goal to improve CVD health in the US by 20% and reduce CVD and stroke deaths by 20% by the year 2020 [360]. The objective is to increase the prevalence of ideal cardiovascular health which consists of 4 ideal health behaviours (not currently smoking, BMI<25kg/m^2, physically very active, consumption of ≥3 servings/day of fruits and vegetables) and 3 ideal health factors (total cholesterol<5.17 mmol/l untreated, systolic blood pressure<120 mmHg and diastolic blood pressure<80 mmHg untreated, fasting plasma glucose<5.55 mmol/l) [360]. In 2011 in a US cohort of 1933 subjects with mean age of 59 years, one subject met all requirements, <10% of the cohort met >5 requirements, 2% subjects had all components of ideal health behaviour and 1.4% had all components of ideal health factors [361]. In 2001, 21.2% of 30-39-year-olds in the Young Finns cohort met 5 requirements though physical activity and diet were ignored due to lack of data. 37.6% had all components of ideal health factors and 41.3% had ideal BMI and did not smoke. In 2007, 21.6% had 5 ideal components, 39.8% had all ideal health factors and 41.2% were non-smoking and had ideal BMI in 30-39-year-olds. Finns seem to meet the ideal requirements for cardiovascular health exceptionally well though the Young Finns cohort was young and the risk factors were measured 4-10 years before the study by Bambs et al. [361]. Nevertheless, between 2001 and 2007 BMI, glucose levels and blood pressure increased in the Young Finns cohort while only total cholesterol decreased. If the adverse trends continue, fewer Finns will meet the requirements of ideal heart health in the future.

Between 2001 and 2007, FMD increased in all age groups except 36-39-year old men. IMT increased in 36-39-year-old women while other vascular changes were nonsignificant. Thus, mean endothelial function has improved in young adults. The

favourable development in other risk factors may have exceeded the vascular effects of increasing obesity, impaired glucose tolerance and blood pressure. However, no consistent trends were observed in IMT and CDist. Therefore, the effect of secular trends on development of subclinical therosclerosis in the Young Finns cohort remains unclear.

6.2.2. Tracking of ultrasound measurements of subclinical atherosclerosis

Examination of tracking of vascular ultrasound measurements revealed that consecutive IMT measurements had the highest 3-month and 6-year tracking. Difference between 3-month and 6-year tracking of IMT was nonsignificant suggesting that measurement error and short-term variability accounted for most of the variability in results and long-term arterial changes had only little effect on tracking. The same results were observed in tracking of CDist and FMD. FMD had the lowest tracking in 3-month and 6-year periods of follow-up. For IMT and CDist, tracking was higher in subjects with baseline 10-year CVD risk above median level according to SCORE risk score. Baseline BMI≥30kg/m2 decreased the tracking of CDist. Obesity may decrease tracking by increasing measurement error. Image quality in ultrasonography can be compromised by obesity [362]. Obesity interferes with ultrasound studies in two ways: the increased tissue layers result in poor penetration of the ultrasound beam beyond the focal depth and thick tissue layers attenuate the ultrasound signal [363]. Suitable methods for improving the image quality in arterial imaging are using the lowest frequency transducer available and applying pressure on the transducer to displace the subcutaneous fat and decrease the distance to the target organ [363,364]. Studies on dyslipidemic subjects have shown that increases in BMI and waist circumference decreases completeness of IMT measurements [365]. Additionally, increased IMT level has been shown to increase the measurement error for IMT in the Tromsø study [366] which can partially explain the observed effect of CVD risk.

Tracking of FMD was not affected by CVD risk or BMI and hypertension did not influence tracking of any examined ultrasound method. FMD was least affected probably due to its high physiological variability which can compensate for potential interference by the examined factors. Obesity may not have interfered with the measured enough to decrease tracking of FMD. CVD risk has previously been assessed with conventional risk factor profiles and studies have shown that tracking of these risk factor measurements is largely independent of interfering factors and differences between sexes [367]. Our study indicated that tracking of ultrasound measurements was influenced by age, sex and BMI and 10-year CVD risk at baseline at varying degrees.

The Tromsø study showed that interfering factors and sex had limited effect on tracking of conventional risk factors [367]. This suggests that ultrasound measurements might be less suitable for assessment of CVD risk than conventional risk factor measurements from the aspect of tracking. Accordingly, there are currently clinical guidelines for IMT [19,271] but measurement of arterial stiffness and FMD are not recommended for CVD risk estimation in asymptomatic adults outside research settings [19,314].

Older men had higher tracking than younger men. Older men have longer exposure to risk factors and higher stage of atherosclerosis than younger men. Therefore, subjects with low and high CVD risk are more likely to maintain their fractile in measurements of atherosclerosis at old age than at young age.

Low tracking of CDist and FMD can be attributed to the complexity of the methods. Tracking of CDist is affected by ultrasound measurement error and variability of blood pressure. Variability of blood pressure has been shown to decrease the reliability of CDist measurements [368]. Reproducibility of FMD has been low in previous studies [369] since FMD is affected by both measurement error and notable physiological variability of endothelial function [370]. Järvisalo et al. showed a 28% hourly variability and a 27% weekly variability in FMD [371].

6.2.3. Cardiovascular risk scores in prediction of subclinical atherosclerosis in young adults

In clinical practice, estimation of overall CVD risk is often based on risk score algorithms. Several studies have developed a number of risk scores based on different study cohorts, risk factors and clinical outcome variables. This demonstrated that all the examined risk scores had practically the same abilities in predicting subclinical atherosclerosis. Despite the fact that the risk scores were developed to predict clinical outcomes, based on comparison of our and previously published data, some risk scores performed equally when predicting IMT as the subclinical outcome. Framingham seems to possess discrimination in prediction of high IMT or carotid plaque in our cohort (AUC=0.735) that is comparable to that for 10-year CVD prediction in previous studies (AUC=0.66) [372]. Reynolds risk score seemed to have similar or slightly better performance in predicting 10-year CVD events in both sexes (AUC in males=0.708; AUC in females=0.808) [232,233] than in prediction of IMT in our study (nonstratified AUC=0.729). Apparently, addition of high-sensitivity CRP and parental history of myocardial infarction into risk score model increases only prediction of clinical events rather than subclinical changes. Exclusion of HDL-cholesterol in SCORE, addition of

LDL-cholesterol and triglycerides in PROCAM and addition of family history of CVD in PROCAM and Reynolds did not seem to have a notable effect on the prediction of subclinical atherosclerosis.

Moreover, risk scores were developed with different study cohorts, of which only the Finrisk cohort was Finnish. Finrisk, SCORE and PROCAM were European and Reynolds and Framingham were from the US. Nevertheless, the origin of the risk score did not affect discrimination or reclassification. Thus, risk assessment of subclinical atherosclerosis in young Finnish patients can be performed with any of the examined risk scores and the choice is limited by only the availability of laboratory results on lipids or knowledge of family risk of CVD.

6.2.4. Metabolic profiling in prediction of subclinical atherosclerosis

In our study, metabolic profiling with NMR improved risk stratification for IMT compared to conventional CVD risk factors with improved discrimination and reclassification. Highest prediction was achieved with a model including lipoprotein lipids (LDL-cholesterol$_{NMR}$ and IDL-cholesterol), lipoprotein subclasses (IDL and medium HDL) and tyrosine and docosahexaenoic acid levels.

Decreased CDist was associated with medium, small and very small VLDL, total, VLDL and IDL triglycerides, lactate, pyruvate, free cholesterol, omega-9 and saturated fatty acids, free cholesterol, lactate, pyruvate and average methylene groups per double bonds. Average double bonds in fatty acid chains and ratio of bisallylic groups to total fatty acids were inversely associated with low CDist prevalence. Metabonomics data improved slightly to no degree the prediction of prevalence of low CDist. Low FMD prevalence was nonsignificantly associated with free cholesterol and no other associations were observed with metabonomics variables. High IMT progression was predicted by small VLDL, large, medium and small LDL, medium and small HDL, LDL-cholesterol, HDL-cholesterol, urea, tyrosine, omega-3 fatty acids, ratio of bisallylic to double bonds and ratio to total fatty acids. Urea displayed an inverse association with IMT progression which might be accounted for the significant direct association with IMT in 2001 and nonsignificant association in 2007. Thus, the result on urea is not fully generalizable. Prediction of high IMT progression was nonsignificant or little when metabonomics data was included in the prediction models.

In our data, single metabolites did not improve risk discrimination and previous studies have shown that CVD risk stratification is rarely improved with addition of single biomarkers [373,374]. Atherosclerosis is a complex pathway with interrelating promoters and inhibitors and thus, metabonomics, with the ability to measure a vast array of

metabolites in samples, may be more suitable for examination of the overall metabolic status of atherosclerosis [24]. In addition, the examined NMR method has similar costs as conventional lipid measurements. In conclusion, metabonomics offers a more thorough and equally economical approach in CVD risk stratification compared to conventional lipid measurements. Clinical use of metabonomics is restricted by lack of clinical studies and very low number of metabonomics machinery in hospitals.

Significant associations were observed between low CDist prevalence and medium, small and very small VLDL particles, total, VLDL and IDL triglycerides, omega-9 and saturated fatty acids, free cholesterol, lactate, pyruvate and average methylene groups per double bonds. Pyruvate acts in several metabolic pathways including the citric acid cycle. Thus, its aherogenic role is unclear. However, pyruvate can be reduced to lactate. Lactate is also a metabolite in numerous pathways. Concentration of lactate is regulated by its constant production from pyruvate by lactate dehydrogenase and its removal. Lactate is also produced in muscle cells during anaerobic exercise. Thus, further studies would be needed to clarify the role of these observations. Glycoprotein and VLDL triglycerides were nonsignificantly associated with low CDist. Average double bonds in fatty acid chains and ratio of bisallylic groups to total fatty acids protected from low CDist. Thus, Atherogenic glycoproteins can be apolipoprotein(a), the glycoprotein component in atherogenic Lp(a) particles [375,376], or adhesion molecules or their ligands in monocyte infiltration in inflammatory atherosclerotic processes [377,378]. However, the measured glycoprotein is unspecific and the actual role of glycoprotein in atherogenesis is unclear. Low FMD prevalence was nonsignificantly associated with free cholesterol similar to low CDist. Associations between metabolites and CDist and FMD were relatively low and inconsistent with findings on IMT and metabolic profiling partly due to the high variability in these atherosclerosis markers.

Data on metabonomics variables did not significantly increase prediction of low CDist. Models that included data on lipoprotein subclasses, LMWMs and lipid metabolites slightly increased discrimination according to IDI, but the improvement was not significant according to AUC and NRI. Lack of improvement may be a result of variation in measurements of CDist. Reclassification in prediction of FMD was not studied due to lack of significant associations between low FMD and metabonomics data, which again may be due to the inherent variation observed for FMD. However, prediction of IMT progression was sligthly improved with addition of both lipoprotein subclasses and LMWM data. These findings suggest that measurement of IMT might be more suitable for studies examining associations between novel risk factors and subclinical atherosclerosis.

In a model with all covariates and metabolites with significant associations with low CDist in 6 years, average double bonds in fatty acid chains was the only metabolite that remained significantly inversely associated. Unsaturated fatty acids, especially polyunsaturated omega-3 fatty acids, seem to be vital in prevention of subclinical atherosclerosis as shown in previous studies [379,380]. Prevention of subclinical atherosclerosis by fatty acids with double bonds is supported by the inverse association between incident high IMT and docosahexaenoic acid, an omega-3 fatty acid. However, another polyunsaturated fatty acid, linoleic acid, was directly associated with IMT. Linoleic acid is an omega-6 acid and the observed protective nature of double-bonds in fatty acids might be due to the double bonds omega-3 acids.

Clinical utility of measurement of lipoprotein subclasses is currently under debate [197,210]. Previous studies have linked lipoprotein subclasses and especially small LDL with CVD risk [381]. Our study showed no higher atherogenicity for small LDL compared to other LDL subclasses. However, as shown in previous studies [382,383], small VLDL and IDL had significant associations with incident high IMT. Conventional lipid measures were replaced by metabonomics lipoprotein measures in our prediction models suggesting that conventional lipid testing could be replaced by NMR in clinical and research use. Previously, a small cross-sectional study found no improvement in prediction of subclinical atherosclerosis with lipoprotein subclasses [384]. Our study had larger cohort, longitudinal data and similar results when only lipoprotein subclasses were used in the models. Nevertheless, a large study on females indicated that NMR subclassing added no incremental value to conventional lipids in prediction of CVD end-points [385]. The observed controversies between our results and previous studies may be attributed to our lack of clinical end-points and the combination of metabolites used in our models rather than single metabolites.

Our study identified tyrosine and glutamine as new potential biomarkers for high IMT. The findings were confirmed with cross-sectional analysis in an independent population of Health 2000 study. The roles of tyrosine and glutamine in atherogenesis is currently unclear. Glutamine and glutamate levels have been shown to discriminate subjects with CVD from subjects without CVD and tyrosine acts as a part of a factor associated with CVD [24]. These amino acids have also been shown to be part of a principal component promoting insulin resistance and discriminating obese from lean subjects [386,387]. Elevated tyrosine, leucine, isoleucine, valine and phenylalanine levels predict future diabetes [388] which might partially explain the association between tyrosine and high IMT. Circulating amino acids may promote insulin resistance by disrupting insulin signalling in skeletal muscle or cause diabetes by decreasing insulin

secretion [388]. However, the associations tyrosine and glutamine with incident high IMT were significant when the models were adjusted for HOMA-IR in 2001 suggesting that the amino acids might have a role in atherogenesis independently of insulin resistance.

Recently, metabonomics has been introduced as a reasonable method for personalized CVD risk profiling and several metabolites have been introduced as novel risk factors [202,339,389,390]. More thorough risk estimation would also allow a more personalized risk intervention [391]. Therefore, identification of novel risk factors such as the ones in this thesis is essential for early recognition of subjects with high risk who may have low levels of conventional risk factors. These subjects with unidentified risk benefit the most from new risk markers.

Although a recent meta-analysis has shown no significant association with dietary saturated fat and CVD and CHD [392], importance of dietary unsaturated fats in prevention of CVD is widely recognised [393-396]. Previous data on the effect of unsaturated fats on subclinical atherosclerosis has been inconclusive [397-399] though omega-3 and omega-6 fatty acids have been associated with decreased clinical CVD risk [379,400-404] and markers of coagulation and inflammation [405,406]. Our data showed that docosahexaenoic acid had inverse and linoleic acid direct association with high incident IMT. Again, the specific roles in atherogenesis are unknown. Potential pathways may be triglyceride-lowering and anti-inflammatory capabilities of the acids [407,408]. Linoleic acid acts as a precursor of arachidonic acid and increased intake enhances production of leukotrienes promoting inflammation which further leads to atherogenesis [409]. Increased intake of omega-6 fatty acids like linoleic acid may increase CVD risk [410]. According to our results, levels of polyunsaturated fatty acids may be used in prediction of subclinical atherosclerosis in young adults.

7. LIMITATIONS

In study I, the risk factor trends of the subjects who participated in at least one of the follow-ups were similar to the trends of those who participated in both of the follow-ups. Blood pressure was measured with a random zero sphygmomanometer which usually indicates the blood pressure level to be lower than the actual value [411]. Therefore, the prevalence of hypertension and MetS might be lower in the current study than in other similar studies. In addition, the low prevalence of MetS in the cohort of 33-year-old women in 2007 was already observed in the follow-up in 2001 when the subjects were 27-year-old [412]. These observations may indicate that the unexpected prevalence is induced by the deviant structure of the concerned cohort. Therefore, the decrease in the prevalence of MetS between 2001 and 2007 in 33-year-

old women according to the NCEP and IDF classifications was likely a result of the cohort with low prevalence of MetS rather, than by an actual change.

In the analyses concerning the changes in risk factor levels between 2001 and 2007, some values in different study years were obtained from the same subjects. Therefore, statistical tests were performed separately for subjects aged 30–33 and 36–39 years to avoid analyses using data from same subjects. However, sampling biases may have affected the observations. Altogether, the results in study I were similar to Finrisk study [413] suggesting that these biases did not have a remarkable effect on the findings.

In study I, results on secular trends of ultrasound variables are unreliable since the effect of measurement error on the observed trends was not taken into account. This limits the interpretation of the trends in ultrasound measurements.

In study II, only 57 subjects had their ultrasound measurements re-examined. The limited number of subjects for these analyses needs to be considered in the interpretation of these findings. However, no difference in long-term tracking was found between re-examined subjects and the rest of the cohort. It is likely, therefore, that tracking in the re-examination sub-group was representative of tracking in the total cohort. Specific techniques to manage analysis based on grouped data, correlation analyses comparing tracking coefficients between different groups, were also performed between mean values in study II.

The effect of CVD risk on tracking was examined by comparing subjects with lower and higher than median values of SCORE. However, this method did not specify which confounding factors in the risk score influenced tracking. Age and sex were included in SCORE and they both independently influenced tracking. Therefore, the combined effect of CVD risk factors was not able to be fully elucidated in this study.

In study III, the risk scores were originally designed for prediction of clinical events (myocardial infarction, CHD or CVD) over a 10-year period. Data on CVD end-points was unavailable in the Young Finns cohort. Instead, markers of subclinical atherosclerosis from the 2007 follow-up were used as outcome variables. Of these, the most consistent data concerning associations with CVD events is available for IMT.

Reynolds risk score for females included hemoglobinA_{1C} levels for diabetics. However, hemoglobinA_{1C} was not measured in Young Finns study and thus, effect of diabetes was omitted from analyses. Effect of exclusion, if anything, is likely to be small due to the number of diabetic men and women (N=6 in 2001 and N=11 in 2007). In addition, Reynolds and PROCAM risk scores define parental history of CVD as myocardial infarction <60 years in either parent. In Young Finns, parental history of

CVD was classified as myocardial infarction <55 years in either parent in 2001 and <55 years in males and <65 years in females in 2007.

There are no official cut-points for IMT, CDist or FMD. Therefore, the results based on ultrasound cut-points in studies III and IV are not fully generalisable. However, these analyses were performed with different cut-points with similar results. In addition, there are multiple methods for measuring IMT, Cdist and FMD and the current results might be generalised only to the used methods.

Associations between clinical events and carotid IMT has been shown to attenuate when within study reader variability is not accounted for [414] and it has been recommended that the same ultrasonographer performs all measurements in follow-up studies [415]. In Young Finns study, all ultrasound data was analysed by the same reader. However, the best protocol for IMT measurement considering reproducibility is mean IMT where the near and far walls are measured at multiple angles [416,417] while in Young Finns IMT was measured at one angle.

Serum samples from the follow-ups prior to 2001 were not suitable for metabolic profiling. Thus, it was not possible to determine the efficacy of knowledge of metabonomic data during childhood and adolescence.

8. CLINICAL IMPLICATIONS

Risk factor levels and secular trends

The observed increase in obesity is consistent with the global trend in adult population and obesity-associated risk factors, blood pressure and glucose intolerance, displayed the increasing trend in cardiometabolic risk caused by MetS. However, no significant secular trends were found in IMT or CDist and the nonsignificant 6-year increase in IMT was extremely low compared to the CVD risk increase shown by Hodis et al. [263] and Lorenz et al. [264]. Moreover, FMD showed an unexpected improvement in both sexes between 2001 and 2007 that may have reflected the observed improvement in lipid profile of the cohort. Current data supports stronger involvement in diagnostics, treatment and prevention of obesity and obesity-related components of MetS in young Finnish adults in basic health care.

CVD risk factors are often left unidentified and untreated in primary health care [422-428]. Results from this thesis suggest that CVD risk factors should be screened more widely and intensively among those with no apparent risk and whose primary illness might not be CVD-related. Costs for conventional risk factor assessment are lower than for the treatment of clinical CVD caused by undiagnosed prolonged risk exposure.

Examination of risk factor levels and 6-year changes revealed that although lipid profiles seem to have improved, factors like blood pressure and prevalence of MetS seem to have increased. This might suggest that more efforts be targeted toward risk factor profiles in young Finnish adults to prevent CVD in the future.

Prediction of subclinical atherosclerosis

According to our results, all the examined CVD risk scores have somewhat the same performance in prediction of subclinical atherosclerosis. Due to the lack of official cut-points for ultrasound markers of atherosclerosis and clinical end-points, the presented results are currently not valid for use in clinical practice. According to AHA guidelines in 2010, IMT is recommended for risk estimation in subjects at intermediate risk while CDist and FMD are limited to research settings [19]. Results in studies II-IV suggest that IMT tracks higher and is more related to conventional and novel risk factors than CDist and FMD. Therefore, of nonivasive ultrasound measurements of atherosclerosis, these data suggest IMT would provide clinicians with the most realistic and reproducible data on the arterial status of the patient.

In asymptomatic patients, carotid IMT and plaques revise CVD risk in subjects with low Framingham risk score and outperform coronary artery calcium in risk revision [429]. This lends support to the clinical use of IMT in screening asymptomatic subjects. Screening studies with IMT are recommended due to the incremental value over and above Framingham risk score especially in intermediate- and possibly low-risk subjects, better matching intensity of treatment to estimated risk and potential improvement in patient commitment to prevention [430]. As an inexpensive and radiation-free method, IMT could be used as a selective diagnostic test in asymptomatic patients prior to coronary artery calcium scoring which has better relative prognostic value compared to IMT [430]. According to data from this thesis, carotid IMT could be applied in screening for subclinical atherosclerosis in the Finnish population. CDist and FMD are less suitable due to their high variability.

Lack of fit was observed in risk scores predicting subclinical atherosclerosis in young adults. This finding likely reflects that the scores were designed for use in much older populations for the prediction of clinical events. However, similar goodness of fit was found in prediction of CVD in adults with Framingham and SCORE risk scores [431] and Framingham, SCORE and PROCAM have displayed overrestimation of risk [432,433] which has not limited the use of risk scores in clinical practice. More suitable risk scores for subclinical atherosclerosis in young adults are needed for better calibration, discrimination and earlier risk intervention. Novel risk scores could be developed with

existing data on ultrasound measurements, conventional risk factors and metabonomic variables in Young Finns study. Due to the findings in study IV, metabonomic variables could be included in some of the models. The new scores could be compared with existing scores in cohorts of the Young Finns study and validated in other similar studies.

Risk stratification for subclinical atherosclerosis was improved with metabolic profiling. Compared with conventional risk factor assessment, measurement of the metabolic profile offers a more thorough view on the metabolic status and cardiovascular risk of a patient. Thus, metabonomics may offer a new individually designed approach in prevention and treatment of CVD in the future.

Guidelines by AHA [19] may encourage Finnish and European health care officials to include IMT measurements in CVD risk assessment in subjects with identified risk. Vascular ultrasound equipment and training are widely available in special health care and present no obstacles for the clinical use of IMT. However, there are no randomized controlled trials showing reduced morbidity and mortality due to screening of IMT. Clinical use of CDist and FMD is limited due to lack of tracking and the complexity of the measurement of vascular function compared to the anatomic marker IMT. In study I, trends in conventional risk factors were inconsistent and the overall change in CVD risk was unclear. However, significant trends were observed in FMD suggesting that ultrasound measurements might add incremental information to conventional methods. In terms of tracking, IMT is reproducible and tracks high enough to meet the same standards as conventional risk factors. Ultrasound is noninvasive, relatively inexpensive and widely available in specialist health care and could thus be used in those with CVD risk to potentially refine their risk status.

9. FUTURE RESEARCH NEEDS

To gain further information on the development of CVD in Finland, future studies should investigate the ongoing temporal trends in cardiovascular risk factor levels in young adults in order to prevent CVD among young Finns.

Baseline BMI decreased tracking of CDist, whereas IMT and FMD were not affected by obesity estimated by elevated BMI. Future studies are needed to reproduce these results to confirm whether the tracking of noninvasive ultrasound measurements are mostly unaffected by obesity. Moreover, high baseline CVD risk estimated with SCORE risk score seemed to increase tracking of IMT and CDist. Additional research should assess if the observed effect was caused by better tracking in subjects with higher state of subclinical atherosclerosis or if CVD risk or components of the risk

score have a direct effect on tracking of subclinical atherosclerosis. Although tracking of ultrasound was mostly not affected by BMI, increasing obesity may interfere with image quality in ultrasound measurements in the future suggesting the need for optional imaging methods unaffected by obesity if imaging of asymptomatic atherosclerosis becomes standard. Thus, tracking of noninvasive ultrasound could be compared to tracking of other nonivasive imaging methods (for instance CT coronary artery calcium score) for quantification of subclinical atherosclerosis.

In 2009, the state of Texas approved a legislation that mandates insurance coverage for coronary artery calcium scanning or IMT measurement every 5 years for 45-75-year-old men and 55-75-year-old women with diabetes or intermediate or higher CHD risk based on Framingham risk score [431]. This legislation was the first of its kind but no data currently exists showing whether screening of IMT will improve public health. Hackam et al. showed that noninvasive imaging screening for inducible myocardial ischemia, coronary calcification, left ventricular hypertrophy or carotid atherosclerosis had no effect on alteration of primary prevention efforts of CVD [432] and in a recent editorial in the Archives of Internal Medicine, the need for randomized controlled trials for the potential benefits of noninvasive imaging screening of atherosclerosis is recognized due to the current lack of such studies [433]. This thesis could not examine the efficacy of adding IMT measurements to risk stratification. Randomized controlled trials are needed to examine if IMT screening improved clinical outcomes of CVD in populational level and which potential clinical cut-points to use for IMT.

This study suggests that different cardiovascular risk scores predict atherosclerosis estimated by IMT, CDist and FMD with largely similar accuracy independently of their origin, components or the clinical event that the risk scores were designed to originally predict. Thus, these results should be reproduced in different settings to assess possible underlying mechanisms. Additionally, future studies should develop novel risk scores for prediction of subclinical atherosclerosis for application among child, adolescent, and young adult populations and compare their performance with existing adult risk scores.

With increasing age of the study population, data on clinical end-points like myocardial infarction and data acquired in autopsies will be available in future follow-ups. Therefore, the ability to predict clinical events and macroscopic anatomical vascular changes with CVD risk scores and metabolic profiling in young adulthood should be examined in future follow-ups.

Metabonomics data is only available for adult follow-ups in the Young Finns study. Thus, there is further need for metabonomics studies in younger age groups. Moreover,

the results on associations between early atherosclerosis and metabolites require further studies to specify the metabolic pathways in atherogenesis. The cellular-level effects of novel risk markers on atherogenesis can not be studied with epidemiological data. Biomedical studies on animal models and cell cultures could further investigate the mechanisms of the metabolites in atherosclerosis.

Due to the isolated character of the Finnish genome, the results on tracking, risk scores and metabonomics should be reproduced in other populations to control for potential population-based differences in metabolism and in vascular structure and function. Previous work in Young Finns has shown that family history of CVD renders vasculature more vulnerable to cardiometabolic risk factors [136]. Reproduction of the analyses should be performed considering family history of CVD or genomic data on CVD risk. Moreover, the analyses could be reproduced in foreign cohorts to examine the genetic effect on the atherogenicity of metabolites and lipoprotein subclasses.

AHA has set criteria for evaluation of novel markers of CVD risk [332]. Novel markers should differ between subjects with and without a particular outcome, predict development of future outcomes in a prospective cohort, add prediction to conventional risk markers, change predicted risk sufficiently to change recommended therapy and improve clinical outcomes and the improvement should justify the additional costs. In study IV, markers in reclassification models meet the first 3 requirements. However, due to the lack of medical treatment, prevention and data on outcome of treated and untreated subclinical atherosclerosis and lack of treatment methods (i.e. risk caused by amino acids and specific lipoprotein subclasses), the latter 3 requirements could not be studied with the current data and treatment. Therefore, future studies could assess if intervention in subjects at increased risk based on metabonomics data improved outcome in subclinical atherosclerosis compared to subjects with treatment based on conventional risk factors.

10. CONCLUSIONS

1. 6-year changes in total cholesterol, LDL-cholesterol and HDL-cholesterol in young Finns were favourable between 2001 and 2007. However, waist circumference, glucose and blood pressure levels increased. Therefore, continuous efforts are still needed in fighting against cardiovascular risk factors.
2. IMT measurements seemed to track better than CDist and FMD examinations. Baseline BMI decreased tracking of CDist. Tracking of IMT and CDist displayed significant increase in subjects with elevated baseline SCORE risk score. Similar tracking analyses on ultrasound measurements have not been previously published. Our data suggest that further studies on the topic are required for proper validation of ultrasound measurements in clinical and scientific use.
3. CVD risk scores are able to predict future subclinical atherosclerosis in young adults. According to our results, risk of subclinical atherosclerosis in young adults can be assessed with any of the examined risk scores. Since our findings link early adulthood CVD risk to vascular changes, young adults should be motivated to reduce CVD risk at an early stage.
4. Lipoprotein subclass testing with NMR methods did not substantially add to conventional lipids for the prediction of subclinical atherosclerosis. Nevertheless, circulating metabolites markedly improved risk stratification. In addition, tyrosine and docosahexaenoic acid levels were found as potential biomarkers of high carotid IMT. Therefore, NMR metabonomics may benefit patients in individual planning of prevention and treatment of atherosclerosis, particularly carotid IMT, at an early stage.

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"No man is an island, entire of itself; every man is a piece of the continent, a part of the main. If a clod be washed away by the sea, Europe is the less, as well as if a promontory were, as well as if a manor of thy friend's or of thine own were: any man's death diminishes me, because I am involved in mankind, and therefore never send to know for whom the bell tolls; it tolls for thee."
- Ernest Hemingway: For whom the bell tolls

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APPENDIX

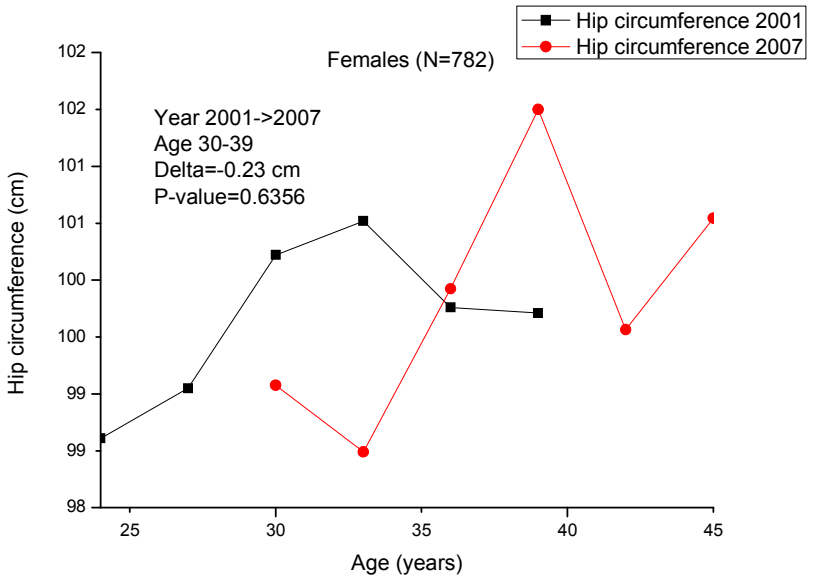

Figure 1. Trend in hip circumference in women between 2001 and 2007.

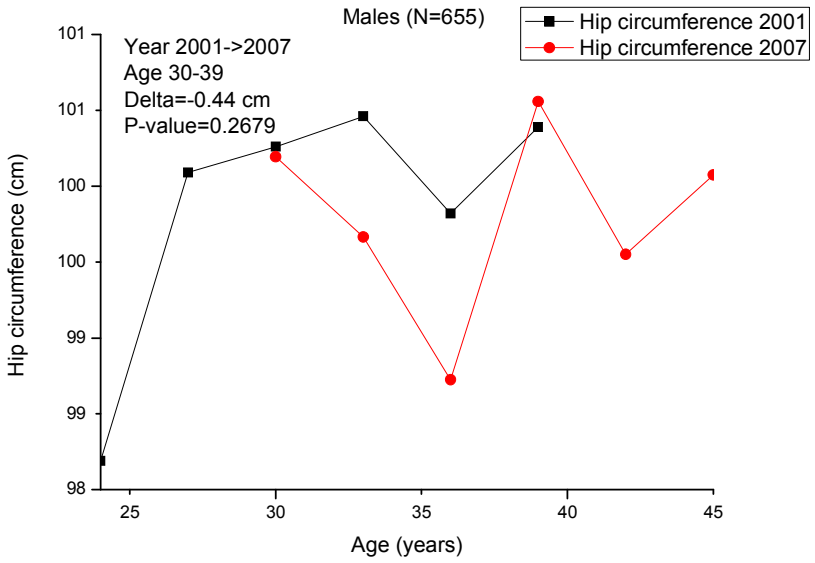

Figure 2. Trend in hip circumference in men between 2001 and 2007.

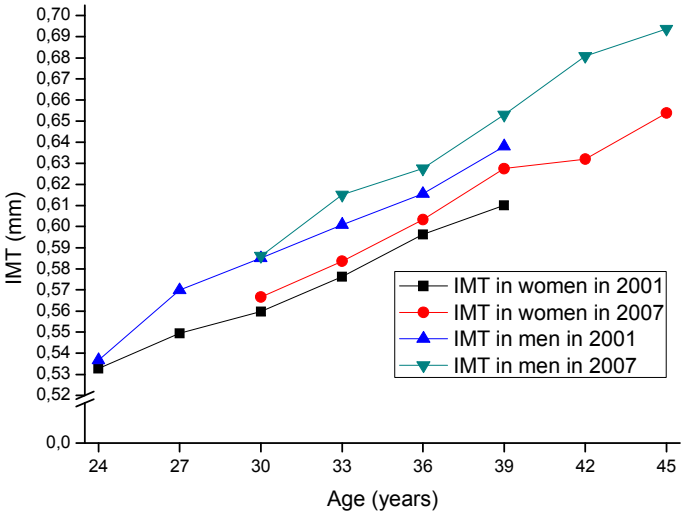

Figure 3. Mean IMT in women and men in 2001 and 2007.

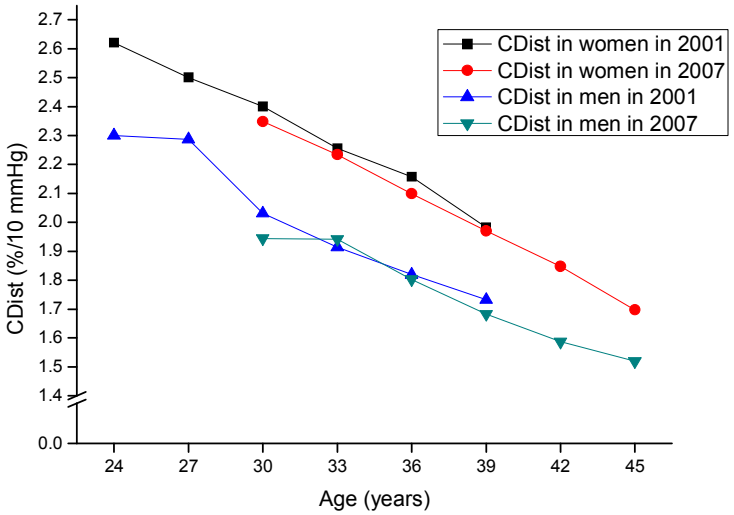

Figure 4. Mean CDist in women and men in 2001 and 2007.

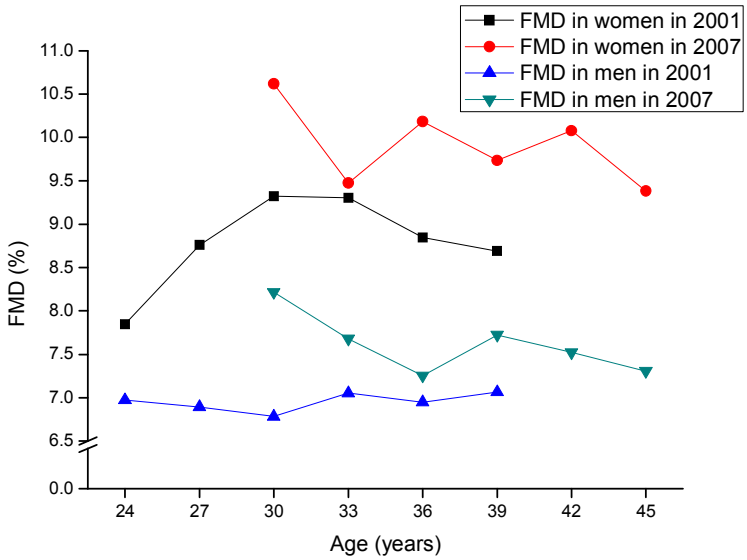

Figure 5. Mean FMD in women and men in 2001 and 2007.

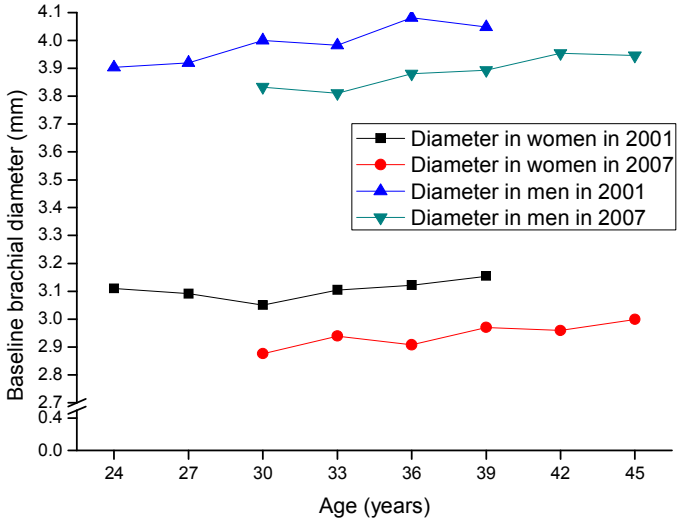

Figure 6. Mean baseline brachial diameter in women and men in 2001 and 2007.

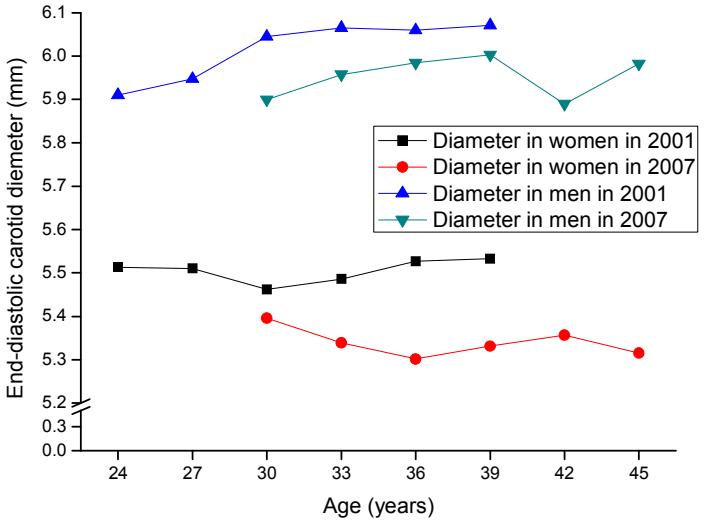

Figure 7. Mean end-diastolic carotid diameter in women in 2001 and 2007.

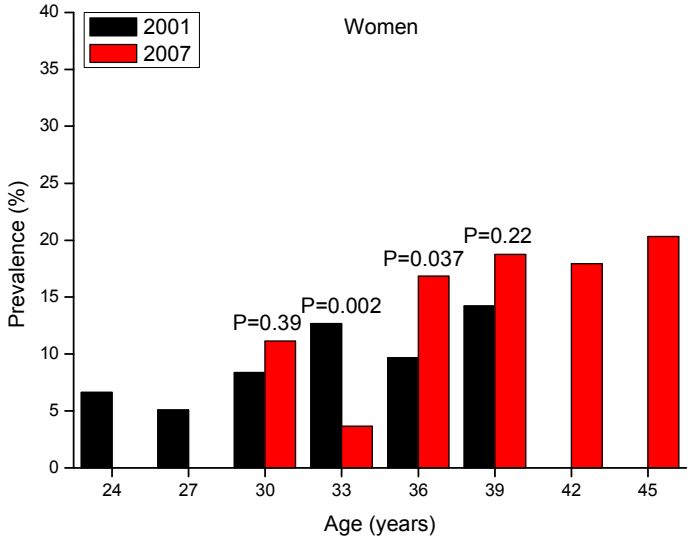

Figure 8. Prevalence of MetS according to the NCEP classification in women in 2001 and 2007.

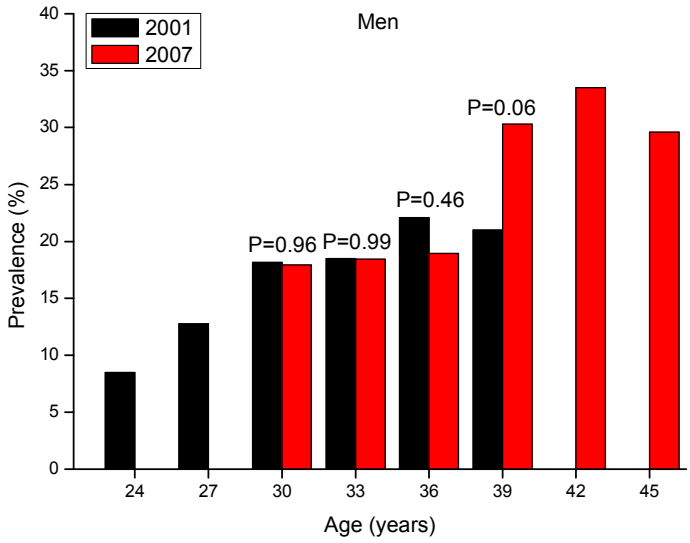

Figure 9. Prevalence of MetS according to the NCEP classification in men in 2001 and 2007.

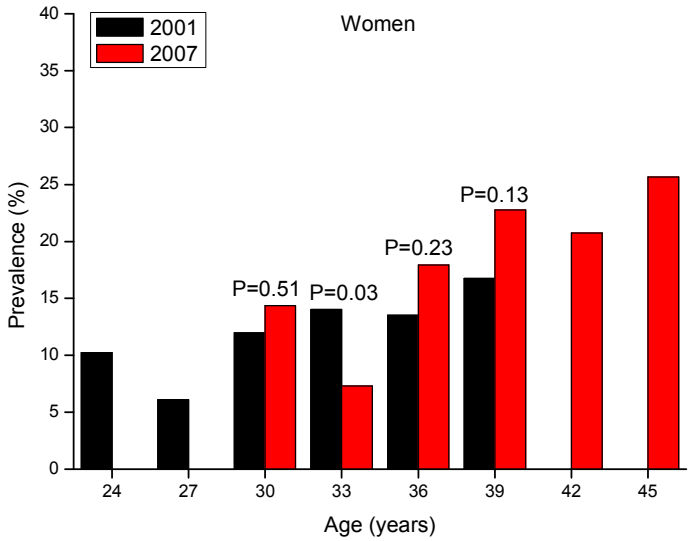

Figure 10. Prevalence of MetS according to the IDF classification in women in 2001 and 2007.

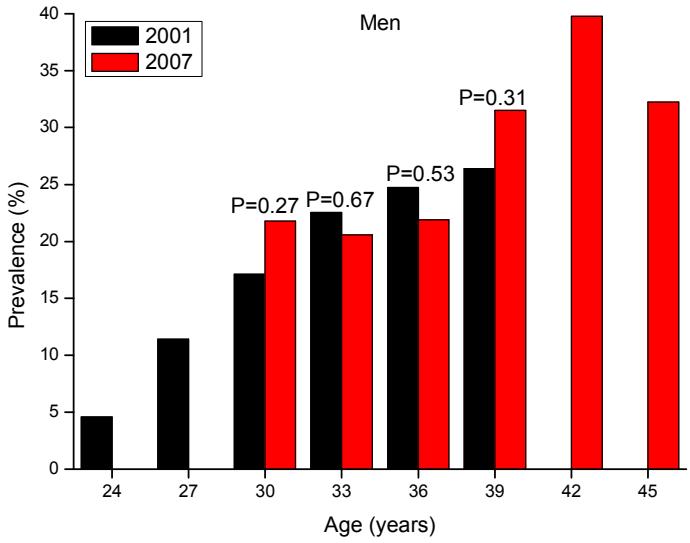

Figure 11. Prevalence of MetS according to the IDF classification in men in 2001 and 2007.

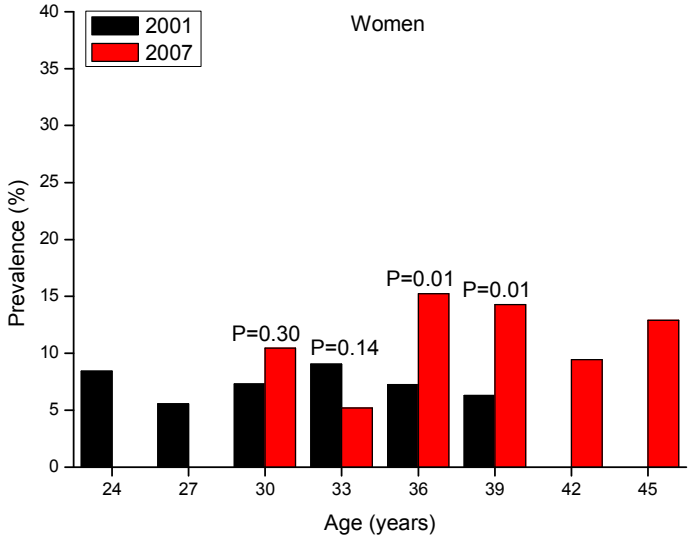

Figure 12. Prevalence of MetS according to the EGIR classification in women in 2001 and 2007.

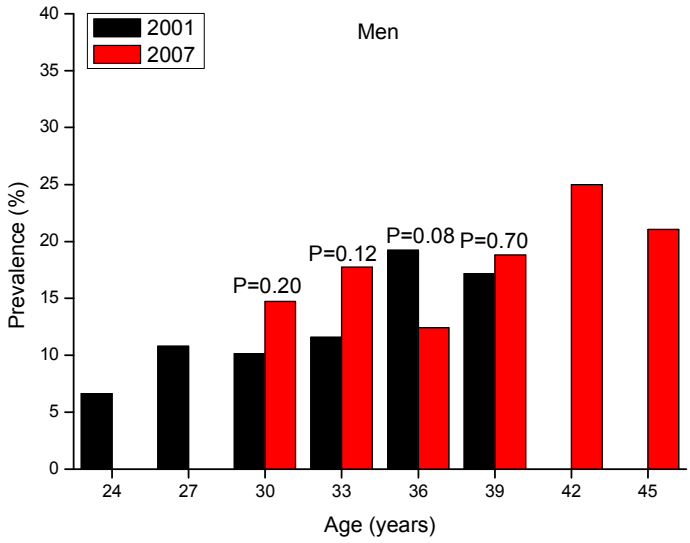

Figure 13. Prevalence of MetS according to the EGIR classification in men in 2001 and 2007.

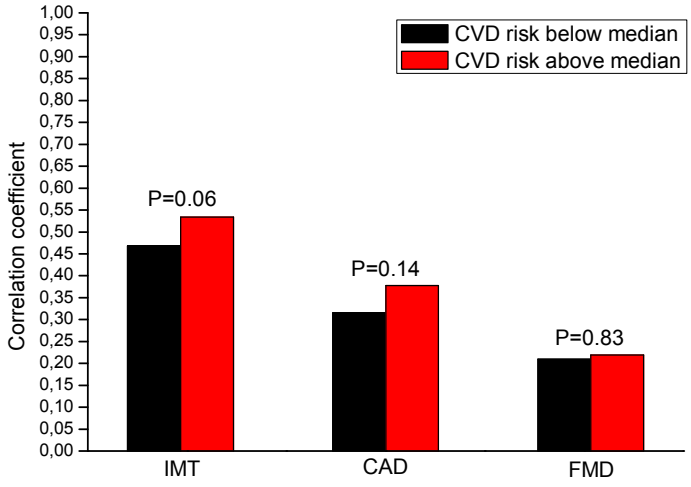

Figure 14. Effect of CVD risk based on Framingham risk score on tracking of ultrasound measurements in 2001 and 2007.