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The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

†Deceased

ISBN 978-951-29-6834-3 (PRINT)
ISBN 978-951-29-6835-0 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama Oy - Turku, Finland 2017

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ABSTRACT

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Cardiovascular risk factors and cardiovascular risk prediction from childhood to adulthood. The Cardiovascular Risk in Young Finns Study.

Background: In the late 1960s, Finland had the world's highest coronary heart disease mortality. Atherosclerosis has its roots in childhood. Childhood risk factors are associated with risk factors in adulthood that predict arterial changes, surrogate markers for atherosclerosis. Since the 1960s, coronary heart disease mortality has decreased over 80%. Over two thirds of the reduction in mortality can be explained by change in risk factor levels. Nevertheless, atherosclerotic cardiovascular diseases are still the leading cause of death and disability in Finland and worldwide.

Aims: The aims of this thesis were to: study risk factor levels and their changes in Finnish adult population; investigate how many childhood measurements of lipids and blood pressure are needed to optimize the prediction of adult risk factor levels and arterial changes; and determine if adding information on genetic variants to child lipid levels improves prediction of adulthood dyslipidemia.

Participants and methods: This thesis uses data from the Cardiovascular Risk in Young Finns Study, a prospective cohort of Finnish children initiated in 1980. Over 31 years, cardiovascular risk factors were measured from participants at several follow-ups. In the most recent follow-up performed in 2011, 57% of the original study population participated.

Results: In 2011, the previously observed favorable trends in cholesterol levels had leveled off and over one-third of participants had prediabetes. Two lipid and blood pressure measures in childhood significantly improved prediction of adult dyslipidemia and hypertension over one measurement. Use of genetic risk score approach combined to traditional risk factors significantly enhanced the prediction of adulthood dyslipidemia over conventional childhood risk factors.

Conclusions: Favorable trends in cardiovascular risk factor levels appear to be leveling off in Finland. At least two measures of blood pressure and lipids should be used in childhood assessment for future cardiovascular disease risk. Genetic information can help identify children at risk for adult dyslipidemia.

Keywords: adulthood, atherosclerosis, blood pressure, childhood, dyslipidemia, genetic risk score, hypertension, lipid, lipoprotein, risk factor

TIIVISTELMÄ

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Valtimotaudin riskitekijät ja niiden ennustaminen lapsuuden havaintojen perusteella (LASERI-tutkimus)

Tausta: Suomessa oli 1960-luvulla maailman korkein sepelvaltimotautikuolleisuus. Valtimotauti alkaa lapsuudessa. Lapsuuden riskitekijät ovat yhteydessä aikuisiän riskitekijöihin, jotka taas ennustavat valtimomuutoksia. Sepelvaltimotautikuolleisuus on laskenut Suomessa yli 80 %:lla 1960-luvulta lähtien. Yli kaksi kolmasosaa kuolleisuuden vähentymisestä selittyy riskitekijätasojen muutoksilla. Valtimotauti on yhä yleisin kuolinsyy sekä toimintakyvyn menetyksen aiheuttaja Suomessa ja maailmalla.

Tavoite: Väitöskirjatyön tavoitteena oli tutkia valtimotaudin riskitekijätasoja ja niiden muutoksia suomalaisessa aikuisväestössä. Toisena tavoitteena on ollut selvittää, kuinka monella lapsuuden aikaisella kolesteroli- tai verenpainemittauksella voidaan tehokkaasti ennustaa aikuisiän riskitekijätasoja ja valtimomuutoksia, sekä tutkia parantaako geneettinen tieto aikuisiän rasva-aineenvaihdunnan häiriöiden ennustamista.

Menetelmät: Väitöskirjatutkimus on osa vuonna 1980 aloitettua Lasten Sepelvaltimotaudin Riskitekijät (LASERI) – seurantatutkimusta. Koko tutkimuksen ajan tutkittavilta on mitattu sydän- ja verisuonitautien riskitekijöitä kattavasti. Vuonna 2011, yli 30 vuoden seurannan jälkeen, oli mukana edelleen 57 % alkuperäisestä tutkimusväestöstä.

Tulokset: Vuonna 2011 todettiin vuosikymmeniä jatkuneen myönteisen trendin veren kolesteroliarvoissa tasoittuneen. Lisäksi kolmanneksella väestöstä oli diabeteksen esiaste. Kahdella lapsuuden aikaisella mittauksella saatiin luotettava ennuste aikuisiän rasva-aineenvaihdunnan häiriöistä ja kohonneesta verenpaineesta, kolmannesta mittauksesta saatava hyöty jäi vaatimattomaksi. Geneettinen tieto alttiudesta rasva-aineenvaihdunnan häiriöille paransi merkittävästi aikuisiän rasva-aineenvaihdunnan häiriöiden ennustamisen tarkkuutta.

Johtopäätökset: Suomalaisten sydän- ja verisuonitautien riskitekijätasojen myönteinen kehitys näyttää tasoittuvan. Lapsuuden verenpaine- ja kolesterolitasojen arvioinnissa on syytä käyttää vähintään kahdesti toistettua mittausta. Geneettistä tietoa käyttämällä voidaan tarkemmin tunnistaa jo lapsena henkilöt, joilla on kohonnut riski rasva-aineenvaihdunnan häiriöihin aikuisena.

Avainsanat: aikuisikä, dyslipidemia, geneettinen riski, lapsuus, lipoproteiini, rasva-aine, riskitekijä, valtimotauti, verenpaine, verenpainetauti

CONTENTS

ABBREVIATIONS

AUC	area under the receiver operating characteristic curve
ApoE	apolipoprotein E
BMI	body mass index
BP	blood pressure
CHD	coronary heart disease
CI	confidence interval
CVD	cardiovascular disease
DBP	diastolic blood pressure
GWAS	genome-wide association study
HbA1c	glycated hemoglobin
HDL-C	high-density lipoprotein cholesterol
IDI	integrated discrimination improvement
IMT	intima-media thickness
LDL-C	low-density lipoprotein cholesterol
MetS	metabolic syndrome
non-HDL-C	non-high-density lipoprotein cholesterol
NPV	negative predictive value
NRI	net reclassification improvement
OR	odds ratio
PPV	positive predictive value
SD	standard deviation
SBP	systolic blood pressure
SNP	single-nucleotide polymorphism
T2D	type 2 diabetes mellitus
TC	total cholesterol
TG	triglycerides
wGRS	weighted genetic risk score
Young Finns Study	Cardiovascular Risk in Young Finns Study

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. Additional unpublished data are also presented.

I **Nuotio J**, Oikonen M, Magnussen CG, Jokinen E, Laitinen T, Hutri-Kähönen N, Kähönen M, Lehtimäki T, Taittonen L, Tossavainen P, Jula A, Loo BM, Viikari JS, Raitakari OT, Juonala M. Cardiovascular risk factors in 2011 and secular trends since 2007: The Cardiovascular Risk in Young Finns Study. *Scand J Public Health* 2014;42:563-571

II Oikonen M, **Nuotio J**, Magnussen CG, Viikari JS, Taittonen L, Laitinen T, Hutri-Kähönen N, Jokinen E, Jula A, Cheung M, Sabin MA, Daniels SR, Raitakari OT, Juonala M. Repeated blood pressure measurements in childhood in prediction of hypertension in adulthood. *Hypertension* 2016;67:41-47

III **Nuotio J**, Oikonen M, Magnussen CG, Viikari JSA, Hutri-Kähönen N, Jula A, Thomson R, Sabin MA, Daniels SR, Raitakari OT, Juonala M. Adult dyslipidemia prediction is improved by repeated measurements in childhood and young adulthood. The Cardiovascular Risk in Young Finns Study. *Atherosclerosis* 2015;239:350-357

IV **Nuotio J**, Pitkänen N, Magnussen CG, Buscot MJ, Jokinen E, Laitinen T, Taittonen L, Hutri-Kähönen N, Lyytikäinen LP, Lehtimäki T, Viikari JS, Juonala M, Raitakari OT. The prediction of adult dyslipidemia using genetic and childhood clinical risk factors: The Cardiovascular Risk in Young Finns Study. (submitted to *Circulation: Cardiovascular Genetics)*

1. INTRODUCTION

Atherosclerotic cardiovascular disease (CVD) is the leading cause of death and disability worldwide.(Murray et al. 2012, Lozano et al. 2012) In Finland, coronary heart disease (CHD) incidence and mortality were very high in the 1960s.(Puska et al. 1998) Since then, mortality has notably diminished and cardiovascular risk factor levels have improved.(Vartiainen et al. 2010, Vartiainen et al. 2000) The observed decline in prevalence of CHD has primarily been attributed to lower cholesterol and blood pressure (BP) levels and decreased smoking prevalence in the population. Thus, the Finnish experience has provided a worldwide model of successful cardiovascular prevention. (Valsta et al. 2010, Borodulin et al. 2015)

Atherosclerosis has its roots in childhood with its progression related to the presence and intensity of known CVD risk factors such as obesity, high BP and dyslipidemia. (McGill et al. 2000, Zieske et al. 2002) Risk factor levels in childhood have a tendency to track, or persist, into adulthood (Mahoney et al. 1991, Juhola et al. 2011, Porkka et al. 1994, Webber et al. 1991), resulting in a sustained increase to the risk of experiencing a cardiovascular event later in life. (Perk et al. 2012)

Dyslipidemia is a disorder of lipoprotein metabolism and is commonly defined as high total cholesterol (TC), high low-density lipoprotein cholesterol (LDL-C), or high triglyceride (TG) levels, or low high-density lipoprotein cholesterol (HDL-C) levels. Likewise, hypertension, a systemic condition accompanying high BP, is generally defined as elevated systolic blood pressure (SBP) or diastolic blood pressure (DBP) levels. Both conditions are major modifiable risk factors for atherosclerotic CVD. (Cullen 2000, Smith 2007, Grundy 1998, Chobanian et al. 2003) Childhood dyslipidemias and elevated BP levels have been associated with adult markers of early atherosclerosis, such as carotid intima-media thickness (IMT) (Magnussen et al. 2009, Juonala et al. 2008, Juhola et al. 2013, Raitakari et al. 2003) that have been shown to predict future cardiovascular events. (Lorenz et al. 2007)

Current pediatric guidelines (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute 2011) for primary prevention of CVD recommend a two-level approach to lipid screening: individual-level, and universal, or population wide, level. For example, the guidelines recommend obtaining a fasting lipid profile on children from the age of 2 years onward, who have history of CVD or dyslipidemia in their family or any other risk factors present (e.g. obesity, hypertension or diabetes). Universal screening

of lipids is suggested for all children aged 9-11 years and again when aged 17-21 years. The guidelines also recommend annual measurements of BP in children aged between 3 and 17 years, elevated BP should be interpreted based on national age-, sex-, and height-specific percentiles. (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute 2011)

Moreover, in recent genome-wide association studies (GWAS), several genetic loci have been identified that are associated with CVD and blood lipid levels. Prior studies have shown that an approach using a combination of weighted genetic risk scores (wGRS) based on a large amount of single-nucleotide polymorphism (SNP) and clinical risk factors adds incremental value to lifetime CHD risk prediction when compared to approach using only traditional risk factors. (Abraham et al. 2016, Khera et al. 2016) Recent study, which used a multistage design in 188,557 subjects, identified 157 loci significantly associated with blood lipid levels of TC, LDL-C, HDL-C and TG, including 62 novel loci.(Global Lipids Genetics Consortium et al. 2013) However, the utility of loci in improving prediction of adult dyslipidemia over and above clinical risk factors in childhood remains unknown.

The Cardiovascular Risk in Young Finns Study (Young Finns Study) is an on-going, multicenter follow-up of 3,596 participants that began in 1980 to study the risk factors of CVD and their determinants in children and adolescents. In this thesis, the main objectives were to: report cardiovascular risk factor levels in 2011 and 4-year-changes in risk factor levels between 2007 and 2011; determine whether repeated childhood lipid and BP measurements add incremental value to the prediction of adult dyslipidemia and hypertension compared to a single measurement; and examine whether the addition of newly identified genetic variants for blood lipid levels improve the prediction of adult dyslipidemia over and above childhood lipid levels.

2. REVIEW OF THE LITERATURE

Atherosclerotic CVDs remain the major cause of premature death in Europe, although CVD mortality has decreased significantly over recent decades in many European countries, including Finland.(GBD 2013 Mortality and Causes of Death Collaborators 2015, Laatikainen et al. 2005) In contrast, CVD mortality is increasing in many economically developing countries and it has been estimated that 80% of all CVD mortality currently occurs in developing countries.(GBD 2013 Mortality and Causes of Death Collaborators 2015) In addition, CVDs are also the leading somatic cause of loss of productivity (Murray et al. 2012) and are a huge economic burden.(Leal et al. 2006)

CVDs are preventable in multiple ways. (Perk et al. 2012) Results from The Multinational Monitoring of Trends and Determinants in Cardiovascular Disease Study demonstrated that the relationship between changes in classic risk factor levels explained almost half of the variation in coronary-event rates in men but proportionally less in women. (Kuulasmaa et al. 2000) In Finland, coronary disease mortality decreased over 80% in working aged men and women from 1969 to 2012. Over two thirds of the observed reduction in mortality over this time period has been attributed to changes in BP, serum cholesterol levels and prevalence of smoking. (Jousilahti et al. 2016) In 2015, CHD still caused every fifth death and over 10,000 persons died of it in Finland. (Statistics Finland, 2016) Among patients with CHD, at least one of the four conventional risk factors (hypertension, dyslipidemia, smoking, type 2 diabetes mellitus (T2D)) is present in four out of every five patients. (Khot et al. 2003) The contribution of these major coronary risk factors to CHD is consistent across different population samples varying in CHD frequency. (Menotti et al. 1996)

2.1. Cardiovascular risk factors

CVD is usually a product of multiple interacting risk factors, including environmental, physical and genetic risk factors. In the mid-20th century, several large epidemiological studies, such as the Framingham Heart Study, Seven Countries Study, and British Medical Doctors Study, reported that tobacco smoking, high serum cholesterol and high BP were associated with increased risk for CHD. (Doll, Hill 1954, Kannel et al. 1961, Keys et al. 1966). As early as 1981, over 200 risk factors were suggested to be associated with CVD. (Hopkins, Williams 1981) Later, other risk factors such as glucose homeostasis, nutrition, and excess adiposity

were associated with CVD. (Perk et al. 2012) In addition, the potential causal role of these risk factors with CVD has been shown in observational studies, clinical trials. (Lewington et al. 2002, Doll et al. 2004, Prospective Studies Collaboration et al. 2007) More recently, causal role of CVD risk factors has also been suggested by results from Mendelian randomization studies, where genetic variants with known effects on risk factor levels such as blood lipid levels that mimic those produced by modifiable exposures are used to estimate the causal effect on disease in presence of confounding factors. (Ference et al. 2012)

2.1.1. Blood pressure

High BP is one of the major risk factors for CVD. (Perk et al. 2012) Elevated BP raises the mechanical stress of blood vessels and increases the workload of the heart. Increased hemodynamic burden decreases the elasticity of blood vessels, promotes formation of atheromas, and induces structural changes in the heart.(Chobanian, Alexander 1996) It has been estimated that each 20 mmHg increment in systolic blood pressure (SBP) levels, and 10 mmHg increment in diastolic blood pressure (DBP) levels above 115/75 mmHg is associated with approximately two-fold difference in death from stroke, CHD, and other vascular causes.(Lewington et al. 2002) Lowering elevated BP levels with medication has been demonstrated to be beneficial in randomized trials, with antihypertensive therapy associated with a 30-39% reduction in stroke incidence and a 21-28% reduction in major cardiovascular events. (Neal et al. 2000) In addition, larger reductions in BP levels have been shown to further reduce risk.(Turnbull, Blood Pressure Lowering Treatment Trialists' Collaboration 2003) Lowering BP provides a similar reduction in risk at all baseline cardiovascular risk levels, though absolute risk reductions become greater as baseline risk increases.(Blood Pressure Lowering Treatment Trialists' Collaboration et al. 2014) However, the most appropriate targets for SBP to reduce cardiovascular morbidity and mortality remain uncertain. Recent results from 9,361 individuals in the SPRINT study demonstrated that targeting a SBP below 120 mmHg, as compared with current treatment recommendations with target levels below 140 mmHg, in patients at high risk for cardiovascular events resulted in lower rates of fatal and nonfatal major cardiovascular events and death from any cause (**Figure 1**). (SPRINT Research Group et al. 2015)

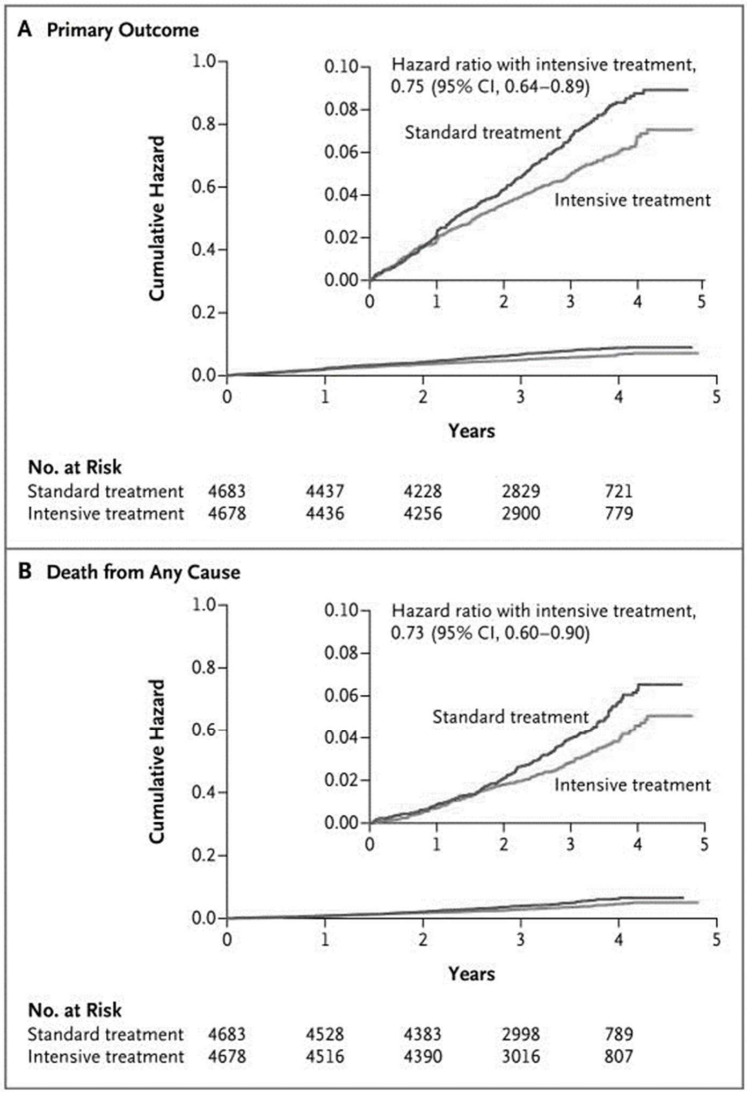


Figure 1. The cumulative hazards for the primary outcome (a composite of myocardial infarction, acute coronary syndrome, stroke, heart failure, or death from cardiovascular causes (Panel A), and for death from any cause (Panel B)). The inset in each panel shows the same data on an enlarged y-axis. Reproduced with permission from (SPRINT Research Group et al. 2015), Copyright Massachusetts Medical Society. Systolic blood pressure target was below 120 mmHg in the intensive treatment group and below 140 mmHg in the standard treatment group.

It is known that BP measurements are affected by many environmental and patient-related factors such as posture of patient, talking during BP measurement, prior smoking, disturbance, and emotions. (Campbell et al. 1994, Handler 2009) For example, an earlier study observed that if the medical staff was talking during BP measurement and patient was actively listening, these actions resulted in a 10 mmHg increase in SBP. (Le Pailleur et al. 1998) Furthermore, cuff-size, auscultation method and calibration can notably affect the measured BP levels. (Kantola et al. 2005, O'Brien et al. 2003, de Greeff et al. 2010) Generally, mercury sphygmomanometer has been considered as a golden standard for BP measurement. In a prior study, BP readings obtained using the random-zero device were observed to be significantly lower than those obtained with the standard mercury sphygmomanometer. (Yang et al. 2008) These relatively small inaccuracies in BP measurements can lead to serious consequences at population level. It has been approximated earlier that in United States an underestimation of 5 mmHg would incorrectly diagnose more than 20 million Americans with prehypertension when actually true hypertension is present. An estimation has been made extending results from a large meta-analysis to predict that the consequences of an untreated 5 mmHg of excessive SBP would lead to a 25% increase over current levels of fatal strokes and fatal myocardial infarctions for these individuals. (Lewington et al. 2002, Handler 2009)

2.1.2. Serum lipids and lipoproteins

In blood plasma, lipids such as cholesterol and TG are transported in lipoprotein particles. In humans, the lipoprotein transport system serves multiple functions such as the transportation of dietary fats from the intestine to the liver and processed cholesterol particles to peripheral tissues for membrane synthesis and steroid hormone production, and the processing of free fatty acids which all have an essential role in human physiology. (Genest 2003) Each lipoprotein particle includes a set of highly conserved apolipoproteins that provide structural integrity for the complex and a mechanism for receptor binding. Traditionally, lipoproteins are classified according to their size and density, with chylomicrons (diameter 80-1000nm), chylomicron remnants (diameter 50-100nm), and very low density lipoproteins (diameter 30-70nm) being light and large. On the other hand, low-density lipoproteins (diameter 20 nm) and high-density lipoproteins (diameter 10nm) are constantly smaller and heavier. The role of serum lipids and lipoproteins in CVD development throughout life has been established by

numerous genetic, pathological, observational, and intervention studies. (Global Lipids Genetics Consortium et al. 2013, Steinberg, Gotto 1999, Castelli 1984, Newman et al. 1986)

2.1.2.1. Low-density lipoprotein cholesterol

Low-density lipoprotein particles are the main carriers of cholesterol to peripheral tissues where they are internalised through the low-density lipoprotein receptor, which is known to be an essential mediator of plasma LDL-C concentrations. (Goldstein, Brown 2009) LDL-C is a fundamental determinant of vascular risk. The primary initiating event in atherosclerosis is the accumulation of low-density lipoprotein in the subendothelial intima leading to modification of low density lipoprotein particles resulting in an increase in macrophage-foam cells, activation of various inflammatory signals, and to injury of the endothelium and the underlying smooth muscle cells. (Lusis 2000) Furthermore, the accumulation of low-density lipoprotein is greater when circulating levels of LDL-C are elevated. (Pentikäinen et al. 2000, Lusis 2000)

Findings from epidemiological studies have consistently shown that elevated LDL-C concentrations are associated with an increased risk of myocardial infarction and vascular death. (Prospective Studies Collaboration et al. 2007) Also, recent results from Mendelian randomization studies suggest that LDL-C is likely to be a causal agent for plaque initiation and progression. (Ference et al. 2012) Based on the findings from genetic studies, it was estimated that a 1 mmol/L lifetime lower LDL-C level would be associated with a 54% reduction in CHD. In 2010 and 2012, meta-analyses from 27 randomized trials that are part of the Cholesterol Treatment Trialists' Collaborators, reported that statins reduced the risk of major coronary events by 24%, stroke by 15%, and coronary revascularization by 24% for each 1 mmol/L reduction in LDL-C levels.(Cholesterol Treatment Trialists' (CTT) Collaborators et al. 2012, Cholesterol Treatment Trialists' (CTT) Collaboration et al. 2010) The observed benefits were similar for women and men, smokers and non-smokers, in elderly and young people, and across all levels of obesity, BP, and glucose.

2.1.2.2. High-density lipoprotein cholesterol

Definition of high-density lipoprotein includes heterogeneous particles containing approximately equal amounts of lipids and protein and these particles are characterized by high density and small size. (Gordon, Rifkind 1989) High-density lipoprotein particles mediate the reverse transport of cholesterol from cells of the arterial wall to the liver for excretion into bile

as neutral sterol or bile acid. (von Eckardstein et al. 2001) A strong inverse association between HDL-C and CHD was first reported by the Framingham Heart Study (Kannel et al. 1964), and formed the basis of a widely accepted hypothesis that HDL-C protective against CHD. This hypothesis suggests that interventions to elevate HDL-C levels would reduce risk for CHD and was later supported by a series of animal studies. (Badimon et al. 1990, Rubin et al. 1991) However, more recent genetic studies and randomized controlled trials have challenged the HDL-C hypothesis. In Mendelian randomization studies, subjects with mutations in apoA-1, ABCA1 and LCAT causing low HDL-C concentrations had not unequivocally increased risk for premature CHD. (Hovingh et al. 2005, Oldoni et al. 2014) On the other hand, carries of loss-of-function variants in endothelial lipase, who had 0.14 mmol/L higher HDL-C and otherwise similar levels of cardiovascular risk factors as non-carriers, did not seem to have any lower risk for CVD compared to non-carriers. (Voight et al. 2012) These studies challenge the concept that raising of HDL-C would automatically result in a reduction in cardiovascular risk. In addition, data from two placebo controlled trials using extended-release niacin on the background of statin therapy suggest that despite a significant increase in HDL-C concentrations, niacin offered no clinical benefit or reduction of cardiovascular events in neither of the trials.(AIM-HIGH Investigators et al. 2011, HPS2-THRIVE Collaborative Group et al. 2014) Moreover, use of torcetrapib, a cholesteryl ester transfer protein inhibitor, combined to atorvastatin induced an increase of 72 % in the HDL-C levels when compared to atorvastatin-only group in an earlier study. (Barter et al. 2007) Although a significant increase was reported in the HDL-C levels, use of torcetrapib failed to reduce the risk of CHD. (Barter et al. 2007)

Nevertheless, the utility of HDL-C as a predictor of cardiovascular risk remains clear. HDL-C concentration reflects various inflammatory and metabolic conditions, which might explain its inverse association with CVD.(Toth et al. 2013) Numerous prospective studies have demonstrated that low HDL-C is a strong, independent predictor of myocardial infarction and ischemic stroke regardless of sex, race, or ethnicity.(Goldbourt et al. 1997, Emerging Risk Factors Collaboration et al. 2009) Furthermore, low HDL-C is also a predictor of incident cardiovascular events in individuals who have already been diagnosed with CVD. (Goff et al. 2014, Toth et al. 2013) Current consideration is that functionality of high-density lipoprotein, especially cholesterol efflux capacity may prove to be more relevant and attractive therapeutic

target to evaluate than HDL-C levels in reducing CHD risk via high-density lipoprotein. (Siddiqi et al. 2015)

2.1.2.3. Triglycerides

Most of the TG in blood are absorbed from the small intestine, although liver also produces and secretes a small amount of triglycerides. Triglyceride-rich lipoproteins play a crucial role in delivering free fatty acids to tissues such as heart and skeletal muscle as source of energy and for storage into adipose tissue. Several metabolic pathways affect the TG levels including intestinal uptake from dietary fat and hepatic production and removal of TG. (Hassing et al. 2012)

Elevated TG levels are a marker for elevated remnants rich in cholesterol that enter into the intima, inducing foam cell and plaque formation and low-grade inflammation.(Goldberg et al. 2011) The cholesterol content of triglyceride-rich lipoprotein remnants is perhaps the more plausible explanation of its role in atherosclerosis and CVD rather than raised TG per se. This is because TG, but not cholesterol, can be degraded by human cells. At mild–to–moderately raised triglyceride concentrations (2–10 mmol/L), apolipoprotein B particles are small enough to enter the arterial wall and have the potential to accumulate and increase the risk of atherosclerosis. Mild-to moderately elevated TG concentrations have been associated with increased risk for CVD and concentrations over 10mmol/L are regarded also as a risk factor for pancreatitis.(Hokanson, Austin 1996) In addition, raised TG concentrations are also strongly associated with low HDL-C concentrations.(Nordestgaard, Varbo 2014) Raised TG concentrations in individuals typically result from multirisk factor burden including high alcohol intake, obesity, increased genetic risk, and unmanaged diabetes.

Three studies based on the Women's Health Study and the Copenhagen City Heart Study Women's Health Study reported that risks of myocardial infarction, CHD, ischemic stroke, and all-cause mortality increased as TG levels increased.(Bansal et al. 2007, Freiberg et al. 2008, Nordestgaard et al. 2007) Based on these reports, when women with TG concentrations greater than 5 mmol/L were compared to those with concentrations under 1 mmol/L, the age-adjusted risk was 17 times higher for myocardial infarction, 6 times higher for ischemic heart disease, 5 times higher for ischemic stroke, and 4 times higher for all-cause mortality after up to 30 years of follow-up. For men, the corresponding risk increases were 5 times higher for

myocardial infarction, 3 times higher for ischemic heart disease, 3 times higher for ischemic stroke, and 2 times higher for all-cause mortality. In 2009, meta-analysis from the Emerging Risk Factors Collaboration that included 302,430 individuals from 68 long term, prospective studies, and 12,785 coronary events, suggested that raised fasting and non-fasting TG were associated with an increased risk of CHD at TG concentrations around 2.8 mmol/L and an increased risk of ischemic stroke at TG concentrations around 2.2 mmol/L (**Figure 2**). (Emerging Risk Factors Collaboration et al. 2009) The association with CHD was weakened after adjustment for HDL-C and disappeared after additional adjustment for non-high-density lipoprotein-cholesterol (non-HDL cholesterol). This observation is consistent with the idea that the cause of CVD is the cholesterol content in apolipoprotein B containing remnant particles, and therefore not raised TG per se.(Varbo et al. 2013, Varbo et al. 2014, Chapman et al. 2011)

In TG-lowering fibrate trials that included post-hoc subgroup analyses for participants with baseline TG of at least 2 mmol/L, a 1 mmol/L reduction in TG reduced coronary events by 54% overall and by 43% in those with high TG. The risk reduction in those with high TG was statistically significant in the individual studies, which included the use of fibrate as an add-on to statin treatment. (Manninen et al. 1988, Scott et al. 2009, Rubins et al. 1999, Bezafibrate Infarction Prevention (BIP) study 2000, Carlson, Rosenhamer 1988)

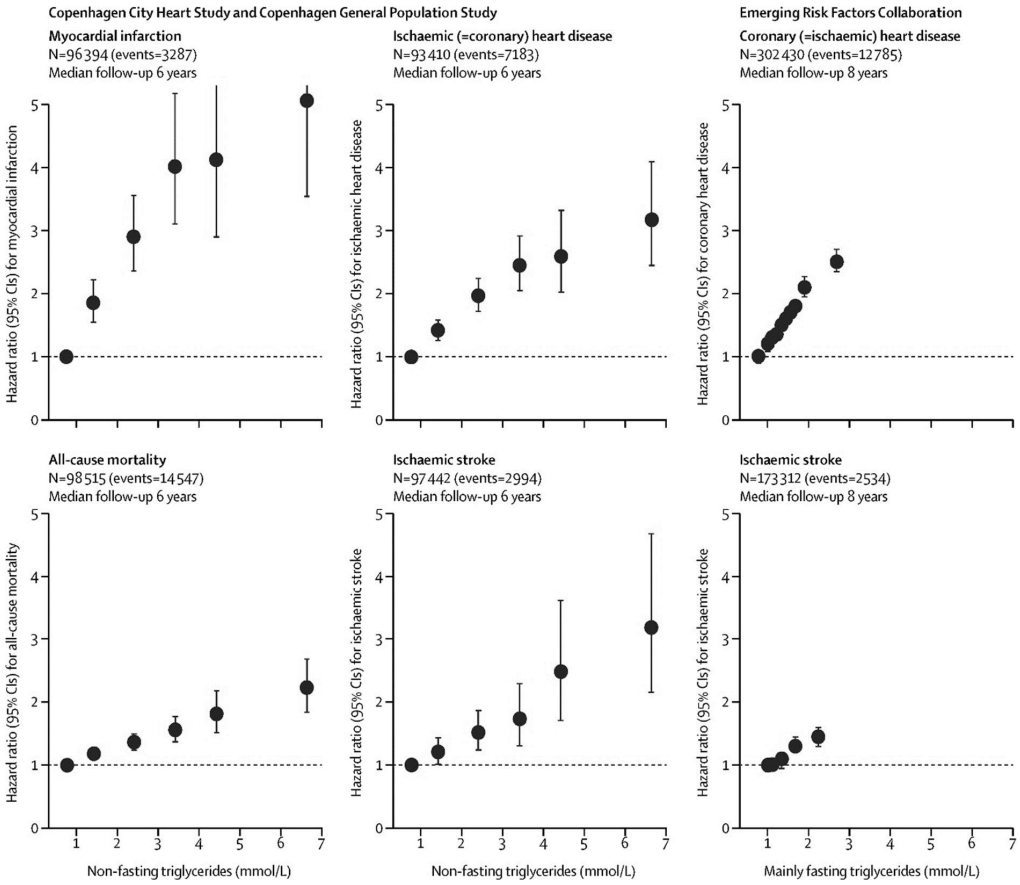


Figure 2. Observational associations between raised concentrations of triglycerides, and cardiovascular disease and all-cause mortality, in the Copenhagen City Heart Study and Copenhagen General Population Study combined (left and middle sections) and in the Emerging Risk Factors Collaboration (right section). Hazard ratios were estimated by Cox proportional hazard regression models and were adjusted for age, sex, and trial group. Right section adapted from Di Angelantonio and colleagues. Reprinted from Nordestgaard BG, Varbo A, Triglycerides and cardiovascular disease. The Lancet, 384, 626–635, (2014), reproduced with permission from Elsevier.

2.1.2.4. Non-high-density lipoprotein cholesterol

Non-HDL-C concentration can be estimated by subtracting HDL-concentration from TC concentration and is often regarded as a simple measurement of cholesterol content of all atherogenic, apolipoprotein B-containing lipoproteins with no need for additional testing. Non-HDL-C provides several advantages in cardiovascular risk prediction when compared to more conventional LDL-C. Firstly, non-HDL-C has been shown to be a superior predictor of cardiovascular events when compared to LDL-C. (Emerging Risk Factors Collaboration et al. 2009) Furthermore, performance of non-HDL-C and apolipoprotein B has been observed to be quite similar in cardiovascular risk prediction, though apolipoprotein B has been demonstrated to have advantages especially in patients with abnormal cholesterol composition. (Raitakari et al. 2013, Emerging Risk Factors Collaboration et al. 2009, Sniderman et al. 2011) Thus, it has been suggested that lipid assessment in CVD could be simplified by measuring of non-HDL-C, which does not require fasting in contrast to calculated LDL-C levels which rely on fasting TG levels.

2.1.3. Genetic risk markers

The contribution of genetic factors to risk for CHD is about equal to the contribution of environmental risk factors. In Danish and Swedish twin studies the heritability for CHD death varied from 52% to 57% in men and from 38% to 58% in women. (Zdravkovic et al. 2002, Wienke et al. 2001) Recent studies have reported that wGRSs are independently associated with CHD risk, though adherence to healthy lifestyle was associated with substantially reduced risk for CHD despite genetic risk. (Abraham et al. 2016, Khera et al. 2016) Furthermore, the heritability for blood lipids varied from 61% to 71%.(Kettunen et al. 2012) In the last decade, the genetic loci for lipid levels have been comprehensively studied in large GWASs. A recent consortia study of nearly 190,000 individuals described 157 genetic loci that associate with one or more circulating lipid levels (**Figure 3**). (Global Lipids Genetics Consortium et al. 2013) In addition, many of the lipid-associated loci further associate with metabolic traits such as obesity, insulin resistance, and hypertension. Loci associated with LDL-C and TG are in turn also associated with CHD events, supporting their role as a causal component of atherosclerosis. In contrast, loci associated only with HDL-C do not clearly show this relationship, which challenges the HDL-C hypothesis as a potential causal factor, and instead

suggests functionality of high-density lipoprotein as more relevant metrics to investigate the role of high-density lipoprotein in CHD risk assessment. (Siddiqi et al. 2015)

Apolipoprotein E (ApoE) binds to receptors on the liver and mediates clearance of chylomicrons and very low-density lipoproteins and thus has a role in the metabolism of cholesterol and triglycerides. (Eichner et al. 2002) ApoE genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4) have been shown to be linearly related to LDL-C and CHD risk. (Lehtimäki et al. 1995, Bennet et al. 2007) Results from an extend meta-analysis demonstrated that LDL-C levels were approximately 30% lower in people with ε2/ε2 than in people with ε4/ε4 genotypes. (Bennet et al. 2007) Nevertheless, a prior report from the Young Finns Study showed that ApoE genotypes are not independently associated with early atherosclerotic changes such as carotid IMT, brachial flow-mediated dilatation or carotid artery compliance suggesting that the effect on the CHD risk is most likely mediated through long-term effects on plasma lipids. (Grönroos et al. 2008)

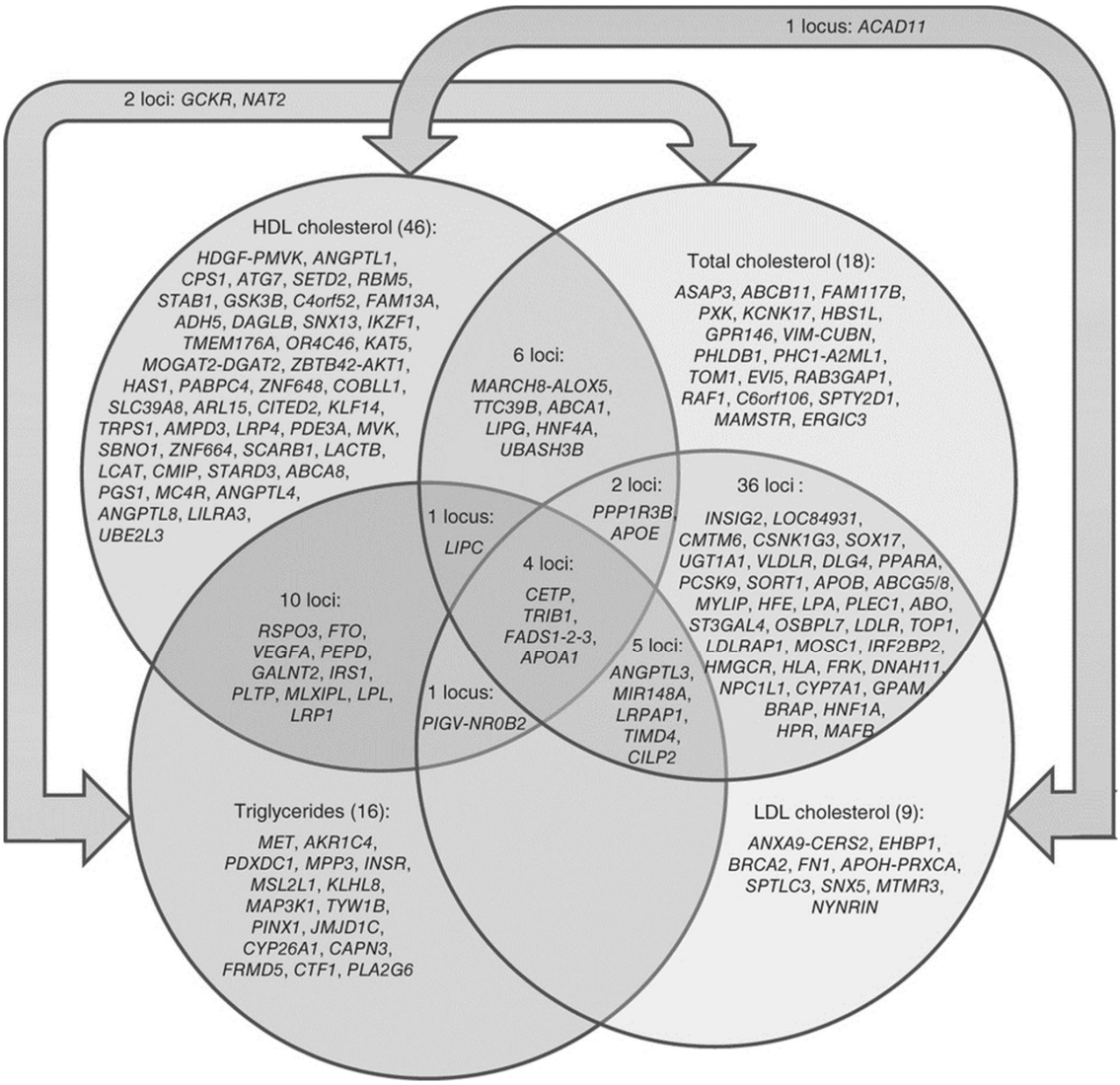


Figure 3. Venn diagram illustrating the overlap of genetic loci associated with LDL-C, HDL-C, TG, and TC. As shown, several loci associate with multiple lipid traits. Reprinted from Nature Genetics (Global Lipids Genetics Consortium et al. 2013) with permission.

2.1.4. Obesity

The relationship between obesity and cardiovascular events has been addressed in multiple studies. Although obesity and overweight are associated with increased CVD mortality and morbidity, the underlying mechanisms through which obesity acts are likely multiple.(Poirier et al. 2006, Twig et al. 2016, Mangner et al. 2014) For example, excess weight is a powerful risk factor for hypertension, dyslipidemia, and insulin resistance already in childhood. (Ylitalo 1981, Dahlstrom et al. 1985, Skinner et al. 2015) Furthermore, childhood obesity is known to associate with increased risk of adult obesity, T2D, and high carotid IMT in adulthood.(Singh et al. 2008, Juonala et al. 2010, Schubert et al. 2009, Juonala et al. 2011) In addition, increased waist circumference is associated with higher incidence of CVD (Flint et al. 2010) and T2D.(Siren et al. 2012) Results even suggest waist circumference may predict CHD risk better than body mass index (BMI) among men and women 60 years of age and older.(Flint et al. 2010) Although childhood obesity strongly associates with many traits in adulthood, encouraging results from cohort studies showed that the risk associated with overweight in childhood and adolescence is reversible.(Gray et al. 2011, Juonala et al. 2011) For example, participants who were overweight or obese as children but were not obese as adults had similar cardiovascular risk profile as those who were never obese.(Juonala et al. 2011) Because obese children are at increased risk of staying obese into adulthood and those who amend their trajectory have a reduced CVD risk factor profile, it has been suggested that intervention should occur whenever an overweight child is seen in a medical settings.(Juhola et al. 2011)

2.1.5. Metabolic syndrome

The metabolic syndrome (MetS) is defined as a cluster of high BP, dyslipidemia including low HDL-C concentrations and high TG concentrations, raised fasting glucose, and central obesity. MetS predicts T2D and CVD in adults and is has also been linked to cardiovascular and all-cause mortality. (Lakka et al. 2002, Wilson et al. 2005) Although there is a general agreement that the term MetS is acceptable for the condition of the presence of multiple metabolic risk factors for CVD and diabetes, no uniform consensus over the diagnostic criteria used has been reached and several clinical definitions have been proposed earlier. (Grundy et al. 2005, Alberti et al. 2005) A major disagreement has been over the defining thresholds for abdominal obesity which is highly correlated with insulin resistance. Definition for the MetS that recognizes that

the risk associated with waist circumference will differ between different populations was released in 2009. (Alberti et al. 2009)

2.1.6. Type 2 diabetes mellitus and prediabetes

T2D is the most prevalent form of diabetes that typically appears later in life. This condition is commonly preceded by MetS and the risk factors that constitute this syndrome contribute independently to CVD risk.(Haffner et al. 1990) Comprehensive epidemiological data have shown that T2D is an independent risk factor for CVD, leading to a two- to three-fold higher risk for CVD compared with the general population.(Fox et al. 2004a, Qazi, Malik 2013, Grundy et al. 1999) In addition, patients with T2D who develop clinical CVD have worse prognosis for survival than do CVD patients without T2D.(Stone et al. 1989, Singer et al. 1989) It has been shown that cardiovascular complications of T2D can be reduced with treatment. For example, an intense intervention including behavior modification and pharmacologic therapy targeting hyperglycemia, hypertension, dyslipidemia, and microalbuminuria, along with secondary prevention of CVD with aspirin, reduced the risk of microvascular and cardiovascular events to approximately half.(Gaede et al. 2003) Lifestyle interventions such as increasing physical activity also play a crucial role in prevention of T2D.(Gillies et al. 2007) For example, lifestyle changes over a three year period in a group of high-risk patients reduced the risk of T2D 58% when compared to a control group with the reduction in the incidence directly associated with the observed lifestyle changes.(Tuomilehto et al. 2001)

According to guidelines by American Diabetes Association, T2D diagnosis is based on either fasting plasma glucose ≥7.0 mmol/l, glycated hemoglobin (HbA1c) ≥6.5% (≥48 mmol/mol), or/and 2-hour value of plasma glucose in oral glucose tolerance test ≥11.1 mmol/l. (American Diabetes Association 2013) As with most diagnostic tests, the test should be repeated to rule out laboratory error, unless the diagnosis is clinically clear, such as a patient with classic symptoms of diabetes. Although, in a situation where results of two different tests are above the diagnostic threshold, the diagnosis of diabetes can be confirmed without need for further testing. Nevertheless, measurement of fasting glucose concentration is not suffient enough to detect abnormal glucose regulation in all patients. In an earlier study more than half of the patients had elevated 2-hour plasma glucose values even though they had normal fasting glucose values. (Saaristo et al. 2008)

Individuals with HbA1c of 5.7–6.4% (39 - 47 mmol/mol), defined as prediabetes, are at increased risk for T2D and CVD.(American Diabetes Association 2013) A prospective study based on 3,854 participants found that a HbA1c cut-point of 5.7% (39 mmol/mol) has a sensitivity of 66% and specificity of 88% to predict incident T2D.(Droumaguet et al. 2006) Therefore, those with prediabetes might benefit from intensive and early intervention, as the duration and preceding phases of T2D affect cardiovascular risk.(Haffner et al. 1990, Fox et al. 2004b)

2.1.7. Smoking

Cigarette smoking is one the major modifiable risk factors contributing to premature morbidity and mortality worldwide.(Centers for Disease Control and Prevention (CDC) 2008) Cigarette smoke is an aerosol that contains >4,000 chemicals including nicotine, carbon monoxide, acrolein, and oxidative compounds.(Csordas, Bernhard 2013) Therefore, exposure to cigarette smoke induces multiple pathological effects on the architecture and function of endothelium.(Ambrose, Barua 2004) Epidemiologic studies have consistently shown that cigarette smoking increases the incidence of myocardial infarction and CHD in comparison to non-smokers.(Jonas et al. 1992, Price et al. 1999, Willett et al. 1987) Active smokers have a 80% increased risk for fatal CHD and those exposed to passive smoking have approximately a 30% increased risk.(Law et al. 1997, Glantz, Parmley 1991) In addition, a meta-analysis concluded that the cardiovascular risk between genders is unequal, and that in women the risk for CHD conferred by smoking was 25% greater when compared with men.(Huxley, Woodward 2011) Numerous studies have demonstrated that cessation of smoking rapidly diminishes the risk for CVD when compared to non-quitters.(Ockene et al. 1990, Wolf et al. 1988) Patients with CHD who quit smoking after initial infarction have a 50% reduction in risk of sudden cardiac death and total mortality.(Salonen 1980, Sparrow, Dawber 1978)

2.2. Development of cardiovascular risk factor levels and mortality from 1970s to 2000s

2.2.1. In Finland

In the late 1960s, Finland had the world's highest CHD mortality rate.(Thom, Epstein 1994) The North Karelia Project, the first community-based CVD prevention project worldwide, was started in 1972 to combat CHD incidence and mortality. Cardiovascular risk factor levels began to improve in the 1970s in Finland.(Vartiainen et al. 2000) This was followed by remarkable

reduction in CHD risk and mortality (**Figure 4 & 5**). From the 1960s to the 1990s the mortality from CHD in Finland amongst working-age men decreased over 70%, and in the whole population by around 60%.(Puska et al. 1998) In a recent study, the reduction in CHD mortality from 1969-1972 to 2012 was estimated to be as high as 82% (from 643 to 118 deaths per 100,000) in working-age men and 84% (from 114 to 17 deaths per 100,000) in working-age women.(Jousilahti et al. 2016) At the same time, smoking prevalence fell from 53% to 29% in men but increased in women from 11% to 19%. In both sexes, TC declined during the first 35 years of the study, but increased slightly between 2007 and 2012. The average decline in TC was 2.4 mmol/L for males and females between 1972 and 2012. Also, SBP decreased considerably in both sexes between 1972 and 2012. The average decline of SBP was 11.2 mmHg in men and 20.1 mmHg in women.(Jousilahti et al. 2016) Meanwhile, incident T2D has increased in the Finnish population by 4.3% on average per year between 1992 and 2001.(Lammi et al. 2008) In a Finnish population subsample aged 25 to 44 years in 2007(Pajunen et al. 2012), 2.4% of the study population had T2D. Furthermore, mean waist circumference and waist-to-height ratio increased in both men and women over the 15-year period from 1992 to 2007.(Lahti-Koski et al. 2012) The upward trends in indices of adiposity were observed in all age groups, especially during the past 10 years. The decrease in mortality has been estimated to be mainly associated with reductions in smoking prevalence, BP levels and blood lipid levels due to favorable changes in dietary habits and lifestyle (effect of over 70%), though improvements in treatment have also had an impact (effect of approximately 25%).(Valsta et al. 2010, Jousilahti et al. 2016) The probability for a 30-year-old man to die of CVD before his 70th birthday had dropped from about 30% in 1970 to 7% in 2013, and for a 30-year-old woman, the corresponding probability had dropped from 13% to about 2%.(Salomaa et al. 2016) In addition, it has been estimated that the decline in cardiovascular mortality has extended life expectancy by about 5 years in males and about 4.5 years in females.

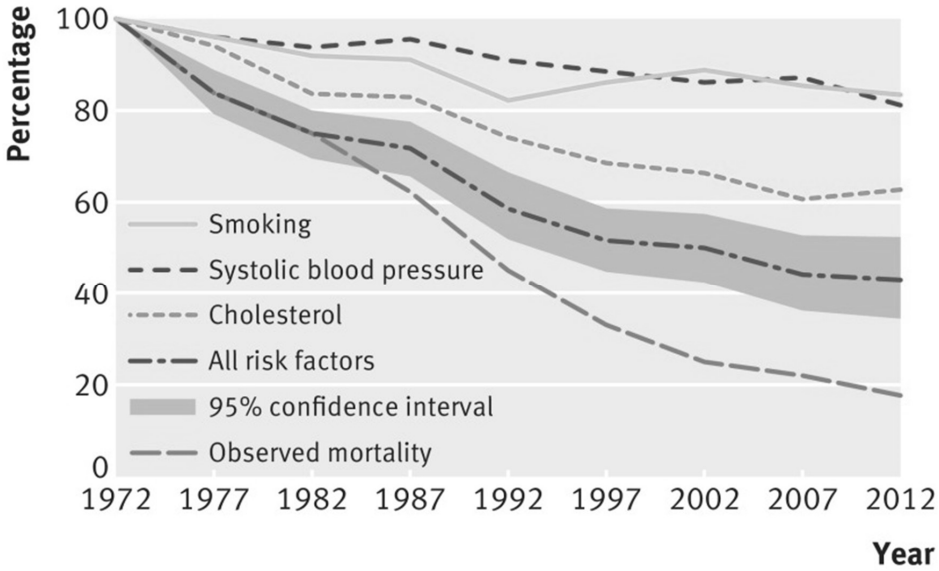


Figure 4. Predicted and observed reduction (%) in coronary heart disease mortality in men aged 35-64 years, 1972-2012. Reprinted from British Medical Journal (Jousilahti et al. 2016) with permission from Elsevier

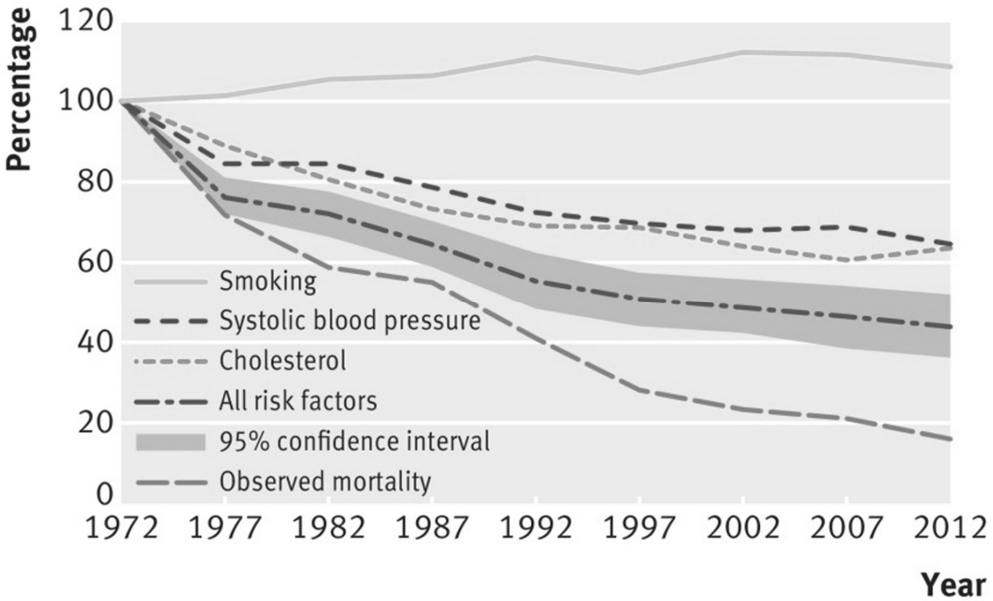


Figure 5. Predicted and observed reduction (%) in coronary heart disease mortality in women aged 35-64 years, 1972-2012. Reprinted from British Medical Journal (Jousilahti et al. 2016) with permission from Elsevier.

2.2.2. Worldwide

As was observed in Finland, a large decline in CHD mortality has been observed since the 1960s in economically developed countries. In the European Union, rates of CHD decreased by a third in men and over a quarter in women from 1985-1989 to 2000-2004.(Nichols et al. 2013)

In Sweden, age specific CHD mortality rates decreased by 53% in men and 52% in women aged 25–84 years between 1986 and 2002. (Bjorck et al. 2009) The Swedish IMPACT analysis model suggested that more than half of the decrease in CHD mortality rate was attributed to changes in smoking prevalence (from 28.9% to 18.6%), TC (-0.64 mmol/L), and SBP levels (-2.6 mmHg). Development of medical and surgical treatments, including secondary prevention, explained only a third of this reduction. (Bjorck et al. 2009) At the same time, adverse trends were seen for diabetes (prevalence had increased from 2.7% to 3.8%) and overweight (average BMI had increased from 24.3 kg/m^2 to 25.4 kg/m^2).

Similar trends were also reported in the United States. Mortality associated with CHD has fallen over 40% in the last decades. (Ford et al. 2007) From 1980 to 2000, the age-adjusted rate of CHD fell from 542.9 to 266.8 cases per 100,000 population among men aged 25 to 84 years and from 263.3 to 134.4 among women aged 25 to 84 years. Estimation was made that 149,635 fewer deaths from CHD were attributable to changes in risk factors, TC levels decreased 0.34 mmol/L, SBP decreased 5.1 mmHg and smoking prevalence decreased by 11.7%. In contrast, increase in BMI of 2.6 kg/m^2 and a 2.9% increase in the prevalence of T2D resulted in approximately 25,905 and 33,465 additional deaths.

Meanwhile, the age-standardized death rates for CVD are highest in the world in the countries that belonged to the former Soviet Union, though death rates have declined in the last decade. The age standardized CVD death rates per 100,000 were 644 in 2005 and 476 in 2013 in the whole former Soviet region. (Roth et al. 2015) In South Asia, estimates of age-standardized rates of CVD deaths have increased from 376 per 100 000 to 398 per 100 000 between 1990 and 2013 and death rates are likely to continue increasing as unfavorable dietary habits and physical activity behaviors are adopted. (Goyal, Yusuf 2006, Roth et al. 2015) Moreover, CVD death rates have been steadily increasing in Sub-Saharan Africa between 1990 and 2013, though CVD mortality remains still relatively low. (Mensah et al. 2015)

2.3. Prediction of adult dyslipidemia and hypertension based on childhood risk factors

The importance of tracking of cardiovascular risk factor levels from childhood to adulthood has been known for over half a decade. As early as in 1965, Johnson et al. outlined that the greatest need is the ability to judge which young person will show progressive elevations of BP or cholesterol with advancing years and which will remain stable. (Johnson et al. 1965)

Serum lipid levels have a tendency to track from childhood to adulthood, resulting in a sustained increase to the risk of experiencing a cardiovascular event later in life.(Juhola et al. 2011, Porkka et al. 1994, Mahoney et al. 1991, Webber et al. 1991, Magnussen et al. 2011) Obesity acquired in adolescence and the young adult years, oral contraceptive use, reduced cardiorespiratory fitness, no upward social mobility, and cigarette smoking have been shown to affect tracking by adversely effecting adult lipid levels.(Lauer et al. 1988, Morrison et al. 1979, Magnussen et al. 2011) Childhood dyslipidemias have been associated with adult dyslipidemias (Magnussen et al. 2008) and surrogate markers of atherosclerosis such as carotid IMT and coronary artery calcification (Hartiala et al. 2012, Juonala et al. 2008, Magnussen et al. 2009), which have been shown to predict future cardiovascular events. (Lorenz et al. 2007, Detrano et al. 2008) Earlier studies from the Young Finns Study cohort showed moderate 27-year tracking for lipids and lipoproteins (for TC r=0.52 in men and r=0.53 in women, for LDL-C r=0.56 in men and r=0.52 in women, for HDL-C r=0.51 in men and r=0.46 in women, and for TG r=0.27 in men and r=0.30 in women) , with the correlation coefficients not considerably affected by baseline age or gender (Juhola et al. 2011) and have suggested that use of two separate childhood measurements increased the amount of adult lipid variability explained in the 12-year follow-up up to 50 percent.(Porkka et al. 1994) In the 15-year follow-up of Bogalusa Heart study, the prediction of adult dyslipidemia was significantly improved by multiple measures of LDL-C.(Bao et al. 1996) Although the prediction of adult dyslipidemia would be improved by multiple child or adolescent measures, results from Lauer et al. (Lauer, Clarke 1990) showed that 25% to 45% of children with TC levels greater than the 90th percentile on two successive occasions did not meet the criteria for intervention suggested by NCEP ATPIII (Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. The Expert Panel. 1988) when they reached adulthood. Results from the Muscatine Study also suggest that the accuracy of prediction of clinically significantly elevated adult cholesterol using three

measurements did not significantly differ from using two measurements. (Mahoney et al. 1991) However, there is paucity of specific evidence from longitudinal studies with a longer period of follow-up that would demonstrate if obtaining multiple measurements in childhood and young adulthood improves prediction of adult dyslipidemias. In addition, whether the prediction of adult dyslipidemia could be improved by inclusion of novel genetic variants remains unknown.

As with blood lipids, BP has also shown the tendency to track from childhood to adulthood, (Chen, Wang 2008) with approximately 10% of elevated BP in adulthood attributable to elevated BP in childhood. (Kelly et al. 2015) High BP in young adulthood is an independent risk factor for high carotid artery IMT and coronary artery calcification (Raitakari et al. 2003, Juhola et al. 2013, Hartiala et al. 2012, Davis et al. 2001, Vos et al. 2003), vascular markers of atherosclerosis that predict future cardiovascular events. (Lorenz et al. 2007, Detrano et al. 2008) Longitudinal studies have shown that prediction of adult hypertension improves with information about multiple childhood risk factors including parental education and hypertension, childhood BMI, and wGRS.(Juhola et al. 2012) Results from a large meta-regression analysis suggest that taking multiple BP measures per visit only marginally increases BP tracking (Chen, Wang 2008) and it has been argued that the number of visits, days or weeks apart, is at least as important as the number of readings per visit.(Gillman, Cook 1995) Results from the Bogalusa Heart Study have demonstrated the value of repeated BP measurements in prediction of later BP status. In 1,501 children, a significant correlation (of 0.41 for SBP and 0.35 for DBP) was observed between SBP and DBP levels at baseline and after an 8-year follow-up. In addition, three serial measurements in the upper BP quartile significantly increased the probability of being in the uppermost quartile in the follow-up from 41.4 % up to 68.4% for SBP and from 34.4% up to 62.0% for DBP compared to a single BP measurement. (Shear et al. 1986) Nevertheless, it is not known how many repeated BP measurements taken over several years can improve prediction of adult hypertension and vascular changes related to atherosclerosis.

Current guidelines for pediatric hypertension recommend the use of age-, sex-, and height-specific 90th or 95th percentiles cut-off points for BP classification and hypertension diagnosis. (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute 2011) No routine

measurements of BP in children under 3 years of age are recommended for primary prevention of CVD. In children aged between 3 and 17 years, annual measurements should be performed and abnormal BP interpreted by the age-, sex-, and height-specific percentiles. In the guidelines, pediatric hypertension is defined as BP above the 95th percentile and prehypertension as BP over the 90th percentile but below the 95th percentile in children aged 3 to 11 years. Similar cutoffs are used for older children aged 12 to 17 years, while also applying the adult limits for prehypertension (120/80 mmHg). Finnish guidelines for child welfare clinics and school health care recommend measurement of BP for the first time in children aged 4 years. (Mäki et al. 2016) Furthermore, BP measurement should be repeated at least in children aged 7-8 years, 10-11 years and 14-15 years. Annual measurements are not recommended for normotensive children.

As for blood lipids, current guidelines recommend both a selective and a universal approach for screening of lipid and lipoprotein levels in children.(Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute 2011) The selective approach suggests that a fasting lipid profile should be obtained from children who have either a family history for CVD, dyslipidemia in either parent, or any other risk factors present (e.g. hypertension, obesity and diabetes), from the age of 2 years onward. The universal, population-wide, approach recommends screening of non-fasting lipid profile among all children aged 9–11 years and repeated among young adults aged 17–21 years – with the decision for a fasting lipid profile assigned by non-HDL-C levels above or equal to 3.75 mmol/l. Finnish Current Care guidelines for dyslipidemia recommend that fasting lipid profile should be obtained from children who have a positive family history for premature CHD (e.g. familial hypercholesterolemia) or other risk factors present (such as type 1 diabetes, obesity, and heart or kidney transplant). (Working group set up by the Finnish Medical Society Duodecim and Finnish Society of Internal Medicine, 2013) No universal screening of blood lipids is recommended among children in Finland.

3. AIMS OF THE STUDY

This thesis is based on findings from the Young Finns Study. The purpose was to examine cardiovascular risk factor levels and their 4-year-changes amongst Finnish adults in 2011 and whether repeated childhood measurements of BP and lipid levels or novel genetic variants in the case of lipid levels enhance the prediction of adult hypertension and dyslipidemia (**Figure 6**).

The specific aims were to:

1. report cardiovascular risk factor levels in 2011 and 4-year-changes in risk factor levels between 2007 and 2011 (**Study I**);

2. determine whether repeated childhood BP measurements enhance the prediction of adult hypertension compared to a single measurement in childhood (**Study II**);

3. study whether repeated childhood lipid measurements improve the prediction of adult dyslipidemia compared to a single measurement in childhood **(Study III**);

4. determine whether newly identified genetic variants affecting blood lipid levels enhance the prediction of adult dyslipidemia compared to clinical childhood risk factors (**Study IV**).

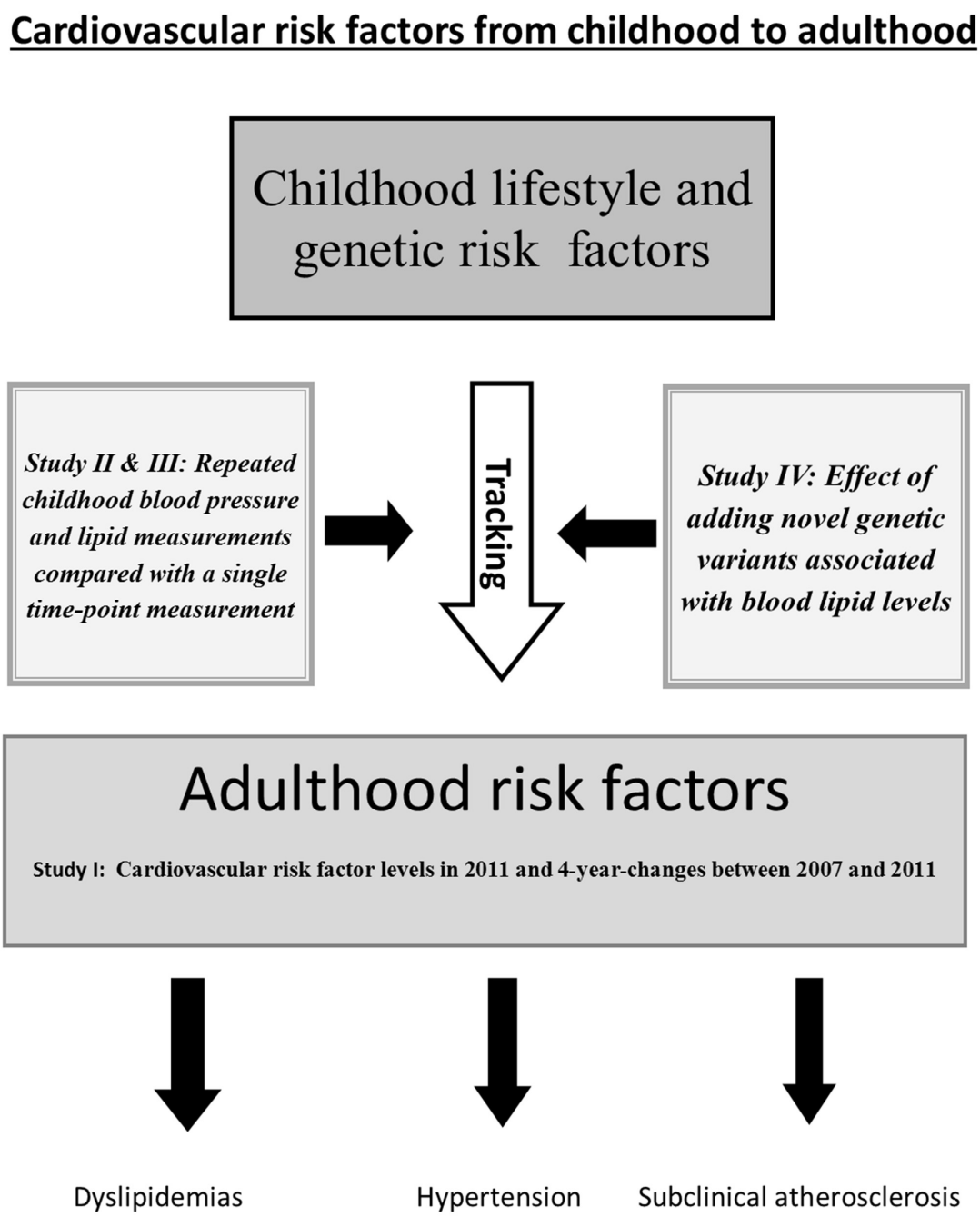


Figure 6. Schematic presentation of the aims of the thesis

4. SUBJECTS AND METHODS

4.1. The Cardiovascular Risk in Young Finns Study

The study population comprised participants of the Young Finns Study, a population-based prospective follow-up study on cardiovascular risk factors in Finland.(Raitakari et al. 2008) It has been carried out in all five Finnish university cities with medical schools and their rural surroundings. The first cross-sectional study was conducted in 1980. Altogether 4,320 children and adolescents aged 3, 6, 9, 12, 15 and 18 years were randomly recruited from the population register of these areas to produce a representative subsample of Finnish children. Of these individuals 3,596 (83%) participated in the original study. Since then, regular follow-ups have been performed **(Figure 7**) and the study group has been dynamic. In 2011, 104 of the original participants had died. In this thesis, data from the 1980, 1983, 1986, 2001, 2007 and 2011 follow-ups were used. The Young Finns Study has been approved by the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital and has been conducted according to the guidelines of the Declaration of Helsinki, and informed consent was obtained from all participants or their parents.

N	Year	Age cohorts														
3596	1980	3	6	9	12	15	18									
2991	1983		6	9	12	15	18	21								
2779	1986			9	12	15	18	21	24							
2737 *	1989				12	15	18	21	24	27						
2730 *	1992					15	18	21	24	27	30					
2283	2001								24	27	30	33	36	39		
2204	2007										30	33	36	39	42	45
2063	2011-2012											34	37	40	43	46	49

Figure 7. Study design and participation rates at each stage of the Young Finns Study.
*Follow-ups were partly questionnaire follow-ups and all of the study population was not clinically examined in the years 1989 and 1992.

4.2. Study design and participants

Study I examined cardiovascular risk factor data from the latest follow-up of the Young Finns Study in 2011 among 34- to 49-year old men and women. The study population comprised 2,063 Finnish adults. The main aim was to study cardiovascular risk factor levels in 2011 and

4-year-changes between 2007 and 2011. Lipid and BP levels, glucose and anthropometry were measured and lifestyle risk factors were examined with questionnaires.

In **Study II**, the aim was to examine whether repeated observations of abnormal BP in childhood improved the prediction of hypertension and subclinical atherosclerosis in adulthood compared with a single observation. The study was based on 1,927 participants (54% women, aged 6-21 years in 1983) who had SBP and DBP measurements performed in 1980, 1983, and in 1986 and had at least one follow-up visit in later adulthood (2001, 2007, or 2011).

Study III examined if the prediction of abnormal lipid levels in adulthood becomes more accurate with repeated measurements in childhood or young adulthood compared with a measurement obtained at a single time-point. The sample comprised 1,912 subjects (54% women, aged from 6-21 years in 1983) who had fasting lipid and lipoprotein measurements collected at three time-points in childhood or young adulthood (1980, 1983, and 1986) and had at least one follow-up visit in later adulthood (2001, 2007, or 2011).

In **Study IV**, the main aim was to examine whether newly identified genetic variants for blood lipid levels enhance the prediction of adult dyslipidemia over clinical childhood risk factors. The sample comprised 2,422 participants (54% women) who had participated at baseline (aged 3-18 years in 1980) and/or the 1986 follow-up study and had participated at least once in a follow-up study in adulthood (2001, 2007, or 2011).

Table 1. Inclusion criteria of participants for studies I-IV.

	Inclusion criteria of participants	
	Childhood (1980–1986)	Adulthood (2001-2011)
Study I		Participation in the 2011 follow-up study, (Participation in both of the 2007 and 2011 follow-up studies to be included in secular trend analyses)
Study II	Blood pressure measures from 1980 and 1983 and 1986	Blood pressure measures from 2011 or 2007 or 2001 or reported use of antihypertensive medication or carotid IMT ultrasound measures from 2007
Study III	Lipid measures from 1980 and 1983 and 1986	Lipid measures from 2011 or 2007 or 2001 or reported use of lipid-lowering medication
Study IV	Lipid measures from 1980 and/or 1986	Lipid measures from 2011 and/or 2007 and/or 2001 or reported use of lipid-lowering medication and genotyping performed

4.3. Data acquisition in The Young Finns Study

4.3.1. Physical examination

Weight was measured with weighing scales to the nearest 0.1 kg and height with anthropometer to the nearest centimeter. BMI was calculated as weight(kg)/height(m)^2. Waist circumference was measured using an anthropometric tape at the midpoint between the iliac crest and the lowest rib to the nearest 0.1 cm. The average of two measurements was used. BP was measured from the right brachial artery with a standard mercury sphygmomanometer in 1980 and 1983 and with a random-zero sphygmomanometer (Hawksley & Sons Ltd, Lancin, UK) in 1986, 2001, 2007 and 2011. BP was measured in the sitting position after a 5-minute rest. The Korotkoff first phase was used as the sign of SBP, and DBP was determined from the fifth phase. Readings were performed to the nearest integer of millimeters of mercury 3 times on each participant and the average of 3 measurements was used in the analysis. From participants 3 years old at baseline, only SBP was collected by using an ultrasound device.

4.3.2. Questionnaires

Physical activity was assessed by a self-report questionnaire. Subjects answered the questions themselves, with their parents' assistance as necessary. The physical activity questionnaire consisted of the following variables: intensity of physical activity, frequency of moderate or vigorous activity, and hours spent on moderate or vigorous activity per week.(Telama et al. 1985) Information on cigarette smoking was collected using a questionnaire. In childhood, smoking was assessed by a questionnaire in subjects aged ≥12 years. In study I, smoking was defined as positive if participants had smoked daily in year 2011. In studies II and III, childhood smoking was defined as positive if participants smoked daily in year 1980. In study IV, smoking in childhood and adolescence was defined as positive if participants had smoked daily at some stage before or at age 24 years. Use of lipid-lowering and antihypertensive medication in adulthood was examined by self-administrated questionnaires.

4.3.3. Biochemical analyses

In childhood, venous blood samples were drawn after a 12-hour fast from the right antecubital vein with the subject lying recumbent. Venipuncture was attempted only once. An aliquot for serum lipid analyses was stored at -25°C until thawed for the first time for the analyses. All

lipid determinations were done in duplicate and in the same laboratory. In 1980, TC concentrations were measured using a fully enzymatic CHOD-PAP method (Boehringer Mannheim, Mannheim, Germany) with OLLI 3000 and Kone CD analyzers (Kone Co., Espoo, Finland). In 1980, serum HDL-C concentrations were measured from the supernatant after precipitation of very low density lipoprotein, intermediate-density lipoprotein, and low-density lipoprotein particles with dextran sulphate-$MgCl_2$ (Pharmacia, Uppsala, Sweden).(Kostner 1976) In 1980 the precipitation was done in the laboratory, whereas in 1983 and 1986 the precipitation was done at the blood collection site. The time between blood collection and precipitation was thus longer in 1980 and may have caused slightly biased (presumably lower) HDL-C concentrations in 1980. In 1980, serum TG were determined by using a fully enzymatic method (Boehringer Mannheim). Non-HDL-C was calculated as TC – HDL-C. All analyses were performed as simultaneously as possible in the laboratory of Rehabilitation Centre of the Social Insurance Institution, Turku, Finland. The concentration of LDL-C was calculated using the Friedewald formula in a following way. (Friedewald et al. 1972)

$$LDL - cholesterol = Total\ cholesterol - HDL - cholesterol - \frac{Triglycerides}{2.2}$$

In adulthood, venous blood samples were drawn from the right antecubital vein of recumbent subjects after a 12-hour fast and serum was separated, aliquoted and stored at -70°C until analysis. TG concentration was determined using the enzymatic glycerol kinase–glycerol phosphate oxidase method (Triglyceride reagent, Beckman Coulter Biomedical, Ireland). TC levels were measured by the enzymatic cholesterol esterase–cholesterol oxidase method (Cholesterol reagent, Beckman Coulter Biomedical). The same reagent was used for estimating HDL-C levels after precipitation of very low density lipoprotein, intermediate-density lipoprotein, and low-density lipoprotein particles with dextran sulphate-$MgCl_2$. (Kostner 1976) All the above assays were performed on an AU400 instrument (Olympus, Japan) and the same methods were used both in 2007 and 2011. LDL-C was calculated by the Friedewald formula as shown earlier. (Friedewald et al. 1972) Participants with TG levels above 4.0 mmol/L were excluded from this analysis (n=32 in 2001, n=46 in 2007, and n=47 in 2011). Also, LDL-C concentrations were measured in 2007 by a direct enzymatic method. Non-HDL-C was calculated as TC – HDL-C. The analysis methods for TC and TG have been accredited by the Finnish Accreditation Service according to standard ISO/IEC17025. All analyses were performed as simultaneously as possible in the laboratory of the Research and Development

Unit of the Social Insurance Institution (Turku Finland) in 2001, and in the laboratory for Population Research of the National Institute for Health and Welfare (Turku, Finland) in 2007 and 2011.

Because of changes in determination methods and kits during study years, lipid levels from 1980, 1983, and 1986 and TG from 2007 were corrected by using correction factor equations to correspond to the samples taken in 2001 **(Table 2**). These equations were determined with linear regression analysis utilizing standardized principal component adjustments. For example, in 2007 the reagent for TG analyses had been altered by the manufacturer. The correction equation for triglycerides was determined comparing 250 samples with wide range of TG concentrations. TG concentrations were determined with both the original and the altered method, and results were used to calculate the correction equation. No correction equations were needed for the 2001 values, the 2007 TC, LDL-C and HDL-C values and the 2011 values.

Table 2. Correction factor equations used for total cholesterol, HDL-cholesterol, triglycerides, and glucose.

Year	Correction equation		Original value (example)	Corrected value (example)
1980, 1983, 1986	Total cholesterol	1.091 * TC – 0.271 mmol/L	5.00	5.18
	HDL-cholesterol	1.068 * HDL-C – 0.0277 mmol/L	1.00	1.04
	Triglycerides	1.00756 * TG + 0.0582 mmol/L	1.50	1.57
2001	No correction equations			
2007	Total cholesterol	No correction equations		
	HDL-cholesterol	No correction equations		
	Triglycerides	(TG + 0.03226 mmol/L)/0.9811	1.50	1.56
	Glucose	(glucose – 0.0235)/ 0.9471	5.0	5.25
2011	No correction equations			

Serum glucose concentration was determined by the enzymatic hexokinase method (Glucose reagent, Beckman Coulter Biomedical). Due to changes in methods or reagents from 2001 to 2007 glucose levels were corrected by using a correction factor equation as shown in **Table 2**. The concentration of HbA1c was assayed with an immunoturbidimetric method (Hemoglobin A1c assay, Abbott, USA) on an Architect ci8200 analyzer (Abbott) in 2011.

4.3.4. Genetic analyses

In the genetic analyses 58 SNPs associated with LDL-C levels, 71 SNPs associated with HDL-C and 40 SNPs associated with TG levels identified recently in a large GWAS were used.(Global Lipids Genetics Consortium et al. 2013) wGRS were calculated for each of the three lipids using the published effect estimates as weights. Genotyping of variants included in the lipid wGRSs was performed with the Illumina Human 670K Bead Chip, and imputation was performed with the 1000 Genomes Project.

For LDL-C, also APOE allele combinations of SNPs rs7412 and rs429358 (coded as 1= ε2/ε2, 2=ε2/ε3, 3=ε2/ε4, 4=ε3/ε3, 5=ε3/ε4, 6=ε4/ε4were added into the model including the genetic risk score. (Bennet et al. 2007) ApoE genotyping was performed by using Taqman SNP Genotyping Assays (rs429358 assay C 3084793_20; rs7412 assay C_904973_10) and the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). No discrepancies emerged in the genotyping results of duplicate samples. (Tolonen et al. 2011)

4.3.5. Ultrasound imaging of carotid IMT

A high-resolution ultrasound system (Sequoia 512, Acuson, CA, USA) with 13.0 MHz linear array transducer was used to perform carotid ultrasound studies in 2001 and 2007. Ultrasound studies were performed for 2,264 participants in 2001 and for 2,197 participants in 2007. Physicians and ultrasound technicians performed all studies simultaneously in the five cities of the multicenter study (Turku, Tampere, Helsinki, Kuopio and Oulu). Carotid artery IMT was measured approximately 10 mm proximal to the bifurcation on the left common carotid artery focusing the image on the posterior wall and recording images from the angle showing the greatest distance between the lumen-intima interface and the media-adventitia interface. At least four measurements were taken at each scan of the common carotid artery incident with

the R-wave of the continuously monitored electrocardiography to derive mean carotid IMT. The scans were analyzed by one reader (same reader in 2001 and 2007) blinded to participants' details. High-risk IMT was defined as IMT ≥90th percentile or the presence of carotid plaques. Presence of carotid plaque was defined as a distinct area of the carotid vessel wall protruding into the lumen >50% of the adjacent intima-media layer.

4.4. Definition of abnormal blood pressure levels, dyslipidemia, metabolic syndrome, prediabetes and type 2 diabetes

4.4.1. Definition of abnormal blood pressure levels and dyslipidemia in childhood

According to current pediatric guidelines, abnormal BP in childhood was defined as pediatric hypertension or prehypertension based on either SBP or DBP being in the uppermost 90th or 95th percentile of the age-, sex- and year-specific distribution.(Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute 2011) The definition of dyslipidemia in childhood based on either TC, LDL-C, non-HDL-C or TG being in the uppermost quintile of the age- and sex-specific distribution, or HDL-C in the lowest quintile.

4.4.2. Definition of abnormal blood pressure levels and dyslipidemia in adulthood

In adulthood, hypertension was defined as BP at or above 140/90 mmHg, or the self-reported use of antihypertensive medication (**Table 3**).(Chobanian et al. 2003) In addition, reimbursement for antihypertensive medication in Finland is issued by The Social Insurance Institute of Finland according to following criteria: SBP >200 mmHg or DBP >105 mmHg, and/or DBP >95 mmHg and positive family history for CVD, dyslipidemia, T2D, or target organ damage present and/or pulmonary hypertension. To define dyslipidemia in adulthood, European cut-points were used to denote abnormal serum lipid values (**Table 3**). (Perk et al. 2012) Use of lipid-lowering medication was considered as an indication of elevated TC, LDL-C and non-HDL-C levels. Lipid and BP measurements from year 2011 were used except in case of missing data when values from 2007 or 2001 were used.

Table 3. Definition of abnormal blood pressure and lipid levels in adulthood

Measure	Cut Point
Elevated systolic blood pressure	≥ 140 mmHg or use of antihypertensive treatment
Elevated diastolic blood pressure	≥ 90 mmHg or use of antihypertensive treatment
Elevated total cholesterol	≥ 5.0 mmol/l or use of lipid-lowering medication
Elevated LDL-cholesterol	≥ 3.0 mmol/l or use of lipid-lowering medication
Reduced HDL-cholesterol	≤ 1.0 mmol/l in men and ≤ 1.2 in women
Elevated non-HDL-cholesterol	≥ 3.8 mmol/l or use of lipid-lowering medication
Elevated triglycerides	≥ 1.7 mmol/l or use of lipid-lowering medication

4.4.3. Definition of the metabolic syndrome, prediabetes and type 2 diabetes in adulthood

The MetS was defined according to the Harmonized criteria (**Table 4**).(Alberti et al. 2009) The use of antihypertensive medication was considered as an indication of hypertension. A diagnosis required that any 3 of the 5 criteria be present. Pregnant women were excluded from analyses involving the MetS (in 2007, n = 37 and in 2011, n = 13). Participants were classified as having increased risk for T2D (prediabetes) if they had fasting glucose from 5.6 mmol/L to 6.9 mmol/L or glycated hemoglobin (HbA1c) from 5.7% to 6.4% (39 to 47 mmol/mol). (American Diabetes Association 2013) The diagnosis of T2D included participants with fasting glucose ≥ 7mmol/L or HbA1c ≥ 6.5% (≥ 48 mmol/mol) or self-reported diabetes or use of medication. (American Diabetes Association 2013)

Table 4. Definition of the metabolic syndrome, prediabetes and type 2 diabetes in adulthood

	Measure	Cut Point
Metabolic syndrome	Elevated waist circumference	≥102 cm in men and ≥88 cm in women
	Elevated triglycerides	≥ 1.7 mmol/L
	Reduced HDL-cholesterol	≤ 1.0 mmol/L in men and ≤ 1.3 mmol/L in women
	Elevated blood pressure	≥ 130 mmHg systolic blood pressure or ≥ 85 mmHg diastolic blood pressure
	Elevated fasting glucose	≥ 5.6 mmol/L or treatment for elevated glucose levels
Prediabetes	Fasting glucose	5.6 mmol/L to 6.9 mmol/L
	Glycated hemoglobin	5.7% to 6.4% (39 to 47 mmol/mol)
Type 2 Diabetes	Elevated fasting glucose	≥ 7mmol/L or self-reported diabetes or use of medication
	Elevated glycated hemoglobin	≥ 6.5% (≥ 48 mmol/mol)

4.5. Statistical methods

Continuous variables are expressed as mean ± standard deviation (SD) and categorical variables as percentages unless stated otherwise. The normality assumptions were assessed by examining histograms and normal probability plots. The values of serum TG were $\log_{10}$-transformed before analyses due to skewed distributions. The statistical tests were performed with SAS versions 9.3 and 9.4, STATA version 10.1 and R version 3.2.2. Statistical significance was inferred at a two-tailed P-value <0.05.

4.5.1 Study I

The effect of age on risk factors was examined using linear regression analysis for continuous variables and logistic regression analysis for categorical variables. Multivariable linear and

logistic regression models were used to examine whether the associations between age and risk factor were similar between men and women. The models included each risk factor as the dependent variable and age and age*sex interaction as independent variables. The differences in risk factor level between men and women in all age groups were examined by applying t-test for continuous variables and Chi-squared test for categorical variables. Changes in cardiovascular risk factor levels were assessed from the examinations performed in 2007 and 2011 using t-test for continuous variables and Fisher's exact test for categorical variables. To compare groups of same age, the groups of 33- to 45-year-old participants in 2007 (mean age for women 39.0 years (n=1038) and for men 39.1 years (n=831)) were compared to the groups of 34- to 43-year-old participants in 2011 (average age among women 38.9 years (n=731), and men 38.7 years (n=609)).

4.5.2. Study II

The studied outcomes were hypertension, high-risk IMT and reimbursed antihypertensive medication in adulthood. Proportions of participants with outcomes in adulthood were stratified according to the number of times (between 0 and 3) abnormal BP had been observed in childhood/youth.

Tracking of BP was studied with Pearson correlation analyses between mean adulthood BP and age-, sex- and year-specific Z-scores in childhood/youth (one, mean of two, or mean of three measurements). Mean adulthood BP was calculated as the average of three measurements in adulthood (2001, 2007, and 2011 transformed into age-, sex- and study year-specific Z-scores). To negate any confounding introduced as a result of different length of follow-ups between the childhood/youth BP measures and those collected in later adulthood, year 1983 was used to represent the single measurement, the mean of 1980 and 1986 was used to represent two measurements, and the mean of 1980, 1983 and 1986 was used to represent three measurements (**Figure 8**). Statistical significance of the differences between correlations coefficients was determined by the Fisher r-to-z transformation.(Lowry 2013) The effect of age was further explored by stratifying participants according to baseline age into two categories: the very young (aged 3 to 9 years at baseline in 1980) and the young (aged 12 to 18 years at baseline in 1980). To study possible modification by BMI, smoking status and physical activity in childhood, the analyses were repeated adjusting for the length of follow-up, baseline smoking,

physical activity, and the mean of three childhood/youth BMI measurements (transformed into age-, sex- and study year-specific Z-scores).

Improved prediction of adult hypertension outcomes was studied by assigning participants a score of 2 if above the 95th percentile which is the standard definition of elevated BP, a 1 if above the 90th but below the 95th percentile, and a 0 if below the 90th percentile at each childhood/youth BP measurement. The ability of child/young adult measurements to predict hypertension risk in adulthood was estimated by calculating the area under the receiver operating characteristic curve (AUC). (Pencina et al. 2008) To account for an effect of increase in BMI, analyses were additionally adjusted for the mean of all three childhood/youth BMIs transformed into age-, sex-, and study year-specific Z-scores.

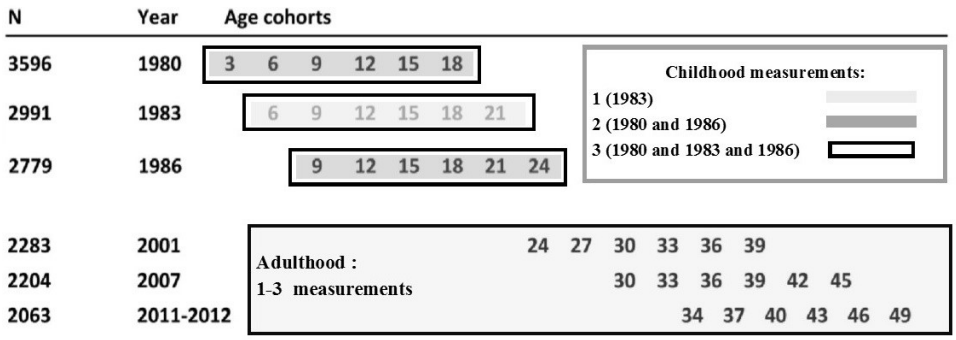


Figure 8. Blood pressure and lipid measurement combinations used in childhood and adulthood.

4.5.3. Study III

Childhood and young adulthood lipid measurements performed in 1980, 1983, and 1986 were transformed into age-, sex-, and study year-specific Z-scores. Year 1983 was used to represent the single measurement, the mean of 1980 and 1986 was used to represent two measurements, and the mean of 1980, 1983, and 1986 was used to represent three measurements as shown in **Figure 8**. This approach was used to assign the single and combined measurements to negate any confounding introduced as a result of different length of follow-ups between the child/young adult measures and those collected in later adulthood. In adulthood, analyses of

absolute values were performed using measurements from year 2011, except in cases of missing data when values from 2007 or 2001 were used. The mean of all adulthood measurements from 2001, 2007 or 2011 (between 1 and 3 measurements) transformed into age-, sex, and study year-specific Z-scores was used to represent the adulthood lipid level in calculation of quintiles and in correlation analyses.

Correlations between the one, two or three measurements in childhood/young adulthood and the mean of all adulthood measurements were calculated using Pearson correlation. To study possible modification by smoking status and physical activity in childhood, the analyses were repeated adjusting for childhood smoking and childhood physical activity. Significant differences between correlation coefficients were calculated using the Fisher r-to-z transformation. (Lowry 2013) Participants who reported use of lipid lowering medication (n=59) in 2001, 2007, or 2011 were excluded from the correlation analyses. Differences in correlation coefficients between men and women were tested using normal probability test for difference between Z-transformed correlation coefficients.

Sensitivity rate was calculated as true positives/(true positives + false negatives), and the specificity rate was calculated as true negatives/(true negatives + false positives). Negative predictive value (NPV) was calculated as true negatives/ (true negatives + false negatives) and positive predictive value (PPV) was calculated as true positives/(true positives + false positives). The ability of child/young adult measurements to predict dyslipidemia risk in adulthood was estimated by calculating AUC and the category-based NRI (net reclassification improvement). (Pencina et al. 2008). For the category-based NRI, participants were assigned to one of two categories that reflected their risk of adult dyslipidemia based on each model.(Pencina et al. 2008) A two-category NRI with cut-off at 50% for TC and non-HDL-C, 60% for LDL-C, 25% for HDL-C, and 20% for TG that roughly paralleled the proportion of those with dyslipidemia in study sample was used. It is acknowledged that the cut-offs are not categorized according to recommendations for the ideal proportion of the adult population with dyslipidemia. However, the choice of cut-off was made to ensure there were a non-zero number of samples in the two categories. It was not possible to increase the number of categories because the predictor variable only had two covariate patterns (dichotomous, 0=no child dyslipidemia vs. 1=yes child dyslipidemia), already considered age and sex in its definition, and the model did not consider any additional covariates.

Participants were also stratified according to the number of times (between 0 and 3) they had been at risk in childhood/young adulthood and reported the proportion that met the respective abnormal lipid or lipoprotein level in adulthood.

4.5.4. Study IV

The mean of age- and sex-specific z-scores of childhood lipid levels and BMI measured in 1980 and 1986 were used to represent childhood risk factor levels. If either measurement was missing, a z-score of a single measurement was used. For physical activity during childhood an age- and sex-specific z-score was calculated. Association of lipid-specific wGRS with the risk of adult dyslipidemia was analyzed using logistic regression. Multivariable logistic regression models, with and without wGRS, were constructed for all outcomes separately including the following childhood risk factors: BMI, smoking status and physical activity.

The additional value of wGRS in prediction of adult dyslipidemia was examined using the R packages PredictABEL(Kundu et al. 2011), Hmisc and pROC(Robin et al. 2011) to estimate fit, calibration and the differences in predictive abilities of the models. The discrimination performance of each model was estimated by calculating the AUC.(Hanley, McNeil 1982) Youden's J statistic was used to determine the optimal cut-off value for sensitivity and specificity in each model.(Youden 1950) The improvement of prediction models was assessed using the continuous NRI and IDI and model calibration was tested using the Hosmer-Lemeshow (H-L) goodness of fit test.(Hosmer, Lemeshow 1989, Pencina et al. 2008)

5. RESULTS

5.1. Characteristics of the participants

Clinical and biochemical characteristics of the study population at baseline (1980) are shown in **Table 5**. The representativeness of participants at follow-up was examined by age-adjusted comparison of their baseline characteristics (1980) with those who did not participate, were missing data, or did not fulfill the inclusion criteria. (**Table 1**) Male participants were older than non-participant males and female participants had lower BMI than non-participant females at baseline. Otherwise, no substantial differences were observed. LDL-C concentrations measured with direct method in 2007 were highly correlated with the estimated LDL-C values (r=0.97). Mean estimated LDL-C value was 3.09 mmol/L (n=2141), and mean direct LDL-C value was 3.14 mmol/L (n=2187) in 2007.

In **Study II**, no participants were currently using antihypertensive medication in childhood/youth. In adulthood, hypertension was observed in 368 participants (19%; BP>140/90 or self-reported antihypertensive medication). Reimbursed antihypertensive medication, indicating a confirmed hypertension diagnosis was detected in 80 (4.2%) participants.

In **Study III**, lipid-lowering medication was used by 59 participants (3.1% of the study population) in adulthood. The mean level of LDL-C in 1983 among those who used lipid-lowering medication in adulthood was 3.77 mmol/L. Concurrently, the mean LDL-C in the whole study population was 3.17 mmol/L in childhood. In adults, an abnormal lipid profile for TC >5mmol/L was observed in 940 (49%) participants, LDL-C >3mmol/L in 1087 (57%) participants, non-HDL-C >3.8mmol/L in 875 (46%) participants and TG >1.7mmol/L in 383 (20%) participants, and HDL-C was <1.2mmol/L in 253 (25%) women and <1.0mmol/L in 228 (26%) men.

In **Study IV**, lipid-lowering medication was used by 89 participants (3.7% of the study population) in adulthood. In adults, an abnormal lipid profile for LDL-C was observed in 1390 (57.5%) participants, abnormal HDL-C in 585 (24.1%) participants, and abnormal TG in 502 (20.7%) participants.

Table 5. Baseline (1980) characteristics of 2,761 participants and 835 non-participants in adult follow-ups of the Cardiovascular Risk in Young Finns Study (53.7% female).

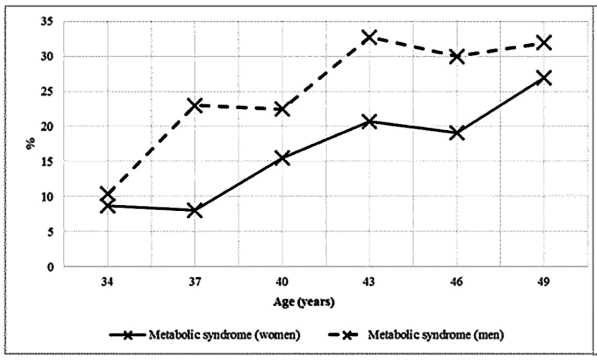
	Women			Men		
	Participants	Non-participants	P-value for difference	Participants	Non-participants	P-value for difference
n	1484	348		1277	487	
Age (years)	10.6	10.4	0.47	10.5	10.0	0.04
Total cholesterol (mmol/L)	5.38	5.29	0.10	5.22	5.22	0.72
LDL-cholesterol (mmol/L)	3.51	3.42	0.10	3.38	3.37	0.59
non-HDL-cholesterol (mmol/L)	1.56	1.55	0.13	1.56	1.57	0.61
HDL-cholesterol (mmol/L)	3.82	3.74	0.56	3.67	3.65	0.72
Triglycerides (mmol/L)	0.69	0.69	0.66	0.64	0.63	0.36
Systolic blood pressure (mmHg)	112	112	0.46	113	112	0.59
Diastolic blood pressure (mmHg)	69	69	0.49	69	69	0.84
Body mass index (kg/m2)	17.8	18.0	0.04	17.9	17.6	0.66
Smoking prevalence (%)	8.2	8.6	0.60	10.3	9.2	0.11

5.2. Cardiovascular risk factor levels in 2011 and their changes since 2007

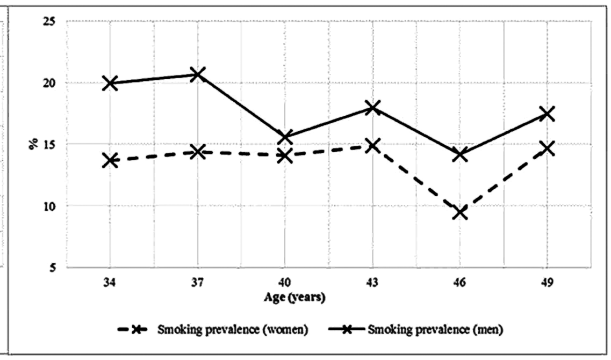
5.2.1. Cardiovascular risk factor levels in 2011

The levels of cardiovascular risk factors in 34- to 49-year-old men and women are shown in **Figures 9** and **10 (Study I**). Men had higher (P <0.0001) total cholesterol, LDL-C, triglycerides, BP, BMI and waist circumference compared with women, whereas women had higher HDL-C. In both sexes TC, LDL-C, TG, BP, BMI and waist circumference increased

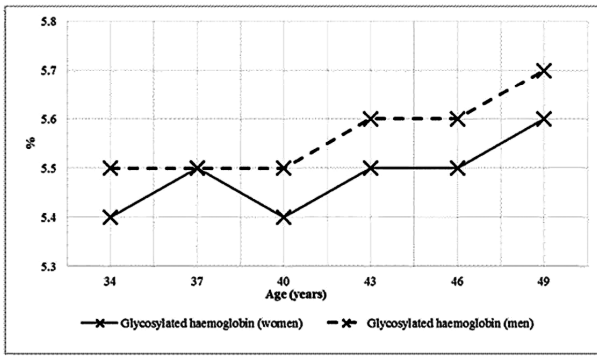
with age (P <0.05). In men, HDL-C also increased with age. As indicated by a statistically significant interaction, the association between SBP and age was greater in women than in men.



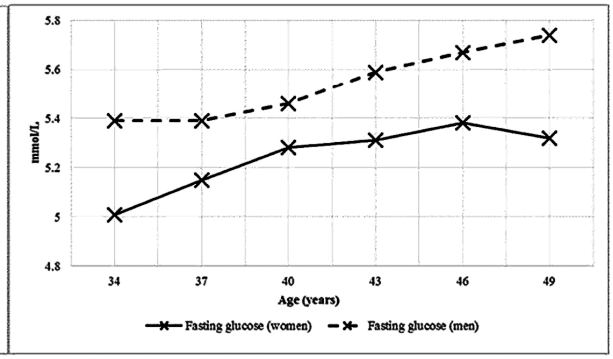
Effect of age was significant in both sexes (P<0.0001) and difference between sexes was significant (P<0.0001).



Age had no effect on smoking prevalence. Difference between sexes was significant (P=0.01).

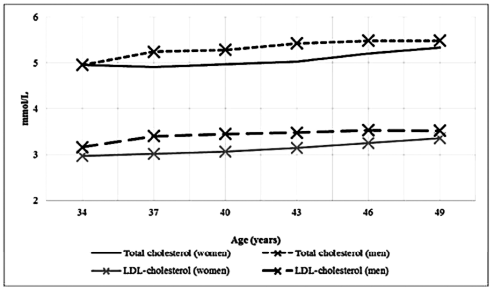


Effect of age was significant in both sexes (P<0.01) and difference between sexes was significant (P<0.0001).

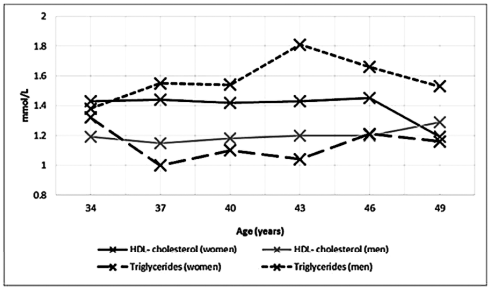


Effect of age was significant in both sexes (P<0.01) and difference between sexes was significant (P<0.05).

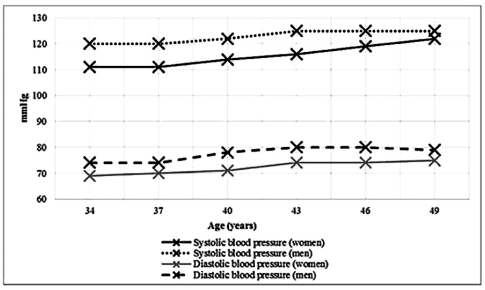
Figure 9. Prevalence of metabolic syndrome and smoking and fasting glucose and glycosylated haemoglobin levels in Finnish women and men aged 34-49 years in 2011 stratified by age.



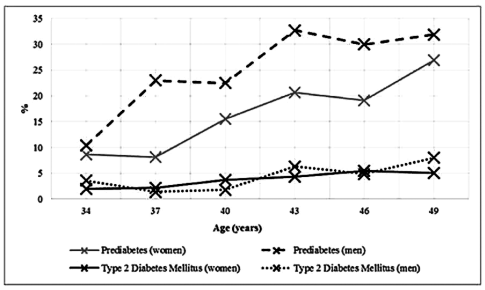
Effect of age was significant in both sexes for total cholesterol and LDL-cholesterol ($P<0.05$) and difference between sexes was significant in total cholesterol and in LDL-cholesterol ($P<0.0001$).



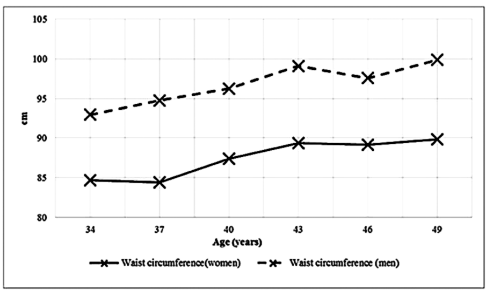
Effect of age was significant in both sexes for triglycerides and in men for HDL-cholesterol ($P<0.01$) and difference between sexes was significant in triglycerides and in HDL-cholesterol ($P<0.0001$).



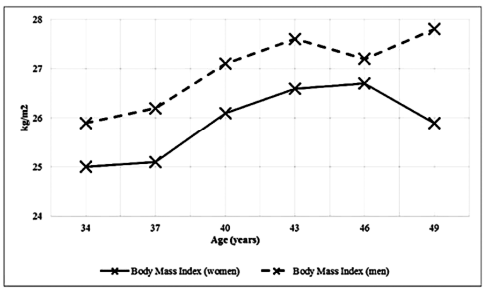
Effect of age was significant in both sexes for both systolic and diastolic blood pressure levels ($P<0.0001$) and difference between sexes was significant in both systolic and diastolic blood pressure ($P<0.0001$).



Effect of age was significant in both sexes for prediabetes and T2DM ($P<0.01$). Difference between sexes was significant in prediabetes ($P<0.0001$) but not in T2DM ($P=0.58$).



Effect of age was significant in both sexes ($P<0.0001$) and difference between sexes was significant ($P<0.0001$).



Effect of age was significant in both sexes ($P<0.0001$) and difference between sexes was significant ($P<0.0001$).

Figure 10. Total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, systolic and diastolic blood pressure, body mass index, and waist circumference levels and prevalence of type 2 diabetes mellitus and prediabetes in Finnish women and men aged 34-49 years in 2011 stratified by age.

The average TC in the participants was 5.19 mmol/L, with 51.3% (57.3% of men and 46.3% of women) of participants having TC over 5.0 mmol/L and 58.0% having LDL-C level over 3.0 mmol/L (**Study I)**. The prevalence of overweight (BMI 25-30 kg/m²) was 29.8% in women and 43.5% in men. The prevalence of obesity (BMI >30 kg/m2) was 20.2% in women and 21.0% in men. Daily smoking prevalence was higher among men (17.6%) than women (13.5%), and was highest in the age group of 37-year-old men (20.7%) and 43-year-old women (14.9%) in 2011.

T2D was observed in 4.1% of the participants. Prediabetes was observed among 33.8% of the participants. Men had higher levels of glucose and HbA1c, as well as higher prevalence of prediabetes and MetS compared with women. There was no significant difference in the prevalence of T2D between sexes. Glucose, HbA1c and prevalence of prediabetes, T2D and MetS increased with age for both sexes. The association of glucose and HbA1c with age in men was stronger than in women. Over half (54%) of the T2D diagnoses were self-reported or based on the use of medication for T2D. Fasting glucose was over 7.0 mmol/L in 15% of the T2D cases, 15% had elevated HbA1c levels (>6.5% (>48 mmol/mol)) and 15% had both elevated fasting glucose and HbA1c levels.

5.2.2. The awareness of type 2 diabetes

Participant awareness of having T2D is presented in **Figure 11**. Almost half (46%) of the participants with T2D did not report having T2D. Of the cases with T2D, 54% of the diagnoses were self-reported or based on the use of medication for T2D. Fasting plasma glucose was over 7.0 mmol/L in 15% of the T2D cases, 15% had elevated HbA1c levels (>6.5% (>48 mmol/mol)) and 15% had both elevated fasting glucose and HbA1c levels.

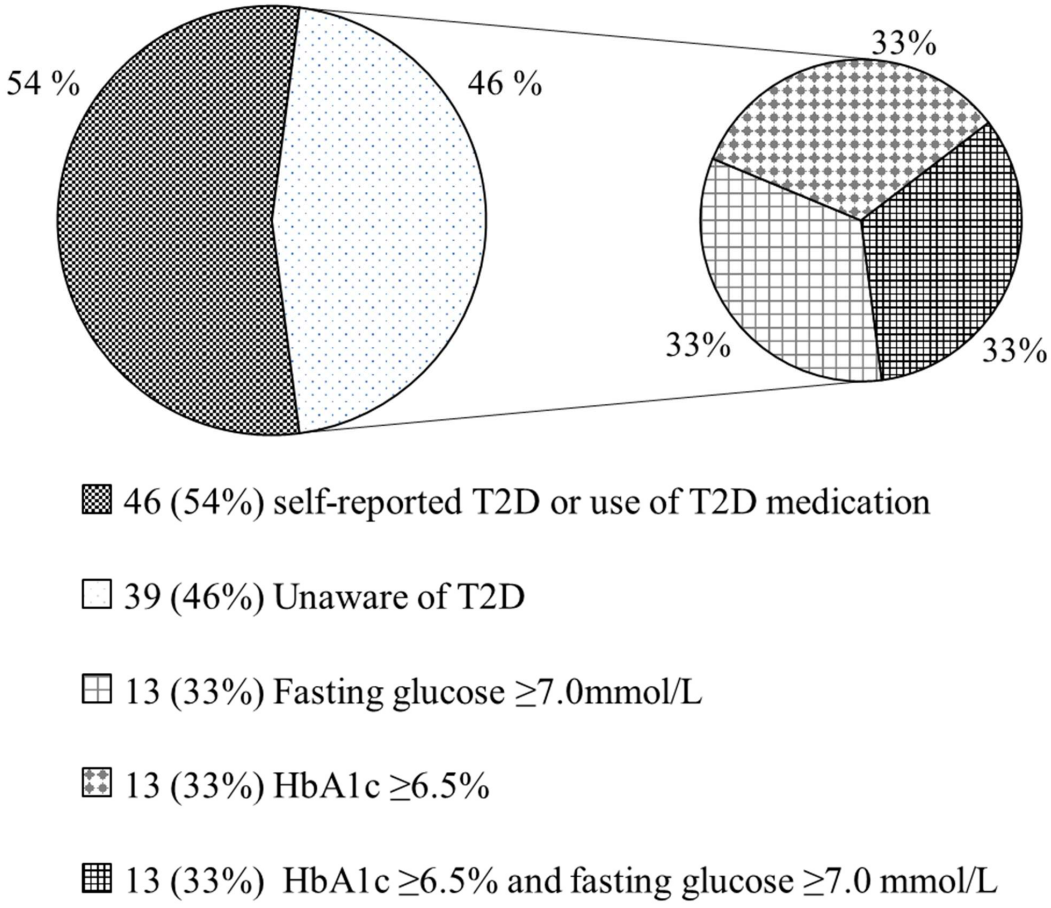


Figure 11. Pie chart displaying participant awareness of having type 2 diabetes including type 2 diabetes diagnoses by criteria

5.2.3. 4-year-change in cardiovascular risk factor levels between 2007 and 2011

Four-year comparisons in cardiovascular risk factor levels between comparable age groups are shown in **Table 6**. Significant decreases were observed in TG and in SBP and DBP for both sexes and a significant increase in waist circumference was observed amongst women.

Table 6. Changes in cardiovascular risk factor levels in 1890 participants (mean age 39 years) in 2007, and in 1334 participants (mean age 39 years) in 2011. N varied in 2007 between 1006-1053 in women and 799-837 in men and in 2011 between 699-724 in women, and 578-607 in men. Modified from Study I.

	Risk factor			**Change between 2007-2011**	
		2007	2011	%	P-value
Men	Total cholesterol (mmol/L)	5.28	5.21	-1.3	0.216
	LDL-cholesterol(mmol/L)	3.36	3.37	0.3	0.830
	HDL-cholesterol(mmol/L)	1.21	1.18	-2.5	0.094
	Triglycerides(mmol/L)	1.68	1.57	-6.5	<0.001
	Systolic blood pressure (mmHg)	126	121	-4.0	<0.001
	Diastolic blood pressure (mmHg)	79	77	-3.3	<0.001
	Body mass index (kg/m2)	26.9	26.6	-1.0	0.294
	Daily smoking (%)	19.7	17.8	-9.5	0.447
	Waist (cm)	95.1	96.5	1.5	0.402
	Glucose (mmol/L)	5.51	5.46	-0.9	0.217
	Metabolic syndrome (%)	23.5	21.3	-9.3	0.399
Women	Total cholesterol (mmol/L)	4.94	4.95	0.2	0.804
	LDL-cholesterol(mmol/L)	2.97	3.04	2.4	0.079
	HDL-cholesterol(mmol/L)	1.44	1.42	-1.4	0.274
	Triglycerides(mmol/L)	1.16	1.12	-3.4	<0.001
	Systolic blood pressure (mmHg)	117	113	-3.0	<0.001
	Diastolic blood pressure (mmHg)	73	71	-3.0	<0.001
	Body mass index (kg/m2)	25.5	25.8	1.4	0.186
	Daily smoking (%)	12.6	13.2	4.9	0.751
	Waist (cm)	84.0	87.0	3.6	<0.001
	Glucose (mmol/L)	5.19	5.2	0.2	0.852
	Metabolic syndrome (%)	15.7	14.1	-10	0.413

Changes in components of MetS were also analyzed. Changes in prevalences of BP and hypertriglyceridemia were significant in both sexes, and change in prevalence of elevated waist circumference was significant in women.

In 2007, according to the self-administrated questionnaires amongst 1869 participants aged 33-45 years, 144 (7.8%) were using anti-hypertensive medication, 44 (2.4%) were using medication for hypercholesterolemia, and 20 (1.1%) were using any treatment for diabetes. In 2011, amongst 1340 participants aged 34-43 years 80 (6%) were using anti-hypertensive medication, 44 (3.3%) were using medication for hypercholesterolemia and 15 (1.1%) were using any treatment for diabetes. The trends in lipid levels, glucose and BP were examined in study participants excluding those with reported use of relevant medication. Trends in lipid levels, BP and glucose remained essentially similar with the trends in the whole cohort.

5.3. Prediction of adult dyslipidemia and hypertension using repeated measurements of childhood lipid and blood pressure levels

5.3.1. Prediction of adult hypertension using repeated measurements of childhood blood pressure levels

Pearson correlation coefficients for BP levels between childhood and adulthood, indicating tracking of BP, stratified by the number of childhood observations of abnormal BP are shown in **Table 7 (Study II)**. There was a significant improvement in the correlation coefficients between one and two or three BP observations in childhood and BP in adulthood. The analyses were repeated adjusting for length of follow-up, childhood BMI and baseline smoking and physical activity with similar results. Childhood/youth SBP was associated with carotid IMT (correlation coefficients between 0.12 and 0.16), but the correlations between DBP measurements in childhood/youth and IMT in adulthood were weaker, and a higher number of childhood/youth observations did not improve prediction of IMT.

Table 7. Correlations between N of measurements of BP in childhood/youth and systolic and diastolic BP and carotid IMT in adulthood. Modified from Study II.

		Systolic BP in adulthood		Diastolic BP in adulthood		Carotid IMT	
		r[a]	P for difference ‖	r	P for difference	r	P for difference
Measurements of DBP in childhood/youth	**1**†			0.17***	ref.	0.06*	ref.
	2‡			0.35***	<0.0001	0.04	0.49
	3§			0.32***	<0.0001	0.06*	0.86
Measurements of SBP in childhood/youth	**1**†	0.35***	ref.			0.12***	ref.
	2‡	0.44***	0.0009			0.16***	0.30
	3§	0.46***	<0.0001			0.16***	0.24

All BP values transformed into age-, sex- and year-specific Z-scores. [a]Pearson correlation coefficients. Stars denote Pearson correlation *** p<0.0001, ** p<0.001 and *<0.05. † Measurement from 1983 was used to represent one measurement; ‡ Mean of measurements from 1980 and 1986; § Mean from years 1980, 1983 and 1986. ‖ Significance of the difference between two correlation coefficients was calculated using Fisher r-to-z transformation.

The AUCs for predicting hypertension, use of reimbursed antihypertensive medication and high-risk IMT in adulthood according to number of BP measurements in childhood are presented in **Table 8 (Study II)**. Two observations of BP significantly improved prediction of hypertension in adulthood compared with one measurement (AUCs 0.63 vs. 0.60, P=0.001 for improved AUC) and three observations improved significantly the prediction of the use of reimbursed medication in adulthood (AUCs 0.69 vs 0.65, P=0.04 for improved AUC). When participants were stratified by age into the very young (aged 3 to 9 years) and young (aged 12 to 18 years) age groups at baseline, significant improvement in hypertension prediction was observed for those aged 12 to 18 years (AUCs 0.63 vs. 0.59, p=0.002 for improved AUC) (**Table 9**). The number of observations of BP did not improve prediction of high-risk IMT and for prediction of use of reimbursed antihypertensive medication, results were non-significantly improved when participants were stratified by age at baseline.

Table 8. Improvement in prediction of adult outcomes according to childhood blood pressure status using one, two or three childhood measurements. Modified from Study II.

Outcome in adulthood	Number of childhood measures	AUC	P for AUC difference
Hypertension *	1 †	0.60	ref.
	2 ‡	0.63	0.003
	3 §	0.63	0.001
High-risk IMT **	1 †	0.59	ref.
	2 ‡	0.59	0.82
	3 §	0.59	0.64
Reimbursed antihypertensive medication	1 †	0.65	ref.
	2 ‡	0.68	0.09
	3 §	0.69	0.04

Models additionally adjusted for mean of BMI Z-scores from 1980, 1983 and 1986. AUC=area under the curve, IMT=intima-media thickness. * BP at or over 140/90 mmHg or self-reported use of antihypertensive medication. ** BP at or over 90th percentile or plaque. † Measurement from 1983 was used to represent one measurement. ‡ Mean of measurements from 1980 and 1986. § Mean from years 1980, 1983 and 1986.

Table 9. Improvement in prediction of adult outcomes according to childhood blood pressure status using one, two or three measurements stratified by age. Modified from Study II.

Outcome in adulthood	Age at baseline	Number of childhood measures	AUC	P for AUC difference
Hypertension *	3-9	1 †	0.62	ref.
		2 ‡	0.64	0.19
		3 §	0.65	0.15
	12-18	1 †	0.59	ref.
		2 ‡	0.63	0.004
		3 §	0.63	0.002
High-risk IMT **	3-9	1 †	0.58	ref.
		2 ‡	0.59	0.83
		3 §	0.59	0.80
	12-18	1 †	0.62	ref.
		2 ‡	0.62	0.97
		3 §	0.63	0.28
Reimbursed antihypertensive medication	3-9	1 †	0.69	ref.
		2 ‡	0.71	0.50
		3 §	0.73	0.27
	12-18	1 †	0.64	ref.
		2 ‡	0.67	0.10
		3 §	0.68	0.05

Models additionally adjusted for mean of BMI Z-scores from 1980, 1983 and 1986. AUC=area under the curve, IMT=intima-media thickness. * BP at or over 140/90 mmHg or self-reported use of antihypertensive medication. ** BP at or over 90th percentile or plaque. † Measurement from 1983 was used to represent one measurement. ‡ Mean of measurements from 1980 and 1986. § Mean from years 1980, 1983 and 1986.

To study the potential clinical utility of repeated BP measurements in childhood, the prevalence of hypertension outcomes in adulthood according to the number of times abnormal SBP and DBP (>90th age-, sex- and year-specific percentile) was observed in childhood/young adulthood was examined (**Table 10** and **11**). The number of times abnormal BP was observed in childhood was directly associated with the prevalence of hypertension and reimbursed antihypertensive medication in SBP and DBP, but had no effect on the prevalence of high-risk IMT in adulthood. Participants with elevated DBP or SBP at three consecutive measurements in childhood and youth had on average 20 mmHg higher SBP and 6 mmHg higher DBP in adulthood compared with those who had never had elevated SBP or DBP in childhood (**Figure 12**). Adulthood blood pressure and IMT according to the childhood deciles of DBP and SBP are presented in **Figure 13** and **Figure 14.**

Table 10. Proportion of participants with outcomes in adulthood according to times high systolic BP was observed in childhood (over 90th age-, sex- and year-specific percentiles).

Outcome in adulthood	High systolic BP observed in childhood	N	Proportion (%) of adults with outcome †
High systolic BP	Never	1531	11.7
	Once	283	24.4
	Twice	84	40.5
	Three times	30	73.3
High IMT or plaque	Never	1251	11.6
	Once	230	20.4
	Twice	68	26.5
	Three times	21	19.1
Reimbursed antihypertensive medication	Never	1525	3.0
	Once	281	6.4
	Twice	84	14.3
	Three times	30	20.0

† Systolic BP at or over 140 mmHg, IMT at or over 90th percentile or plaque, and/or reimbursed antihypertensive medication.

Table 11. Proportion of participants with outcomes in adulthood according to times high diastolic BP was observed in childhood (over 90th age-, sex- and year-specific percentiles).

Outcome in adulthood	High diastolic BP observed in childhood	N	Proportion (%) of adults with outcome †
High diastolic BP	Never	1235	14.8
	Once	245	29.0
	Twice	74	44.6
	Three times	14	28.6
High IMT or plaque	Never	1027	15.1
	Once	191	14.1
	Twice	56	19.6
	Three times	10	30.0
Reimbursed antihypertensive medication	Never	1231	3.6
	Once	244	7.0
	Twice	73	20.6
	Three times	14	21.4

† Diastolic BP at or over 90 mmHg, IMT at or over 90th percentile or plaque, and/or reimbursed antihypertensive medication

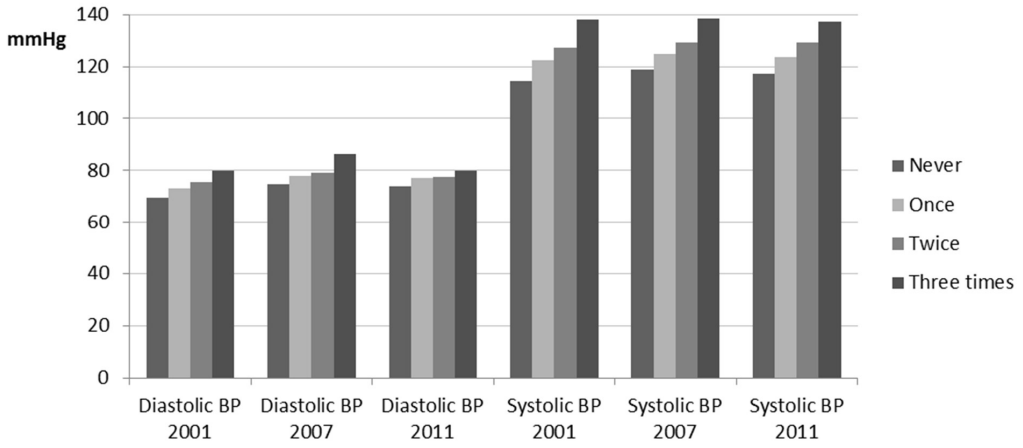


Figure 12. Mean adult diastolic and systolic blood pressures according to the number of times that high diastolic or systolic blood pressure was observed in childhood (over 90th age-, sex- and year-specific percentiles).

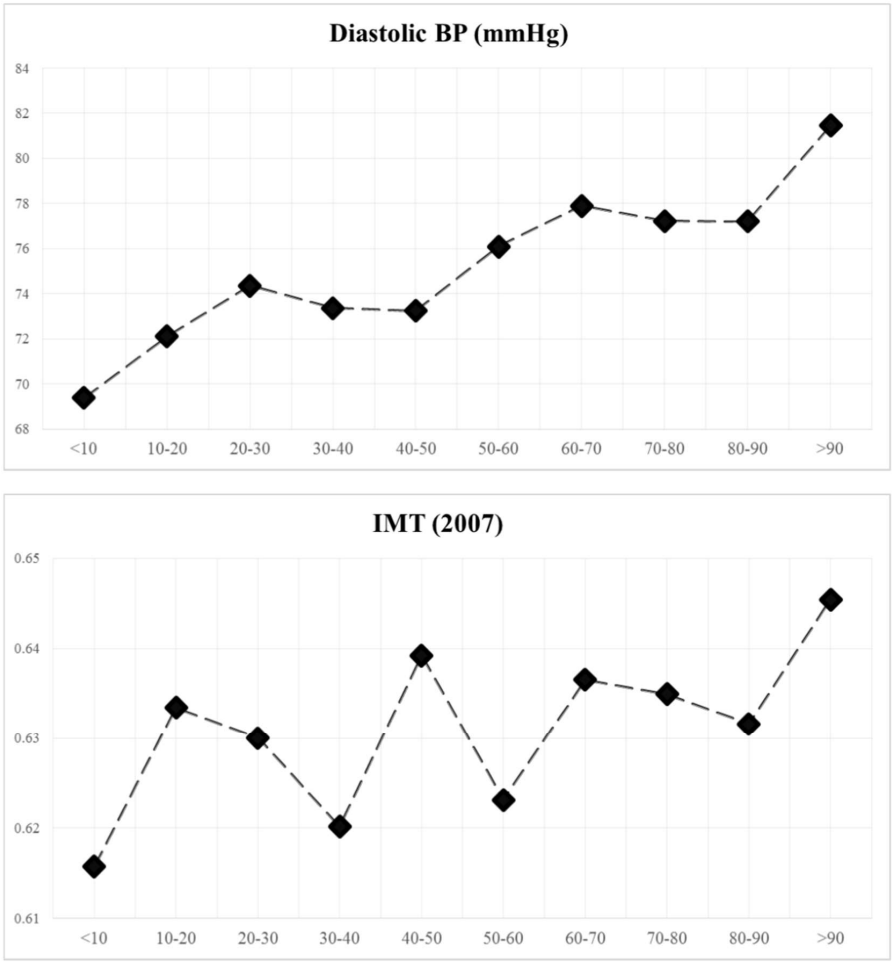


Figure 13. Adulthood diastolic blood pressure and IMT in deciles of diastolic blood pressure in childhood. Adulthood blood pressure measurements were primarily from 2011, but in case of missing data, values from 2007 or 2001 were used.

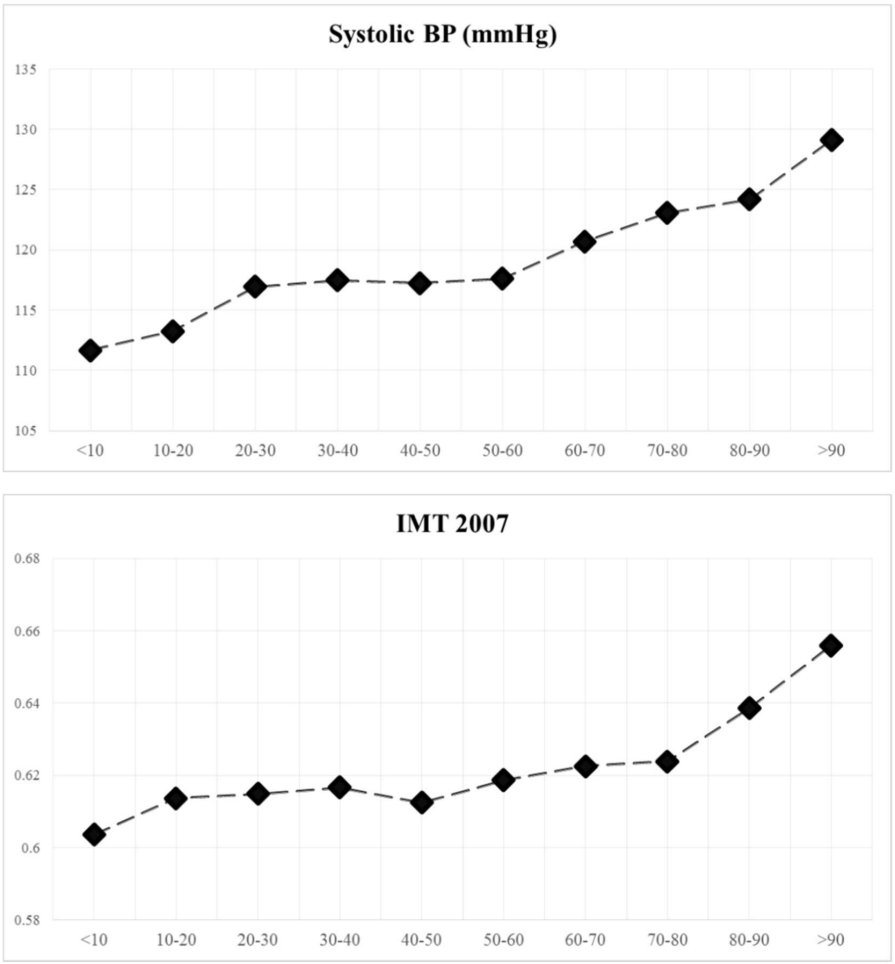


Figure 14. Adulthood systolic blood pressure and IMT in deciles of systolic blood pressure in childhood. Adulthood blood pressure measurements were primarily from 2011, but in case of missing data, values from 2007 or 2001 were used.

5.3.2. Prediction of adult dyslipidemia using repeated measurements of childhood lipid levels

Correlations of lipid levels between childhood and adulthood and the effects of repeated measurements on these correlations are shown in **Table 12 (Study III)**. Pearson correlation

coefficients between childhood and adulthood, indicating tracking of TC values, were between 0.60-0.67 from one to three measurements. The coefficients for LDL-C were 0.64-0.69, for non-HDL-C 0.57-0.64, for HDL-C 0.61-0.67, and for TG 0.34-0.42. Significant improvement in the correlations in all serum lipids was achieved with two childhood measurements compared to one (P<0.05), except in TG where three measurements significantly improved the correlation compared with one measurement (P=0.004). The correlation coefficients for TG (0.49 in men and 0.36 in women) were significantly different between men and women (P=0.001). In other lipids, differences between sexes were not observed (P always >0.05). The coefficients for TC were 0.66 in men and 0.68 in women, for LDL-C 0.68 in men and 0.71 in women, for HDL-C 0.66 in men and 0.62 in women, and for non-HDL-C 0.65 in men and 0.68 in women. Specificity, sensitivity, PPV, NPV, AUC and NRI of childhood measurements predicting adulthood dyslipidemia are shown in original publication of **Study III** in **Table 3.** Significantly improved prediction in AUC-values and in NRI-values of high adult non-HDL-C, LDL-C HDL-C and TG was observed with two childhood measurements (P<0.05). For TC, the improvement in prediction was borderline significant in NRI but not in AUC.

Table 12. Pearson correlation coefficients of lipid levels between childhood and adulthood* by number of childhood measurements in 1,912 participants of the Cardiovascular Risk in Young Finns Study. Modified from Study III.

No. of childhood measurements	Total cholesterol		LDL-cholesterol		HDL-cholesterol		Non-HDL cholesterol		Triglycerides	
	r	P-value‖	r	P-value‖	r	P-value‖	r	P-value‖	r	P-value‖
1†	0.60	ref.	0.64	ref.	0.61	ref.	0.57	ref.	0.34	ref.
2‡	0.65	0.02	0.68	0.03	0.65	0.04	0.62	0.02	0.39	0.11
3§	0.67	0.001	0.69	0.001	0.67	0.004	0.64	0.001	0.42	0.004

All values were transformed into age-, sex- and year-specific Z-scores.
* A mean value of available measurements in 2001, 2007 and 2011.
† 1983, ‡ 1980 and 1986, § 1980 and 1983 and 1986
‖The significance of the difference between two correlation coefficients was calculated using Fisher r-to-z transformation.

Results from additional analyses show that 80% of the participants who had LDL-C >3.5 mmol/L in 1983 had dyslipidemia in adulthood. Meanwhile, 85% of participants who had LDL-C >4 mmol/L in 1983 and 92% of the participants who had LDL-C >4.5 mmol/L in 1983 had dyslipidemia in adulthood.

The prevalence of dyslipidemia in adulthood according to the number of times participants met the at risk levels in childhood is presented in the original publication of **Study III** in **Table 4**. The risk of abnormal lipid levels in adulthood grew incrementally according to the number of times a child had been at risk. Concerning absolute adult lipid levels, participants who had at risk levels on three occasions in childhood had 1.15 mmol/L higher LDL-C in adulthood than those who were never at risk as a child. Similar differences were observed in TC (+1.28 mmol/L), non-HDL-C (+1.25 mmol/L), TG (+1.02 mmol/L) and HDL-C (-0.37 mmol/L) levels ($P<0.0001$ for all) (**Figure 15**). No age-interaction was observed for the association between the number of non-favorable lipid levels in childhood and adult non-HDL-C, HDL-C or triglyceride levels (P always >0.05). AUC-values and 95% confidence intervals (CI) for predicting dyslipidemia in adulthood according to childhood dyslipidemia risk, stratified by age were calculated (data not shown). No major differences were observed between age groups with substantial overlap of CIs.

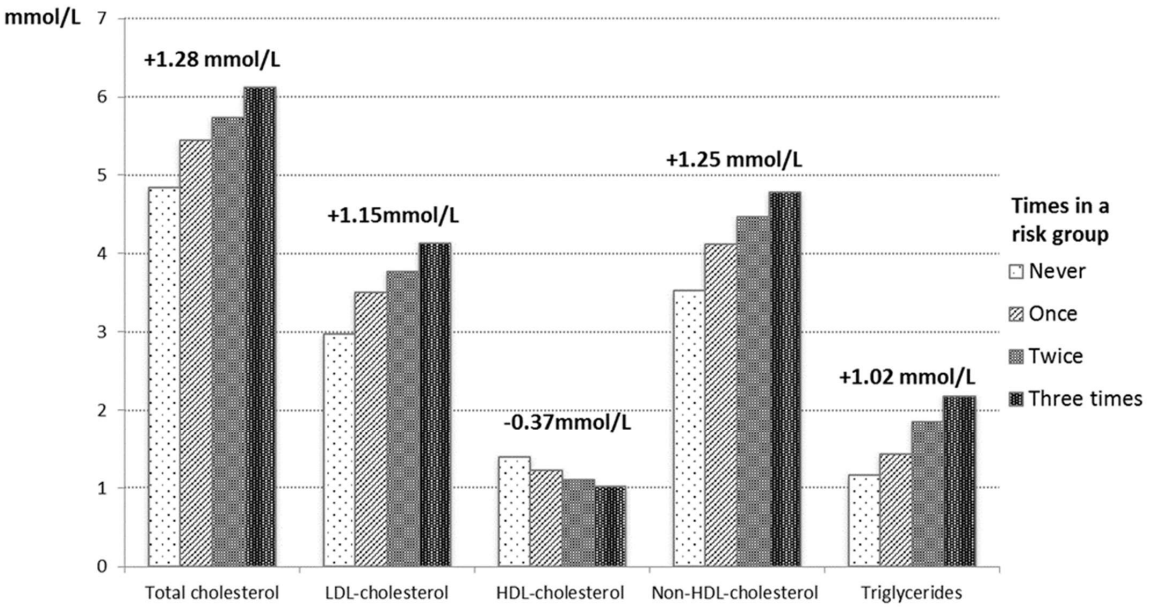


Figure 15. Lipid levels in adulthood (age- and sex-adjusted least squares means) according to times at risk of dyslipidemia defined as age-, sex- and study year-specific Z-scores of total cholesterol, LDL-cholesterol, non-HDL cholesterol or triglycerides >80th percentile, or HDL-cholesterol <20th percentile in childhood (aged 6-21 years) in 1,912 participants of the Cardiovascular Risk in Young Finns Study. P-value for trend was significant (<0.0001) for all lipids for association between times at risk in childhood and lipid levels in adulthood. Adulthood measurements were primarily from 2011 but in case of missing data, values from 2007 or 2001 were used. Absolute lipid values (+/-) indicate the mean difference in lipid level in participants who were three times at risk compared to those who were never at risk in childhood. Reprinted from Atherosclerosis (Study III) with permission from Elsevier.

5.4. Prediction of adult dyslipidemia using genetic and childhood clinical risk factors

Childhood lipid levels and lipid-specific wGRSs were significantly associated with abnormal lipid levels in adulthood (P always <0.001). ORs were respectively 3.82 (3.27 - 4.45) and 1.25 (1.12 - 1.39) for LDL-C, 0.28 (0.24 - 0.33) and 0.80 (0.72 - 0.90) for HDL-C, and 1.85 (1.63 - 2.10) and 1.50 (1.33 - 1.68) for TG. In addition, ApoE genotypes were independently associated (OR 1.20 (1.06-1.36), P < 0.001) with elevated LDL-C levels in adulthood (**Study IV**).

To determine if the effect of the wGRS was similar across all age groups, an age-wGRS interaction term was included in the multivariable models for each lipid. No statistically significant interactions (P always >0.05) were observed. (**Figure 16.)**

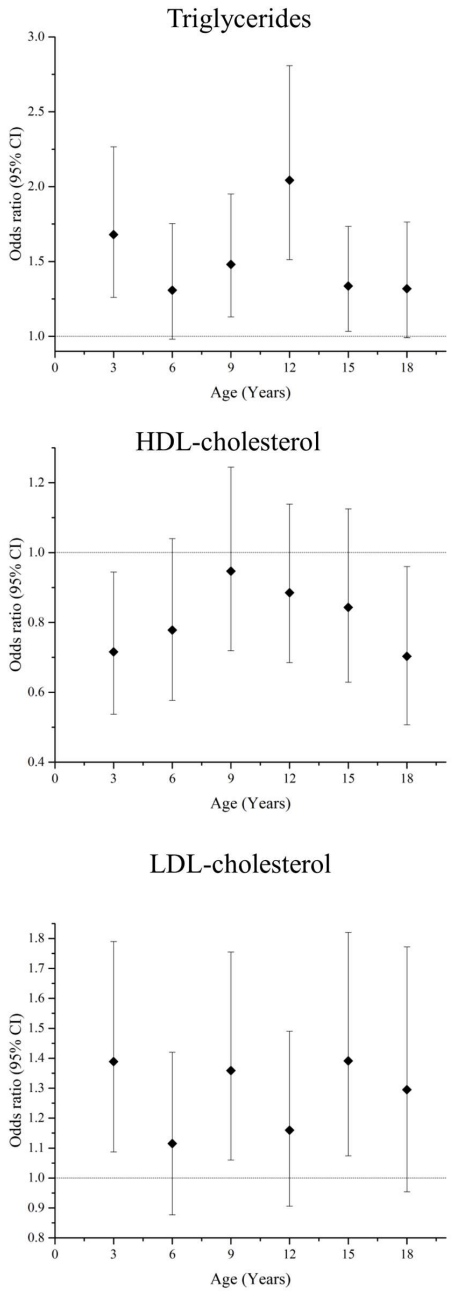


Figure 16. Odds ratio and confidence intervals for the best childhood prediction model (age, sex, childhood lipid level, wGRS) in predicting elevated lipid levels in adulthood for different age groups (3, 6, 9, 12, 15, 18). The age-wGRS interaction term was included in the multivariable models, but statistically significant interactions were not observed. Modified from study IV.

Table 14. Discriminating properties of the pediatric multivariable model for predicting adult dyslipidemia

	(A) Without wGRS			(B) with wGRS			
Adult outcome	AUC (95% CI)	Specificity (%)	Sensitivity (%)	AUC (95% CI)	Specificity (%)	Sensitivity (%)	p-value for difference in AUCs*
LDL-cholesterol over 3 mmol/l or self-reported use of lipid lowering medication	0.806 [0.788 - 0.823]	79.6	66.7	0.811 [0.794 - 0.829]	77.4	69.0	0.01
HDL-cholesterol under 1.2 mmol/l in women or under 1.0 mmol/l in men	0.773 [0.751 - 0.794]	70.2	71.8	0.776 [0.755- 0.798]	72.7	69.5	0.09
Triglycerides over 1.7 mmol/l	0.740 [0.715 - 0.764]	74.6	62.6	0.758 [0.734-0.782]	70.3	69.3	<0.01

wGRS, weighted genetic risk score; AUC, area under the receiver-operating curve

Adjusted for age, sex, childhood BMI z-score, childhood physical activity z-score and smoking status.

LDL-C model additionally adjusted for *APOE* genotype.

*Model with wGRS vs. model without wGRS

Specificity, sensitivity and AUCs for each of the models are shown in **Table 14** (**Study IV**). Adding wGRS to the childhood risk factor model significantly improved the AUC for LDL-C (P=0.01) and TG (P<0.01). For LDL-C, the AUC increased from 0.806 to 0.811, and for TG from 0.740 to 0.758. For HDL-C, the observed improvement with the addition of the wGRS (from 0.773 to 0.776) did not reach statistical significance (P=0.09). When the model without the wGRS and the model with genetic information were compared for LDL-C, the number of false positive adult dyslipidemia cases increased from 194 to 214, whereas the number of false

negatives reduced from 466 to 433 when using the threshold corresponding to the best sum of sensitivity and specificity.

The net percentage of individuals with abnormal lipid levels correctly classified upward (event NRI) was 4.4% for LDL-C, 15% for HDL-C, and 9.8% for TG (**Table 15**) (**Study IV**). Furthermore, the net percentage of individuals without abnormal lipid levels correctly classified downward (non-event NRI) was 17.2% for LDL-C, 6.5% for HDL-C, and 17.6% for TG. These changes resulted in an overall statistically significant improvement in continuous NRI of 0.22 ($p<0.001$) for LDL-C, 0.22 ($P<0.001$) for HDL-C, and 0.29 ($P<0.001$) for TG. Furthermore, the IDI was 0.011 ($p<0.001$) for LDL-C, 0.007 ($p<0.001$) for HDL-C, and 0.022 (<0.001) for TG, indicating that the difference in average predicted risks between the individuals with and without the outcome increased significantly when the lipid-specific wGRS was included in the prediction model

The p-values for the H-L goodness of fit test were not significant (P always >0.05) for all models, indicating no evidence of poor fit.

Table 15. Improvement of reclassification properties of the pediatric adult dyslipidemia prediction models including wGRS compared with models without wGRS.

Adult outcome	Event NRI (95% CI)	Non-event NRI (95% CI)	Overall NRI (95% CI)	IDI (95% CI)	H-L Chi-square
LDL-cholesterol over 3 mmol/l or self-reported use of lipid lowering medication	0.044 (-0.001-0.10)	0.172 (0.11-0.23)	0.22 (0.13-0.30)	0.011 (0.01 - 0.02)	4.01
	p=0.1	p<0.001	p<0.001	p<0.001	p = 0.81
HDL-cholesterol under 1.2 mmol/l in women or under 1.0 mmol/l in men	0.15 (0.07-0.23)	0.065 (0.02 -0.11)	0.22 (0.12 - 0.31)	0.007 (0.003 - 0.01)	9.76
	P<0.001	p<0.001	p<0.001	p<0.001	p=0.28
Triglycerides over 1.7 mmol/l	0.098 (0.01-0.187)	0.176 (0.13-0.22)	0.29 (0.18-0.37)	0.022 (0.02-0.03)	3.36
	p=0.03	p<0.001	p<0.001	p<0.001	p=0.91

wGRS, weighted genetic risk score; NRI, net reclassification index; IDI, integrated discrimination index.

Adjusted for age, sex, baseline BMI z-score, childhood physical activity and smoking status

In order to examine the prevalence of elevated LDL-C levels in adulthood according to childhood LDL-C levels and wGRS, participants were divided into four groups using the 80th percentile of childhood LDL-C levels and the median value of wGRS for LDL-C as the cut-off (**Figure 17**). Of those who had low wGRS and normal childhood LDL-C levels, 45% had high LDL-C in adulthood compared with 92% in those who had both high wGRS and high childhood LDL-C levels.

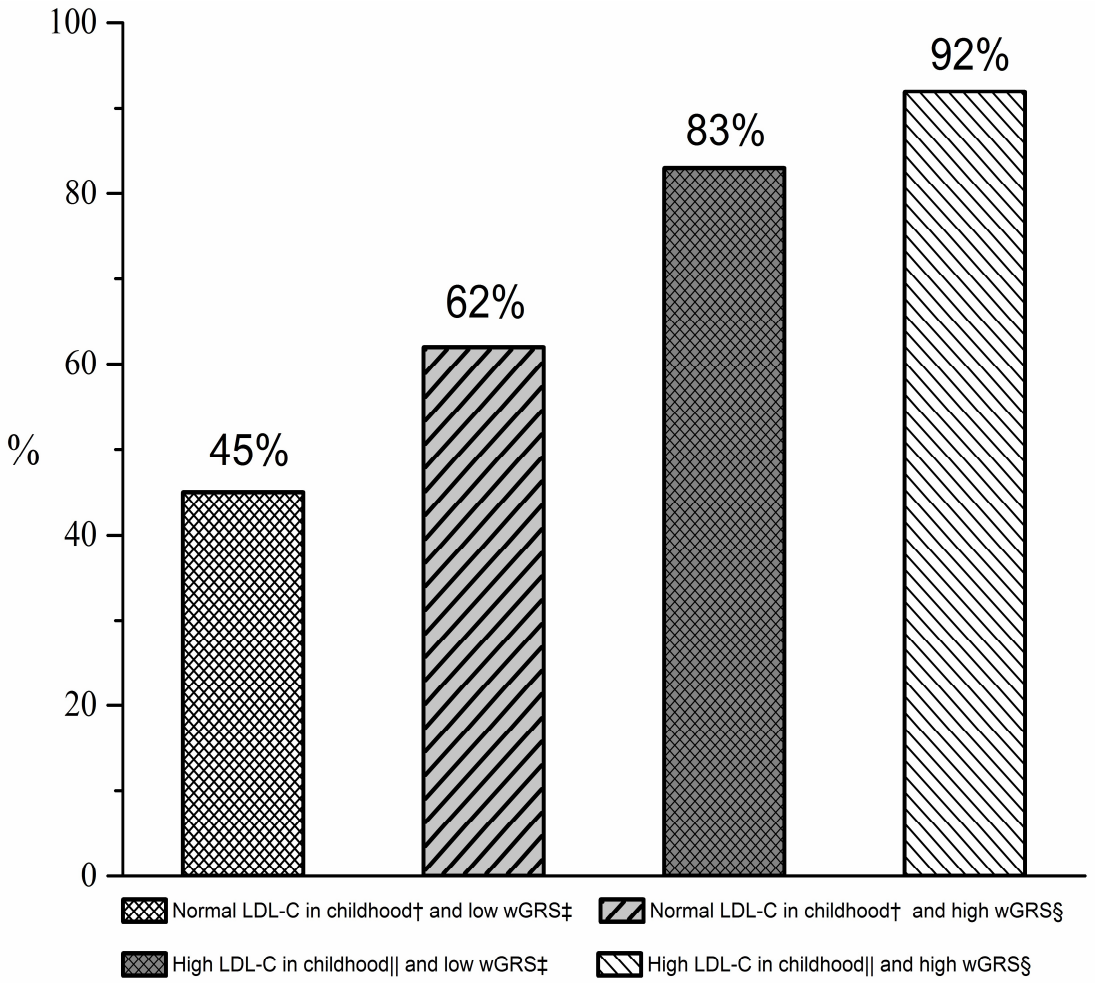


Figure 17. Prevalence of elevated LDL-cholesterol levels in adulthood* according to childhood LDL-cholesterol levels and genetic risk score. Modified from Study IV.

* LDL-cholesterol >3mmol/L or reported use of lipid-lowering medication

† LDL-cholesterol level <80th percentile-point in childhood

‡ wGRS under median value

§ wGRS over median value

|| LDL-cholesterol >80th percentile-point in childhood

6. DISCUSSION

An overview of the main findings from this thesis is shown in **Figure 18**. In the 2011 follow-up, previously observed favorable trends in cholesterol levels have leveled off. In addition, over one-third of the study population had prediabetes (**Study I**). Two lipid and BP measures in childhood significantly improved prediction of adult dyslipidemia and hypertension, incremental value of third measurement was modest (**Study II & III**). Furthermore, the prediction of adult dyslipidemia was significantly improved when taking into account genetic risk scores (**Study IV**).

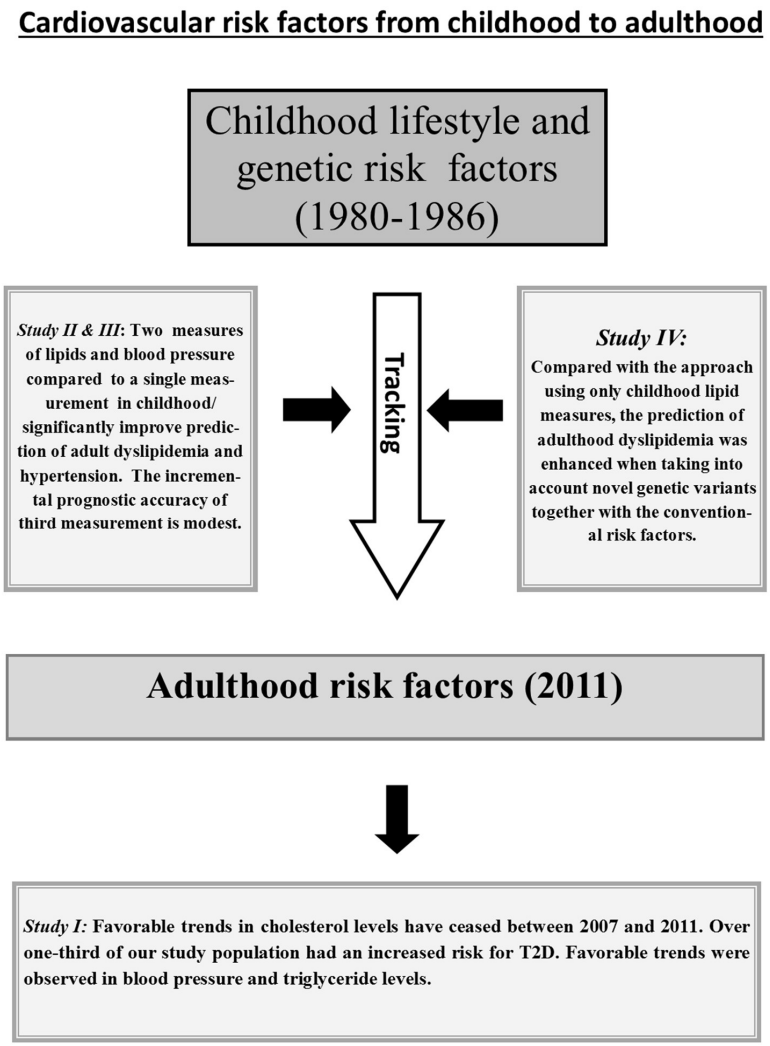


Figure 18. Main findings of this study

6.1. Participants

The participants in this study were from the Young Finns Study; an on-going epidemiological study of CVD risk factors in children and young adults.(Raitakari et al. 2008) The initial sampling in 1980 consisted of 4,320 participants aged 3, 6, 9, 12, 15 and 18 years that were invited to a cross-sectional survey. They were randomly selected from different parts of the country, equally from both genders, and from urban and rural areas. The sampling frame was designed to select a cohort that represented Finnish children and adolescents as closely as possible. A total of 3,596 subjects, 83.2% of those invited, participated in the study in 1980. Participation rate was high in 1980 and replies obtained from the non-participating families revealed no systematic reason for non-participation. Thus, the final sample in 1980 was concluded to be representative of the total random sample.

Of the original study cohort from 1980, a total of 2,283 individuals (63.5%) in 2001, 2,204 individuals (61.3%) in 2007, and 2,063 individuals (57.4%) in 2011 participated. Although a common limitation in longitudinal studies is non-participation at follow-up, particularly when non-participation is differential, the participation of the study group in the follow-up studies has been dynamic. However, male participants were older than non-participants, and female participants had significantly lower BMI than non-participants. Otherwise, no substantial differences in risk factor levels were observed. Thus, the randomly selected study population clearly represented the original study population. Therefore, the results in this study may be generalized to populations consisting of white, apparently healthy individuals. However, several parameters such as geographical area, genetic risk factors and dietary habits could have modulated the outcomes.

6.2. Methods

Because the methods for laboratory measurements including lipid levels are well standardized, the results can be generalized from study to study. All assays were carried out as simultaneously as possible in the laboratory of the Rehabilitation Centre of Social Insurance Institution (Turku, Finland) in 1980, 1983 and 1986, in the laboratory of the Research and Development Unit of the Social Insurance Institution (Turku, Finland) in 2001, and in the laboratory for Population Research of the National Institute for Health and Welfare (Turku, Finland) in 2007 and 2011. Following methods of the laboratory are also accredited by the Finnish Accreditation Service according to standard ISO / IEC17025: TC, HDL-C, and TG,

glucose. The Laboratory of the Rehabilitation Centre of Social Insurance Institution continuously checked cholesterol determinations with the World Health Organization laboratory in Prague. Subsequently, performance of the laboratory methods has been externally evaluated in Labquality's external quality assessment programme and using quality assessment testing provided by National Institute of Standards and Technology. Due to changes in methods or reagents between follow-ups, TC, HDL-C and TG values in 1980, 1983, and 1986, and TG and glucose values in 2007 were adjusted using correction factors determined from linear regression analysis using standardized principal component adjustments. In addition, stability of laboratory methods is suggested by lack of systematic changes in the lipid and glucose levels. Estimated LDL-C values were highly correlated with direct LDL-C values, though estimated LDL-C may have very slightly underestimated the current LDL-C levels of study population when compared to direct measurements. Participants with TG above 4 mmol/L were excluded from the analyses concerning estimated LDL-C as the accuracy of the Friedewald's equation decreases when TG concentrations increase and may lead to misclassification of the subjects. (Marniemi et al. 1995)

Measurement of BMI has remained uniform throughout the study and therefore can be compared from time-point to time-point. Although self-reported questionnaire measures of physical activity and smoking may constitute a potential limitation due to recall or reporting bias, they remain feasible and affordable instruments for monitoring health behaviors, and were measured consistently across follow-ups.

In risk prediction, one of the key questions is how best to assess and quantify the improvement offered by different models. Three statistical measures, AUC, NRI and IDI, to assess the performance of risk prediction models were mainly used. AUC is the probability that given two subjects, one who will develop an event and the other who will not, that the model will assign a higher probability of an event to the former. AUC describes the overall performance of the model in discriminating individuals with and without the outcome, but it is relatively insensitive to change if risk factors with strong associations with the outcome are already included in the initial model. The NRI is a prospective measure which quantifies the correctness of upward and downward reclassification or movement of predicted probabilities between models. The NRI focuses on reclassification tables constructed separately for participants with and without events, and quantifies the correct movement in categories—upwards for events

and downwards for non-events. The improvement in reclassification can be quantified as a sum of differences in proportions of individuals moving up minus the proportion moving down for people who develop events, and the proportion of individuals moving down minus the proportion moving up for people who do not develop events. One potential drawback of the reclassification-based measure defined above is its dependence on the choice of categories. Therefore, it has been suggested that category-less, continuous NRI defined as a measure of event rate increase among those who are reclassified upwards and event rate decrease among those who are reclassified downwards is more objective measure of improvement in risk prediction. The IDI is a summary measure based on the difference in average risks between cases and controls and does not require categories.

6.3. Results

6.3.1. Cardiovascular risk factor levels and secular trends

Since 1970s, levels of cardiovascular risk factors have remarkably improved among Finnish population. (Vartiainen et al. 2000, Borodulin et al. 2015) These changes have resulted in a significant reduction in CHD risk. Recent results from the FINRISK study have shown that CHD mortality in Finland decreased over 80% in men and women from 1969-1972 to 2012.(Jousilahti et al. 2016) This reduction in CHD mortality has been mostly driven by favorable alterations in dietary habits and life style resulting in a decrease in classical CVD risk factor levels, although treatment protocols and secondary prevention have also advanced in the last decades.(Valsta et al. 2010, Salomaa et al. 2016)

This study shows that favorable trends in cholesterol levels ceased between 2007 and 2011 among the Young Finns Study cohort. Our results are in line with the findings from the Swedish Västerbotten County population, where blood cholesterol levels have increased among men and women from 2007 after an initial decrease since 1990. (Ng et al. 2012) Furthermore, the results of the FINRISK survey in 2012 among 7921 Finnish men and women aged 25-74 years suggest that serum cholesterol levels have begun to rise after decades of favorable development. (Borodulin et al. 2015, Vartiainen et al. 2012) TC levels were above 5.0 mmol/L in 62.9% of male participants, and 47.3% of female participants aged 35 to 44 years. In line with the FINRISK results, TC was above 5.0 mmol/L in 57.3% of men and 46.3% of women in this thesis (**Study I**). There is a possibility that popular low-carbohydrate diets and controversial information in television and social media may have impacted adherence to the

national nutrition recommendations as the overall consumption of saturated fat (butter) has increased and the consumption of skim milk has decreased in Finland since 2009. (Helakorpi et al. 2012) Earlier studies suggest that lowering HDL-C levels normally results in rising triglyceride levels. (Brewer 1999) In this study a non-significant decline in HDL-C levels as well as a significant decrease TG levels were observed, with no plausible explanation apparent. Even though, only speculation is possible on the reasons for this observation; one putative explanation could be an altered diet. However, results from an earlier study suggest that increased use of butter would result in an increase in TC, LDL-C and HDL-C levels. (Engel, Tholstrup 2015) In this thesis no significant increases were observed for TC or LDL-C levels, as well as HDL-C levels decreased non-significantly. Furthermore, alcohol consumption is associated with blood lipid concentrations. Generally, moderate ethanol intake tends to raise HDL-C and TG concentrations. (Brinton 2010) In Finland, consumption of pure ethanol decreased from 10.5 l / capita to 10.1 l / capita between years 2007 and 2011. (Virtanen et al. 2008, Varis, Virtanen 2012) This may have partially influenced the decrease in HDL-C and TG levels observed in this study.

Decrease in age-adjusted BP levels has discontinued in Finnish adult population according to results from the FINRISK study in 2012. (Laatikainen et al. 2013) Nevertheless, both SBP and DBP decreased (P always <0.0001) during 4- year follow-up in this study. SBP decreased 3.6% in men and 3.1% in women, and DBP decreased 3.3% in men and 3.4% in women. When participants (n=201, 10.1% of the study population) who were using antihypertensive medication were excluded, the trends were essentially similar (P always <0.0001). A decrease was observed in the prevalence of MetS in this thesis mediated by decreases in BP levels and in TG levels in both sexes. However, these observed decreasing trends in BP levels must be interpreted cautiously as participants who did not participate in the 2011 follow-up study but had earlier participated in the 2007 follow-up study, had higher BP levels and smoking prevalence in 2007 than subjects who had participated in both of the 2011 and 2007 follow-up studies. This loss-to follow-up may have caused a selection bias in the study population.

Despite increasing BMI among Finns, BP levels of all age groups have decreased over the last 30 years, even though they remain still high. (Viikari et al. 2006) The decreases in BP levels can be partly explained by alteration of diet in the Finnish population, especially a reduced sodium intake, increased intake of fruits and vegetables and unsaturated fats, and a decreased

intake of saturated fats.(Laatikainen et al. 2006, Pietinen et al. 1996) Obese individuals consume more salt, and due to hyperinsulinemia, the obese may also be more salt sensitive than leaner persons. (Kotsis et al. 2010) Furthermore, alcohol consumption is significantly related to BP levels. (Marmot et al. 1994) Total consumption of pure ethanol has decreased between year 2007 (10.5 litres per capita) and year 2011 (10.1 litres per capita) which may have beneficially impacted the BP levels.(Virtanen et al. 2008, Varis, Virtanen 2012)

Prevalence of obesity has increased in epidemic manner worldwide for decades(Lobstein et al. 2004, Ahluwalia et al. 2015, Ogden et al. 2014) and approximately 20% of the Finnish adults are obese (BMI >30).(Lahti-Koski et al. 2010) Results from FINRISK 2012 show that prevalence of obesity has not increased between 2007 and 2012 in the Finnish working-age population, though the prevalence of obesity has remained consistently high.(Männistö et al. 2015) Similar trends have been observed worldwide, especially in individuals with high socioeconomic status.(Ogden et al. 2014) No remarkable changes in BMI were observed in this study. Nevertheless, increase in waist circumference was observed among both sexes, though the increase was not significant in men. Earlier studies suggest that increased waist circumference is associated with higher incidence of CVD (Flint et al. 2010) and T2D. (Siren et al. 2012)

In a 9-year period between 1992 and 2001, incidence of T2D has increased by average of 4.3% per year in Finnish population.(Lammi et al. 2008) In 2007, prevalence of T2D was 2.4% in a Finnish population aged 25 to 44 years (Pajunen et al. 2012). In this thesis (**Study I**), 4.1% of the participants had T2D. Furthermore, results show that over one-third of the study population had an increased risk for T2D, as indicated by the presence of prediabetes. In addition, very high T2D risk was observed in 4% of the study population (HbA1c between 6.0% (42 mmol/mol) and 6.5% (48 mmol/mol)). (Shimazaki et al. 2007, Andersson et al. 2012)

Results from an earlier study that was conducted in adults aged between 25 and 54 years suggest that high CVD risk was almost as common in women compared to men, but women received less often preventive medications compared to men at similar age. (Lehto et al. 2012) Significant differences between men and women aged 34-49 years were observed for all risk factor levels except T2D in this thesis.

6.3.2. Tracking of blood pressure levels and prediction of adult hypertension and abnormal intima-media thickness

The tracking of BP from childhood to adulthood has many important public health implications. As growing evidence indicates, hypertension, one of the major modifiable risk factors for CVD, is established early in life with approximately 10% of elevated BP in adulthood attributable to elevated BP in childhood.(Kelly et al. 2015)

Previous meta-regression analysis demonstrated moderate tracking from early life to adulthood for SBP (average r-value 0.38) and for DBP (average r-value 0.28).(Chen, Wang 2008) Moderate tracking was also observed in this thesis as correlation coefficients were 0.35-0.46 for SBP and 0.17-0.35 for DBP (**Study II**). These results are also consistent with results from the Bogalusa Heart Study, where the value of repeated BP measurements in prediction of later BP status was shown in 1,501 children. A remarkable correlation between baseline BP levels and BP levels after an 8-year follow-up was observed, and with three serial measures in the upper BP quartile at baseline resulted in significant increase of the probability of being in the uppermost quartile in the 8-year follow-up.(Shear et al. 1986)

Earlier studies from the Young Finns Study (Raitakari et al. 2003, Juhola et al. 2013) and other studies (Davis et al. 2001, Vos et al. 2003) have previously demonstrated that SBP and DBP measured in childhood are independent risk factors for elevated IMT in adulthood. In this thesis, multiple childhood BP measurements did not improve the prediction of abnormal adult carotid IMT when compared to a single BP measurement.

Observations from epidemiological studies show that BP levels have been increasing in children and adolescents for two decades worldwide which can be partially explained by increasing prevalence of overweight and obesity (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents 2004). Results from a recent report show that although the increasing trends of obesity have ceased in some countries, youth overweight and obesity among 11–15-year-olds remains high in Europe and North America. (Ahluwalia et al. 2015) Childhood overweight tracks strongly into adulthood, and thus a concurrent rise in BP levels can be expected to continue in the younger age groups. Results from this thesis suggest that repeated monitoring of BP in childhood and youth can improve early identification of children and adolescents who have the highest risk of

developing hypertension outcomes by adulthood, which may have implications in targeting clinical prevention strategies.

6.3.3. Tracking of serum lipids and prediction of adult dyslipidemia

The tracking of serum lipids in children and young adolescents is crucial for the assessment of childhood preventive strategies of adult CHD. Earlier studies from this and other cohorts have mainly examined lipid tracking and prediction between single measurements in childhood and adulthood. These prior reports have demonstrated that serum lipid and lipoprotein levels in childhood track strongly into adulthood (Srinivasan et al. 2006, Porkka et al. 1994, Magnussen et al. 2011, Webber et al. 1991). Results from Bogalusa Heart Study have shown that 38.5% of the participants who ranked in the top quintile in childhood remained in the top quintile also in adulthood over 27-year follow-up period and 66% maintained their high ranks by being above 60th percentile.(Srinivasan et al. 2006) Results also suggest that participants who had non-HDL-C ≥3.7mmol/L as a child had significantly increased prevalence of obesity and higher LDL-C and TG levels in adulthood than participants who had non-HDL-C ≤3.2mmol/L in childhood. (Srinivasan et al. 2006) High childhood non-HDL-C was also associated with increased prevalence of hyperglycemia, hyperinsulinemia and low HDL-C levels in adulthood. (Srinivasan et al. 2006) The impact of two versus one measurement in childhood on prediction of lipid levels in adulthood has been considered by three prior studies. In the 12-year follow-up of the Young Finns study, use of two individual childhood lipid measurements increased the amount of adult lipid variability explained by up to 50 % (Porkka et al. 1994), while the prediction of adult dyslipidemia was considerably improved by multiple measurements of LDL-C in the 15-year follow-up of the Bogalusa Heart Study (Bao et al. 1996). Even though, the prediction of adult dyslipidemia would be improved by multiple child or adolescent lipid measurements, results by Lauer et al. (Lauer, Clarke 1990) still suggested that 25% to 45% of children with TC levels greater than the 90th percentile on two successive occasions did not meet the criteria for intervention suggested by NCEP ATPIII (Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. The Expert Panel. 1988) when they reached adulthood. Furthermore, results from the Muscatine Study suggested that the accuracy of prediction of clinically significantly elevated adult cholesterol using three measurements was not significantly improved when compared to approach using two childhood lipid

measurements.(Mahoney et al. 1991) Findings from the present thesis (**Study III**) are consistent with these earlier results but extend them to a longer period of follow-up, consider the current National Heart, Lung, and Blood Institute guidelines for universal screening of lipid and lipoprotein levels twice during childhood and early adulthood, and compare any apparent benefits of a third measurement. These results also demonstrate that elevated lipid levels in childhood have a relatively high chance of predicting dyslipidemia in adulthood. On the other hand, a lack of childhood dyslipidemia does not necessarily exclude development of dyslipidemia in later life. Finally, even though multiple childhood lipid measurements increase the accuracy, even after three measurements the incremental prognostic accuracy is still quite modest as demonstrated by the AUCs both by cut-points (0.62 for LDL-C, 0.66 for HDL-C, and 0.61 for TG) and quintiles (0.71 for LDL-C, 0.68 for HDL-C, and 0.63 for TG).

Prior reports from the Young Finns Study have shown moderate 27-year tracking (r-value for LDL-C 0.50-0.52, for HDL-C 0.46-0.51 and for TG 0.27-0.30) for lipids and lipoproteins, with the correlation coefficients not considerably affected by baseline age or gender. (Juhola et al. 2011) Hence, in this study the focus was on examining whether multiple measurements in childhood would improve the prediction of adult dyslipidemia. For all of the serum lipids, significant improvements in the correlation coefficients, indicating better tracking between childhood and adulthood, were observed with two or three childhood measurements. Significant improvement was achieved with two measurements compared to one ($P<0.05$) in TC, LDL-C, HDL-C and non-HDL-C. Furthermore, for TG, the improvement became significant ($P=0.004$) with three measurements. The prediction of lipid-specific adult dyslipidemia was significantly enhanced for non-HDL-C, LDL-C, HDL-C and TG with two measurements ($P<0.05$ for difference in AUC). In line with these results, the Lipid Research Follow-up study (comprising 2170 white men over 30 years of age) has earlier suggested that single cholesterol measurement may underestimate the risk of CHD because of within-person variability, and that a mean of two or three measurements is also subject to individual variation but the bias is less than with a single measurement (Davis et al. 1990).

In December 2011, the Expert Panel published its Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute 2011). These guidelines concluded that non-fasting non-HDL-C

levels should be measured first at the age of 9-11 years, and again at the age of 17-21 years concerning universal screening. Universal screening recommendation of blood lipids has been widely debated after publication and the adherence to the guidelines has remained low. (Valle et al. 2015) It has been acknowledged that universal screening would identify children with abnormal lipid patterns, approximately every fifth child. (Benuck 2015) Potentially also other family members who may not be aware of their own risk could be identified through reverse cascade as familial hypercholesterolemia is still widely underdiagnosed and undertreated. (Nordestgaard et al. 2013, Benuck 2015) Even though, a lack of long-term studies considering effectiveness, costs and potential harm of the universal lipid screening, and safety of lipid-lowering medication in children has been emphasized by experts. (Gillman, Daniels 2012, Mitka 2012, Newman et al. 2012, Uy, Agawu 2013) In Finland, no universal screening of blood lipids has been recommended in the pediatric primary prevention of CVD.

Results from this thesis are consistent with these recommendations as a single childhood measurement seems not to be sufficient enough for an accurate prediction of adult dyslipidemia. The tracking of adult lipids and the prediction of dyslipidemia were both enhanced when a second or third measurement was taken into account. Based on the overall NRI findings from this study, two or three measurements showed significantly improved reclassification of individuals compared with a single measurement across the observed lipid measures. Even though no direct comparison was made for two versus three measurements, these results show that most of the incremental predictive value over a single measurement was observed when two childhood lipid measurements were used. This differed depending on the lipid or lipoprotein studied. For example, for TC, non-HDL-C, or LDL-C, approximately 80% with elevated lipid levels at two time-points in childhood maintained these into adulthood with the equivalent for HDL-C and TG much lower with 53% and 35% respectively. Furthermore, even with a third measurement, approximately 30-50% still did not maintain low HDL-C or high triglyceride levels into later adulthood. These results call into question the relative utility of HDL-C and triglyceride levels in later prediction of adult levels, which was highlighted by pooled analyses among three prospective cohorts (Magnussen et al. 2008) and may be a result of these lipid levels being more amenable to environmental factors, such as lifestyle changes. The importance of environmental factors was emphasized by earlier results from Childhood Determinants of Adult Health study demonstrating that weight management, physical activity, not smoking, and improvement in socioeconomic status had impact on maintaining normal

blood lipid levels from childhood to adulthood. (Magnussen et al. 2011) However, determination of HDL-C is necessary in order to calculate non-HDL-C, and TG levels are needed to estimate LDL-C levels.

Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents concluded that measurement of apolipoprotein B and apolipoprotein A-1 would not provide any additional advantage over measurement of conventional lipids measures. (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute 2011) Apolipoprotein B measurement reflects the number of atherosclerotic particles circulating, meanwhile conventional cholesterol measurements only take into account the total mass of cholesterol circulating. (Marcovina, Packard 2006) For example, presence of T2D and obesity in patient are known to increase the amount of small dense low-density lipoprotein particles, significantly increasing the amount of atherogenic apolipoprotein B-containing particles, meanwhile LDL-C levels may remain normal. (Kovanen, Jauhiainen 2015) In addition, apolipoprotein A-1-containing particles are known to mediate the reverse cholesterol transport. (Marcovina, Packard 2006) Prior report from the Young Finns study demonstrated that apolipoprotein B and apolipoprotein A1 determined in adolescence were consistently superior to conventional lipid measures in predicting abnormal carotid IMT in adulthood. (Juonala et al. 2008) In adults, apolipoproteins B and A1 have been demonstrated to be superior to conventional lipid measures, especially in patients with metabolic disorders. Still, clinical assessment of cardiovascular risk could also be simplified by using non-fasting non-HDL-C measurements, which reflects cholesterol content in all of the atherogenic apolipoprotein B-containing particles and has been demonstrated to be superior to LDL-C in predicting CVD risk. (Sniderman et al. 2011, Pencina et al. 2015, Raitakari et al. 2013, Emerging Risk Factors Collaboration et al. 2009) Implementation of non-HDL-C and apolipoproteins into clinical practice has advanced slowly and remains a remarkable challenge for clinicians.

6.3.4. Effect of weighted genetic risk scores to adult dyslipidemia prediction

Whether recently discovered novel genetic variants can enhance and sharpen the CVD risk prediction in early life is a clinically relevant question that can be addressed using data from longitudinal studies extending throughout the life-course from childhood to adulthood. In this thesis (**Study IV**), the results suggest that the prediction of adult dyslipidemia can be

significantly enhanced by including lipid-specific genetic risk scores into risk prediction models when compared with prediction models consisting of only the corresponding lipid level in childhood, age, sex and childhood BMI, smoking status in childhood and young adolescence, and physical activity in childhood. However, the improvement observed was modest. Because two measures were found to be sufficient earlier in this thesis (**Study III**) and current pediatric guidelines recommend universal lipid screening with two lipid measures, the mean of two child lipid measures was used. (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute 2011)

Genetic risk scores can add incremental prognostic value to traditional childhood risk factors as their measurement is unequivocal, they remain unchanged during lifetime, and are not confounded by other lifestyle-related risk factors. Earlier results from the Young Finns Study showed that lipid-specific genetic risk scores that considered 95 loci previously found to be associated with lipid levels did not significantly enhance the prediction of adult dyslipidemia over clinical lipid measurements for all but TG. (Teslovich et al. 2010, Tikkanen et al. 2011) In this thesis (**Study IV**), using wGRSs based on 157 loci including also 62 novel loci, the results showed that the lipid-specific wGRSs significantly improved prediction of adult dyslipidemia over clinical childhood risk factors, although improvements in prediction accuracy were modest. Furthermore, earlier study from the Young Finns Study suggested that GWAS-derived genetic risk scores are associated with average lipoprotein levels at all ages, with participants in the lower wGRS quartiles tending to have average lipoprotein concentrations 30 to 45% lower than those in the upper-quartile wGRS beginning at age 3 years and continuing through to age 49 years. (Buscot et al. 2016) Results in this thesis are consistent with these earlier findings showing that there were no major differences between child and adolescent age groups from age 3 to 18 years suggesting the genetic risk score predicts adult dyslipidemia throughout childhood.

AUC, NRI and IDI were used to compare the discrimination properties of different risk prediction models. The AUC values are used to demonstrate the overall performance of the model in discriminating individuals with and without the outcome. Nevertheless, AUC testing is known to be relatively insensitive to change if risk factors with strong associations with the outcome are already included in the initial model. In this study, the increase in AUCs was statistically significant for LDL-C and TG. For LDL-C, the genetic model identified 33 less

false negative cases compared with the model without lipid specific wGRS. On the other hand, there were 20 more false positive cases when the model including wGRS was compared with the model consisting only of clinical childhood risk factors. Even though, the decrease in the number of false negative cases was greater than the increase in the amount of false positive cases resulting in an overall improvement in the prediction accuracy.

For LDL-C and TG, the improvement in the overall NRI was mostly accountable to the non-event NRI indicating that the wGRS correctly decreased the risk estimates for non-events. On the other hand, for HDL-C, the changes were more dominant in event NRI suggesting that the wGRS correctly reclassified the participants with dyslipidemia as adults. The IDI was statistically significant for all models indicating that the difference in average predicted risks between the individuals with and without the outcome increased significantly when the wGRS was included in the models.

Furthermore, data in this thesis suggest that having low genetic risk for dyslipidemia and normal lipid phenotype as a child does not necessarily exclude development of dyslipidemia in later life, as 45 % of participants with low genetic risk and normal LDL-C levels as a child had elevated LDL-C in adulthood. Meanwhile, participants who had elevated LDL-C levels as a child and low genetic risk score had a higher risk for adult dyslipidemia (83%) than participants with high genetic risk but low LDL-C levels (62%). Nevertheless, individuals with both elevated LDL-C levels in childhood and high genetic risk had clearly highest risk for adult dyslipidemia, as 92% of these individuals had elevated LDL-C levels in adulthood.

6.4. Clinical implications

Atherosclerotic CVD is a multifactorial process associated with numerous risk factors, beginning in childhood, and developing throughout the lifespan leading ultimately to complications, such as myocardial infarction and stroke, which are leading causes of death and morbidity in the world.(Perk et al. 2012) This thesis offers observations from a wide sample of free-living Finnish adults followed throughout life and provides insight into development and prediction of atherosclerosis and its' risk factors from childhood to adulthood.

This thesis suggests that the previously observed, favorable trends in cholesterol levels have leveled off for the first time in decades (**Study I**). In addition, over one third of the study

population had an increased risk for T2D. According to these observations, continuous efforts and interventions are still needed in fighting against cardiovascular risk factors.

When a single observation was compared to two or three measurements of abnormal BP in childhood and adolescence, the prediction of adult hypertension was significantly enhanced. Participants with elevated BP measured at three different time points as a child or adolescent had 20 mmHg higher SBP and 6 mmHg higher DBP in adulthood compared with participants who did not have elevated BP in childhood (**Study II**). Based on prior findings, it has been estimated that each increment of 20/10 mmHg in BP levels, commencing at 115/75 mmHg, is associated with more than a twofold difference in the death rates from CHD, stroke, and other vascular causes. (Lewington et al. 2002) These results are relevant for implementing pediatric primary prevention. From a clinical point of view, it is established that multiple measures of BP in childhood, taken over several weeks or months in routine clinical practice, improve prediction over a single measurement. However, this study was able to show that multiple measures taken over several years are able to significantly improve prediction of adult hypertension. In clinical practice, much more than 3 values are usually required to confirm whether a young individual should be placed on antihypertensive treatment. Hypertension may be undertreated in young adults all over the world. In order to diagnose hypertension, Finnish Current Care guidelines recommend use of average of four pair measurements performed in clinical setting or average of pair measurements performed at home in the morning and in the evening from four to seven days. (Working group set up by the Finnish Medical Society Duodecim and the Finnish Hypertension Society. 2014) Earlier study also suggested that young adults with continuously elevated BP levels have slower initiation rates of antihypertensive medication when compared middle-aged and older adults which may result in undertreatment of hypertension and more rapid progression of vascular changes related to atherosclerosis in young adults. (Johnson et al. 2014)

Concerning the clinical impact of multiple childhood lipid measurements, a remarkable increase in the prevalence of adult dyslipidemia was observed when the number of times a participant had abnormal lipid levels as a child increased (**Study III**). For non-HDL-C, of those participants with high non-HDL-C levels at three time-points in early life, approximately 90% had elevated non-HDL-C levels in later life as adults, whereas only ~35% of those having all three non-HDL-C measures within the normal range had high non-HDL-C levels in adulthood.

When evaluating how these results from repeated childhood lipid measurements translate into the clinical cardiovascular risk, results of genetic studies and randomized clinical trials can be utilized. Analyses by Ference et al.(Ference et al. 2012)suggested that a 1.0 mmol/L lower LDL-C level would be associated with a 24% (statin trial data) to 54% (genetic studies) reduction in prevalence of CHD. In this thesis, participants with all childhood measures within the normal range had 1.15 mmol/L lower adult LDL-C levels compared with those who had high lipid levels in all three childhood time points. Extending from the estimates of Ference et al. this difference could be converted to a 30-60% difference in CHD risk later in life (Ference et al. 2012).

For other cardiovascular risk factors, such as adult hypertension (Juhola et al. 2012) and T2D (Pitkänen et al. 2016), earlier findings from the Young Finns Study have shown that use of genetic risk scores provides incremental predictive information in addition to clinical childhood risk factors. In the future, which is headed more to a direction of personalized medicine, novel genetic variants may also help to identify children with high risk for adult dyslipidemia early in life. Results from this thesis are consistent with this hypothesis by demonstrating that genetic information provide incremental information over clinical risk factors in prediction of adult dyslipidemia in childhood (**Study IV**). As atherosclerosis has its roots in childhood, early identification and treatment of dyslipidemia throughout the life span would reduce the risk for later clinical CVD. Earlier findings from the Special Turku Coronary Risk Factor Intervention Project suggest that lower lipid levels in childhood could be achieved by repeated dietary counseling, as the intervention group had significantly lower lipid levels than the control group. (Niinikoski et al. 2012)

6.5. Strengths and limitations

A common limitation in longitudinal studies is non-participation at follow-up. Because baseline risk factor levels were mostly similar among participants and non-participants and the study group has been dynamic, the present study population was probably representative of the original population. However, loss to follow-up was greater in cigarette smokers who also had higher SBP and DBP in the 2007 and 2011 follow-ups in both sexes, which might have differentially affected secular trend analyses in BP levels and the prevalences of smoking and the MetS. To minimize the bias caused by non-participation, only participants who had

participated in both follow-ups in 2007 and 2011 were included in the analyses concerning change in cardiovascular risk factors between 2007 and 2011.

Glucose concentrations were determined from serum samples, although the established diagnostic thresholds have been developed for plasma glucose.(American Diabetes Association 2013) The glucose values determined from serum have been shown to be 1.15% lower than corresponding values determined from plasma which may have resulted in a minor downward bias.(Frank et al. 2012) Although diagnosis of T2D using fasting glucose and HbA1c should be based on repeated measurements to rule out the laboratory error, this was not possible in this thesis. However, a group of participants (15% of the diagnoses) had both their HbA1c and fasting glucose over the diagnostic thresholds which confirms the diagnosis without repeated blood tests. Furthermore, findings from prior studies have suggested that measurement of fasting glucose concentration is not sufficient enough to detect abnormal glucose regulation in all patients as more than half of the patients had elevated 2-hour plasma glucose values even though they had normal fasting glucose. (Saaristo et al. 2008) Therefore, oral glucose tolerance test is recommended for patients with elevated risk for T2D. Oral glucose tolerance tests were not performed in Young Finns Study and this may have resulted in an underestimation of T2D prevalence in the study population.

BP was measured in 1980 and 1983 with a standard mercury sphygmomanometer and in 1986, 2001, 2007, and 2011 with a random-zero sphygmomanometer. Sphygmomanometer was not used in the youngest age group at baseline, instead only SBP was measured using ultrasound device. The use of random-zero sphygmomanometers may have resulted in a downward bias in BP levels. (Yang et al. 2008) Therefore, the prevalence of hypertension and MetS may be lower in this study than in similar studies. Nevertheless, all BP measurements were performed similarly in a sitting position, after a 5-minute rest from the right arm and an average of three measurements was used.

In addition, follow-up examinations in the Young Finns Study were originally designed to maintain three-year differences between the age groups.(Raitakari et al. 2008) However, the follow-up in 2011 was performed 4 years after the 2007 follow-up, so that studying 4-year changes between 2007 and 2011 was only possible by merging age groups to achieve approximately similar average ages.

A decreasing trend in lipid levels has been previously observed between the study years 1980 to 1986 in the Young Finns Study.(Porkka et al. 1997) Hence, age-, sex- and study-year specific cut-points were used, because a uniform cut-point would have caused bias when there is already a decreasing trend present in lipid levels. Another limitation is that only a single measurement in adulthood was used as an outcome as no repeated lipid measurements were available at a single time point. There is a possibility that reduced tracking of lipid levels may be due to changes in lifestyle habits between childhood and adulthood. (Magnussen et al. 2011) Findings from this thesis support an emphasis on lifestyle intervention after the first observation of abnormal lipids in childhood and young adulthood, when there is a chance to encourage more children and young adults to adopt healthier lifestyles. TG and HDL-C levels in particular appear to vary the most from childhood to adulthood, which may lead to the conclusion that worsening lifestyle, weight gain, and metabolic derangement from childhood to adulthood are especially important to address already early in life. Nevertheless, this would probably not affect comparisons differentially regarding the number of child lipid measurements used to predict adult dyslipidemia.

The National Heart, Lung, and Blood Institute guidelines call for confirmation of an at-risk lipid value by repeated fasting lipid profile. In this study, it was not possible to consider a second reading at each of the time-points because data was lacking. Given the biological variability of lipid measurements, it is likely that unavailability of these data may have led to an underestimation of the true predictiveness that could be achieved if the National Heart, Lung, and Blood Institute guidelines were followed completely. Furthermore, there were no clinical end-points available because the participants of the study are still relatively young and progression of atherosclerosis into a clinical condition endures usually for decades. However, prior reports have demonstrated that dyslipidemias associate with adult surrogate markers (Juonala et al. 2008, Magnussen et al. 2009) of atherosclerosis that have been shown to predict cardiovascular events in the future. (Lorenz et al. 2007).

6.6. Future research directions

6.6.1. Linkage with clinical cardiovascular endpoints

Although there is strong evidence for the relationship between CVD risk factor levels and surrogate markers of atherosclerosis, and between surrogate markers of atherosclerosis and

cardiovascular events, the ability to study associations between child risk factors levels and clinical cardiovascular events would lead to an even better understanding of the disease process. As the Cardiovascular Risk in Young Finns study population ages, clinical end points will accumulate to a level that will allow sufficient statistical power to examine these as end-points. Because of the regular follow-ups, this would allow different risk factors and markers of atherosclerosis throughout the life span that lead to clinical cardiovascular events.

6.6.2. Ongoing monitoring and intervention of cardiovascular risk factor levels among Finnish adults

Results from this thesis suggest that previously observed, favorable trends in cholesterol levels have leveled off and over one third of the Finnish population has an increased risk for T2D. Because CVD remains the leading cause of mortality in Finland, these findings highlight the need for ongoing monitoring and intervening of cardiovascular risk factor levels among Finnish adults.

6.6.3. Replication of clinical utility of genetic risk scores in pediatric setting for prediction of future dyslipidemia

Current results suggest that when genetic information is added to childhood risk factor data, there is an improvement in the predictive utility of risk models. Nevertheless, replication of this finding in other longitudinal cohorts would be important to verify its clinical utility so that the knowledge can be incorporated into guidelines for pediatric risk prediction. In addition, genetic risk may be attenuated by adherence to healthy lifestyle habits.

6.6.4. Clinical utility of repeated measures from the same time-point

Although the National Heart, Lung, and Blood Institute guidelines call for confirmation of abnormal lipid and BP values by repeated measures, it was not possible to consider multiple measures from the same time-point in this study because these data were not collected. Instead, two or three lipid and BP measures obtained with a 3 to 6 year interval in childhood were used. These analyses should be replicated following the National Heart, Lung, and Blood Institute guidelines by using one or multiple measures from the same time-point to estimate the true predictiveness that could be achieved with the National Heart, Lung, and Blood Institute guidelines.

6.6.5. Harms and benefits of lipid screening in childhood.

Universal pediatric lipid screening would identify individuals with moderate dyslipidemia who could benefit from lifestyle interventions. Still, most randomized trials of lipid lowering in youth are relatively short and involve treatment of high-risk children with lipid-lowering medication. The extent to which lifestyle intervention reduces long-term risk in those with moderately elevated lipid levels is unclear. Furthermore, the cost of a single lipid measure may appear trivial, but major costs will ensue from aggregating over the population, thorough workups and long-term intervention. Therefore cost-benefit analyses will be needed in the future.

7. SUMMARY AND CONCLUSIONS

Firstly, favorable trends in cholesterol levels have leveled off for the first time in decades during the 4-year follow-up of the Young Finns Study (**Study I**). Furthermore, over one-third of study population had prediabetes and may be at increased risk for T2D. Although favorable trends were observed in BP and TG levels, the data suggest that monitoring of cardiovascular risk factor levels is needed in the future in order to observe the possibly worsening of cardiovascular risk profile.

Secondly, both the tracking of BP from childhood to adulthood and the accuracy of predicting adult hypertension can be enhanced by multiple BP measurements in childhood and youth, compared with prediction models consisting of only a single childhood BP measurement (**Study II**). Tracking of BP indicated by correlations between BP measurements in childhood/youth and adulthood was significantly enhanced by two or three BP measurements in childhood compared with a single measurement. Most of the incremental predictive value over a single measurement was observed when two childhood BP measurements were used.

Thirdly, two or three lipid and lipoprotein measurements in childhood and young adulthood improve both the tracking of lipid levels from childhood to adulthood and the accuracy of predicting dyslipidemias in later life (**Study III**). For the universal screening of serum lipids in children, a single measurement does not provide sufficient information, and at least two measurements should be used. Data from this thesis is consistent with the National Heart, Lung, and Blood Institute's pediatric guidelines that call for measurement both in childhood and early adulthood.

Fourthly, childhood lipid levels and lipid-specific genetic risk scores are independently related to dyslipidemia in adulthood 21 to 31 years later (**Study IV**). Addition of wGRSs significantly improves the prediction of adult dyslipidemia when compared to a prediction model only consisting of clinical lipid measures in childhood. However, participants who have normal lipid levels as a child and have low genetic risk are still at relatively great risk of developing dyslipidemia later in life.

8. ACKNOWLEDGEMENTS

This study was carried out at the Research Centre of Applied and Preventive Cardiovascular Medicine (CAPC) in collaboration with the Department of Internal Medicine and the Department of Clinical Physiology and Nuclear Medicine, University of Turku, Finland, during 2012-2017.

This thesis work was financially supported by the Academy of Finland, the Finnish Government grants to Turku University Hospital, Turku University Foundation, the Juho Vainio Foundation, the Turku University Hospital Foundation, the Maud Kuistila Memorial Foundation, the Finnish Foundation for Cardiovascular Research, the Orion Research Foundation, and the Ida Montin Foundation.

I have been privileged with the opportunity to learn from, and work with three excellent supervisors. I would like to express my sincere gratitude to my supervisor, Professor Markus Juonala for his continuous support and encouragement. I truly admire your expertise and knowledge in both academic work and clinical practice. I owe my deepest gratitude to my supervisor, Docent Costan Magnussen for his vast knowledge, friendly support, and constructive criticism. I also sincerely thank you for reviewing the language of this thesis. I am indebted to my supervisor, Mervi Oikonen, who personally introduced the scientific world to me and always had time to discuss and help me with both major and minor things. Mervi's unexpected and too early departure left us all devastated. I deeply miss your enthusiasm, wide knowledge and passion for science.

I owe my gratitude to professors Olli Raitakari and Jorma Viikari for giving me the opportunity to work in the Cardiovascular Risk in Young Finns Study. Without their enormous knowledge, energy, and efficiency The Cardiovascular Risk in Young Finns Study and its 31-year follow-up would never have been possible to conduct. I would also like to thank you for inspiring discussions and comments that have enriched my thesis. Furthermore, I want to acknowledge the efforts of several other scientists, including former Study Coordinators, during 31 years of The Cardiovascular Risk in Young Finns Study, this thesis would not have been possible without your dedication and hard work. I am grateful to professors Tapani Rönnemaa and Jukka

Marniemi who personally clarified the laboratory methods used in Young Finns Study for me. In addition, I want to express my gratitude to the volunteer study subjects who made this study possible.

I am grateful to the official reviewers, docent Minna Hannuksela and docent Matti Jauhiainen, for providing excellent comments and fruitful criticism, which incented me to improve my thesis.

The co-authors of the original publications in this thesis, Niina Pitkänen, Eero Jokinen, Tomi Laitinen, Nina Hutri-Kähönen, Mika Kähönen, Terho Lehtimäki, Leena Taittonen, Päivi Tossavainen, Antti Jula, Britt-Marie Loo, Mikko Venäläinen, Laura Elo, and Leo-Pekka Lyytikäinen are gratefully acknowledged for their comments and encouragement. In addition, I also want to thank my international co-authors Michael Cheung, Matthew Sabin, Stephen Daniels, Marie-Jeanne Buscot, and Russell Thomson.

I warmly thank Tomi for setting an excellent example of being a young researcher and physician. I am indebted for your generous help with my thesis and advice in numerous matters considering science as well as life now and then. I would also like to thank Lauri for introducing me to the Young Finns Study in the first place and sharing moments of joy and frustration at tennis court. I am very grateful to Irina Lisinen, Ville Aalto, Noora Kartiosuo and Johanna Ikonen for their statistical advice. I also wish to thank Nina Ruotsalainen for helping me whenever I needed assistance with practicalities. I warmly thank all of the researchers of CAPC, especially Jarkko, Juhani, Hanna, Ville, Harri, Katja, Olli, Emmi, Mari, Jonna, Saku, Suvi, Ari, Petri, Juha, Lara, and Kristiina for the pleasant discussions, advices, and support during these years.

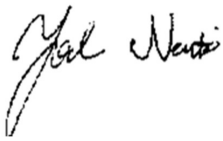
I want to thank all of my dear childhood friends for their great friendship during these and former years. Despite having lived here and there, every time we meet it feels like nothing has really changed. I would also like to express my gratitude to all of my friends I have met in Turku for the privilege of your company, and for sharing and enjoying the past years.

Special thanks go to my parents Annaliina and Kimmo for the enormous and on-going support and encouragement throughout my whole life. I am also fortunate in having two very fine

siblings, Aino and Tuomas, who have always been there to share the ups and downs of life with me.

Finally, my dearest Jasmine has shown enormous understanding and patience despite my peculiar little hobbies. She is an extraordinary lady.

Helsinki, March 2017



Joel Nuotio

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Annales Universitatis Turkuensis

Turun yliopisto
University of Turku

Painosalama Oy, Turku, Finland 2017

ISBN 978-951-29-6834-3 (PRINT)
ISBN 978-951-29-6835-0 (PDF)
ISSN 0355-9483 (Print) | ISSN 2343-3213 (Online)