KALLE SIPILÄ

Cardiometabolic and Genetic Risk Factors for Early Atherosclerosis

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ACADEMIC DISSERTATION
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To my family

TIIVISTELMÄ

Tausta: Ylipainosta on tullut vakava kansanterveydellinen ongelma länsimaisessa nyky-yhteiskunnassa. Lihavuus ja vähän liikuntaa sisältävä elämäntapa ovat kiinteästi yhteydessä metaboliseen oireyhtymään, sen osakomponentteihin ja eriasteisiin glukoosiaineenvaihdunnan häiriöihin. Nämä ovat hyvin tunnettuja sydän- ja verisuonitautien riskitekijöitä, ja ne on liitetty myös varhaisiin valtimotautimuutoksiin. Näiden riskitekijöiden itsenäinen merkitys valtimotaudin kehittymisessä on kuitenkin vielä epäselvä. Tiedetään, että tyypin 2 diabetes lisää sydän- ja verisuonitautien riskiä naisilla miehiä enemmän. Tämän vuoksi on luontevaa esittää hypoteesi, että myös lievempien glukoosiaineenvaihdunnan häiriöiden ja metabolisen oireyhtymän yhteydet varhaiseen valtimotautiin voisivat olla samalla tavalla sukupuoliriippuvaisia. Tutkimustieto on kuitenkin tältä osin vähäistä.

Perintötekijöiden vaikutus sydän- ja verisuonitautiriskiin on toinen runsaasti huomiota viime vuosina saanut osa-alue alan tutkimuksessa. Suuret koko genomin kattavat analyysit ovat paljastaneet useita geenipolymorfismeja, jotka assosioituvat sydän- ja verisuonitautiriskiin. Useissa eri tutkimuksissa on todettu kromosomissa 9p21.3 sijaitsevan geneettisen lokuksen vaikuttavan sepelvaltimotaudin riskiin. Vahvimmin sepelvaltimotautiin tässä kromosomissa on assosioitunut rs1333049-niminen geenivariantti. IL6–174 G>C -nimellä tunnettua polymorfismia, joka sijaitsee interleukiini-6 -geenin välittömässä läheisyydessä kromosomi 7:ssä, on tutkittu lukuisissa kandidaattigeenitutkimuksissa. Tämä polymorfismi on yhdistetty mm. sydäninfarktin riskiin ja useisiin sydän- ja verisuonisairauksien riskitekijöihin, mutta aiemmat tulokset ovat ristiriitaisia. Interleukiini-6 on tärkeä välittäjäaine tulehdusprosessissa, jonka ajatellaan nykyisin olevan metabolisen oireyhtymän, tyypin 2 diabeteksen ja myös itse valtimotautiprosessin taustalla. Näiden kahden yllä mainitun genotyypin rooli varhaisessa valtimotaudissa on epäselvä.

Tavoitteet: Tässä tutkimuksessa tutkittiin metabolisen oireyhtymän ja eriasteisten glukoosiaineenvaihdunnan häiriöiden itsenäisiä yhteyksiä varhaisiin valtimotautimuutoksiin. Erityisesti selvitettiin mahdollisia sukupuolieroja näissä yhteyksissä. Toinen tutkimustavoite oli selvittää joidenkin potentiaalisten geenimuuttujien yhteyksiä sydän- ja verisuonitautien riskitekijöihin ja varhaisiin valtimotautimuutoksiin. Tutkimuskohteiksi valittujen geenien valinnassa käytettiin kahta lähestymistapaa: tarkemman arvioinnin kohteeksi valittiin polymorfismi, joka assosioituu koko genomin kattavissa tutkimuksissa kaikkein vahvimmin sepelvaltimotautiin, ja toisaalta polymorfismi, joka assosioituu kandidaattigeenitutkimuksissa vahvasti sy-

dän- ja verisuonitautien riskitekijöihin ja sepelvaltimotautiin. Polymorfismi kromosomissa 9p21.3 (rs1333049) ja IL6–174 G>C -genotyyppi edustavat näitä kahta lähestymistapaa tässä tutkimuksessa.

Menetelmät: Terveys 2000 -tutkimus on suuri suomalainen terveystutkimus (toteutettiin vuosina 2000–2001), jonka alaotos muodosti tämän tutkimuksen pääasiallisen tutkimuspopulaation (otoksen koko 1353). Tässä populaatiossa tutkimushenkilöt olivat yli 45-vuotiaita (ikäjakauma 46–76 vuotta). Lasten sepelvaltimotaudin riskitekijät (LASERI) -tutkimus on monikeskustutkimus, missä selvitetään valtimotaudin riskitekijöitä lapsilla ja nuorilla aikuisilla. Tämän tutkimuksen populaatiota käytettiin toisena kohorttina selvitettäessä rs1333049-polymorfismin yhteyttä varhaiseen valtimotautiin. LASERI-aineistossa tutkittavat olivat 24–39 vuotiaita (otoksen koko 2251). Molemmissa populaatioissa tutkittaville tehtiin fyysinen tutkimus ja lukuisia mittauksia, joita käytetään varhaisten valtimotautimuutosten arvioinnissa. Ultraäänellä mitattuja kaulavaltimon intima-median paksuutta, kaulavaltimon elastisuutta ja olkavarsivaltimon endoteelitoimintaa sekä impedanssikardiografialla mitattua pulssiaallon etenemisnopeutta käytettiin tässä tutkimuksessa. Molemmissa populaatioissa sairaushistoria, lääkitykset ja elämäntapaan liittyvät tekijät selvitettiin haastattelututkimuksella, ja lisäksi tehtiin edustava joukko verikoemäärityksiä mukaan lukien genotyypitykset kiinnostuksen kohteena olevien geenipolymorfismien osalta. Sokeritasapaino arvioitiin esitietojen ja sokerirasituskokeen perusteella, ja luokittelussa käytettiin ADA:n (American Diabetes Association) kriteerejä. Metabolinen oireyhtymä luokiteltiin käyttämällä NCEP:n (National Cholesterol Education Program) ja IDF:n (International Diabetes Federation) luokituksia. Pääosin analyyseissä käytettiin NCEP-luokitusta.

Tulokset: Metabolinen oireyhtymä oli itsenäisesti, muista sydän- ja verisuonitautien riskitekijöistä riippumatta, yhteydessä kohonneeseen pulssiaallon etenemisnopeuteen ja kaulavaltimon lisääntyneeseen intima-median paksuuteen molemmilla sukupuolilla. Tämä yhteys vaikutti kuitenkin olevan voimakkaampi naisilla erityisesti kaulavaltimon seinämäpaksuuden osalta. Kun metabolisen oireyhtymän osakomponentit otettiin huomioon, yhteys kaulavaltimon intima-median paksuuden ja metabolisen oireyhtymän välillä säilyi merkitsevänä naisilla mutta ei miehillä. Miehillä perinteiset sydän- ja verisuonitautien riskitekijät olivat vahvasti yhteydessä kaulavaltimon seinämäpaksuuteen, eikä metabolinen oireyhtymä vaikuttanut antavan oleellisesti lisäinformaatiota kokonaisriskistä. Naisilla vastaavasti metaboliseen oireyhtymään liittyi lisäriski kaulavaltimon intima-median paksuuden suurentumiselle erityisesti silloin, kun perinteisten riskitekijöiden perusteella määritetty riski oli suhteellisen matala.

Kaulavaltimon intima-median paksuus lisääntyi ja kaulavaltimon elastisuus vähentyi molemmilla sukupuolilla trendinomaisesti glukoosiaineenvaihdunnan häiriön

vaikeutuessa. Tämä trendi heikentyi merkittävästi, kun muut sydän- ja verisuonitautien riskitekijät otettiin huomioon. Glukoositoleranssin yhteys kaulavaltimon elastisuuteen säilyi merkitsevänä vain naisilla muiden riskitekijöiden huomioinnin jälkeen. Kaulavaltimon intima-median paksuuden ja glukoositoleranssin yhteys ei ollut merkitsevä kummallakaan sukupuolella muista riskitekijöistä riippumattomana.

Yhden nukleotidin polymorfismi kromosomissa 9p21.3 (rs1333049) ei ollut yhteydessä kaulavaltimon intima-median paksuuteen kummassakaan tutkimuskohortissa eikä olkavarsivaltimon endoteelitoimintaan nuorilla aikuisilla.

IL6–174 G>C -genotyyppi ei ollut yhteydessä kaulavaltimon intima-median paksuuteen. Se assosioitui kuitenkin kokonaiskolesteroli-, LDL-kolesteroli- ja paastosokeritasoihin sekä painoindeksiin ja systoliseen verenpaineeseen miehillä.

Johtopäätökset: Metabolinen oireyhtymä on yhteydessä varhaiseen valtimotautiin molemmilla sukupuolilla. Tämä yhteys vaikuttaa olevan voimakkaampi naisilla erityisesti silloin, kun sydän- ja verisuonitautiriski perinteisten riskitekijöiden perusteella arvioituna on matala. Varhaiset valtimotautimuutokset lisääntyvät glukoosiaineenvaihdunnan häiriön vaikeusasteen kasvaessa sekä miehillä että naisilla. Tämä yhteys on ainakin osittain riippuvainen muista riskitekijöistä, ja se vaikuttaa naisilla voimakkaammalta kuin miehillä. Yhden nukleotidin polymorfismi kromosomissa 9p21.3 (rs1333049) ei ole yhteydessä varhaisiin valtimotautimuutoksiin, mikä viittaa siihen, että sen vaikutusmekanismi on jokin muu kuin suoraan ateroskleroosimuutosten edistäminen valtimoiden seinämissä. IL6–174 G>C -genotyypin vaikutus sydän- ja verisuonitautien riskitekijöihin saattaa olla erilainen miehillä ja naisilla. Tämä voi osittain selittää aiemmat ristiriitaiset tulokset koskien tämän genotyypin yhteyttä sydän- ja verisuonitautiriskiin. IL6–174 G>C -genotyypin vaikutus voi olla myös riippuvainen kehon rasvapitoisuudesta ja toisaalta elimistön metabolisesta ja inflammatorisesta tilasta. Väestötasolla tällä polymorfismilla näyttää olevan merkitystä geneettisenä riskitekijänä, mutta lisätutkimuksia tarvitaan sen vaikutuksen selvittämiseksi erilaisissa populaatioissa.

Monet tämän tutkimuksen tuloksista korostavat sitä tosiseikkaa, että sydän- ja verisuonitautien kokonaisriskiä arvioitaessa on tärkeää huomioida yksilön sukupuoli kuten myös muut yksilölliset tekijät. Monet tunnetuista riskitekijöistä saattavat vaikuttaa eri tavoin riippuen näistä tekijöistä. Tällainen lähestymistapa on tulevaisuudessa erittäin tärkeä myös sydän- ja verisuonitautien tieteellisessä tutkimuksessa. Jopa monien perinteisten riskitekijöiden vaikutuksia voidaan joutua arvioimaan uudelleen tietyissä populaatioissa.

ABSTRACT

Background: Overweight has become a major health issue in modern Western society. Obesity and a sedentary lifestyle are closely related to metabolic syndrome, its components and different stages of glucose metabolism impairment. These are well-known cardiovascular risk factors and they have also been related to early stages of atherosclerosis. The independent roles of these particular risk factors in the development of atherosclerosis are not, however, fully understood. It is known that type 2 diabetes has a more pronounced effect on cardiovascular risk in women than in men. Therefore, it is reasonable to hypothesize that milder impairment in glucose metabolism and metabolic syndrome might also have similar sex-related differences in their relationships to early atherosclerosis. This has not been studied extensively

Genetics is another field in cardiovascular research that has gained a tremendous amount of attention in recent years. Large genome-wide association studies have revealed several gene polymorphisms relating to cardiovascular risk. Several different studies have identified a genetic locus on chromosome 9p21.3 that has an influence on the risk of coronary heart disease. The single nucleotide polymorphism on chromosome 9p21.3 showing the strongest association with coronary heart disease is known as rs1333049. A polymorphism located in the promoter region of inflammatory cytokine interleukin-6, known as IL6–174 G>C, has been studied in a number of candidate gene studies. It has been associated with the incidence of cardiovascular events, such as myocardial infarction, and several risk factors for cardiovascular disease. Interleukin-6 is closely related to inflammation, which is believed to be the driving force behind metabolic syndrome, type 2 diabetes and the atherosclerosis process in general. Overall, the results regarding IL6–174 G>C genotype and its associations with cardiovascular risk have been very controversial. The role of these two above-mentioned genotypes in the early stages of atherosclerosis is not clear.

Aims: In the present study, the independent associations of metabolic syndrome and different stages of glucose intolerance with the markers of early atherosclerosis were studied. Specifically, possible sex-related differences in these associations were investigated. Another study aim was to evaluate the associations of some contemporary candidate genes with cardiovascular risk factors and the markers of early atherosclerosis. Two approaches were used in the selection of the genetic variants of interest: the polymorphism showing the strongest association with coronary heart disease in genome-wide association studies and a polymorphism

strongly associating with cardiovascular risk factors and coronary heart disease in candidate gene studies were selected to be studied in detail. A single nucleotide polymorphism on chromosome 9p21.3 (rs1333049) and IL6-174 G>C genotype represent these two approaches in the present study.

Subjects and Methods: A subpopulation of the Health 2000 Survey, which is a large Finnish cross-sectional health examination survey carried out in 2000–2001, formed the main population of the current study (sample size 1,353). The subjects were over 45 years old (age range 46–76 years). The population of the Cardiovascular Risk in Young Finns Study, a multi-centre study of atherosclerotic risk factors in children and young adults, was used as a second cohort in the investigation regarding the association of the rs1333049 polymorphism and early atherosclerosis. Subjects in the Young Finns Study were 24–39 years old (sample size 2,251). In both cohorts subjects underwent a physical examination and a variety of measurements used as markers for early atherosclerosis. Carotid artery intima-media thickness, carotid artery elasticity and brachial artery flow-mediated dilatation measured by ultrasound as well as pulse wave velocity measured by whole-body impedance cardiography were used in this study. Medical history, medication and lifestyle-related factors were evaluated with questionnaires, and a comprehensive selection of blood sample measurements, including genotyping for the genetic variants of interest, was carried out for both cohorts. Glucose tolerance status was evaluated using medical history and the oral glucose tolerance test, and it was defined using the American Diabetes Association criteria. Metabolic syndrome was defined using the National Cholesterol Education Program criteria (used in most of the calculations) and the International Diabetes Federation criteria.

Results: Metabolic syndrome was associated with increased pulse wave velocity and carotid artery intima-media thickness independently of other cardiovascular risk factors in both sexes. This association, however, appeared to be stronger in women, which was seen especially regarding carotid artery intima-media thickness. After the components of metabolic syndrome were taken into account, the association between metabolic syndome and carotid artery intima-media thickness remained significant in women but not in men. In men, traditional cardiovascular risk factors were strongly associated with carotid artery intima-media thickness, and metabolic syndrome seemed to offer little additional information. In women, however, metabolic syndrome was associated with an additional risk of increased intima-media thickness, especially when the risk according to the traditional risk factors was relatively low.

There was a trend of increasing carotid artery intima-media thickness and decreasing carotid artery elasticity according to the worsening of glucose tolerance

in both sexes. This trend was weakened markedly after the adjustment for other cardiovascular risk factors. The association of glucose tolerance status and carotid artery elasticity remained significant in women but not in men after the other risk factors were taken into account. The association of glucose tolerance status and carotid artery intima-media thickness was not significant in either sex after these adjustments.

The rs1333049 polymorphism was not associated with carotid artery intima-media thickness in either of the study cohorts, and it was not associated with brachial artery flow-mediated dilatation in the Young Finns Study cohort either.

IL6–174 G>C genotype was not associated with carotid artery intima-media thickness. It was, however, associated with the levels of total cholesterol, LDL cholesterol and fasting plasma glucose as well as with body mass index and systolic blood pressure in men.

Conclusions: Metabolic syndrome is associated with early atherosclerosis in both sexes. This association seems to be stronger in women, especially if the risk for cardiovascular disease defined by traditional risk factors is low. There is a trend of increasing early atherosclerosis according to the worsening of the glucose tolerance in both sexes. This association is at least partly mediated by other risk factors and may be stronger in women than in men. The rs1333049 polymorphism is not related to the markers of early atherosclerosis, suggesting that its effect on the risk of coronary heart disease might be mediated by different mechanism than simply promoting atherosclerotic changes in the vascular wall. The association of IL6–174 G>C genotype with cardiovascular risk factors seems to be different in men and women. This may partly explain the previous controversial results regarding its effect on cardiovascular risk. The effect of this genotype may also depend on factors such as body fat mass as well as the metabolic and inflammatory state. At the population level, this polymorphism seems to have an impact as a genetic risk factor. However, future research is needed to evaluate its effect in different populations.

Many of the results of the present study emphasize the fact that in the evaluation of overall cardiovascular risk, it is important to take the subjects' sex as well as other characteristics into account. Many of the known risk factors may act differently depending on these characteristics. This kind of approach will be extremely important in the future cardiovascular research as well. Some of the traditional risk factors may even have to be re-evaluated in specific populations.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications:

I Sipilä K, Koivistoinen T, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kööbi T, Kukkonen-Harjula K, Majahalme S, Kähönen M. Metabolic syndrome and arterial stiffness: the Health 2000 Survey. Metabolism. 2007 Mar;56(3):320–6.

II Samani NJ, Raitakari OT, Sipilä K, Tobin MD, Schunkert H, Juonala M, Braund PS, Erdmann J, Viikari J, Moilanen L, Taittonen L, Jula A, Jokinen E, Laitinen T, Hutri-Kähönen N, Nieminen MS, Kesäniemi YA, Hall AS, Hulkkonen J, Kähönen M, Lehtimäki T. Coronary artery disease-associated locus on chromosome 9p21 and early markers of atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 2008 Sep;28(9):1679–83. Epub 2008 Jul 3.

III Sipilä K, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kukkonen-Harjula K, Lehtimäki T, Kesäniemi YA, Koivistoinen T, Nieminen MS, Tuomilehto J, Kähönen M. Metabolic syndrome and carotid intima media thickness in the Health 2000 Survey. Atherosclerosis. 2009 May;204(1):276–81. Epub 2008 Sep 4.

IV Riikola A, Sipilä K, Kähönen M, Jula A, Nieminen MS, Moilanen L, Kesäniemi YA, Lehtimäki T, Hulkkonen J. Interleukin-6 promoter polymorphism and cardiovascular risk factors: the Health 2000 Survey. Atherosclerosis. 2009 Dec;207(2):466–70. Epub 2009 Jun 11.

V Sipilä K, Kähönen M, Salomaa V, Päivänsalo M, Karanko H, Varpula M, Jula A, Kaaja R, Kesäniemi YA, Reunanen A, Laakso M, Moilanen L. Carotid artery intima-media thickness and elasticity in relation to glucose tolerance. Submitted.

The final publication is available at http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s00592-011-0291-z.

ABBREVIATIONS

AASI	ambulatory arterial stiffness index
ADA	American Diabetes Association
ADC	arterial diameter change
AGE	advanced glycation end product
AHA	American Heart Association
AIx	augmentation index
apo	apolipoprotein
BMI	body mass index
CAC	carotid artery compliance
CAS	carotid artery stiffness
CCA	common carotid artery
CHD	coronary heart disease
CIMT	carotid artery intima-media thickness
CRP	C-reactive protein
CVD	cardiovascular disease
DAD	diastolic arterial diameter
DBP	diastolic blood pressure
DM	diabetes mellitus
EGIR	European Group for the Study of Insulin Resistance
Ep	Peterson's elastic modulus
FFA	free fatty acid
FMD	flow-mediated dilatation
FRS	Framingham Risk Score
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
hsCRP	high-sensitivity C-reactive protein
ICG_{WB}	whole-body impedance cardiography
IDF	International Diabetes Federation
IFG	impaired fasting glucose
IL-6	interleukin-6
IMT	intima-media thickness
IGT	impaired glucose tolerance
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
MetS	metabolic syndrome
NCEP	National Cholesterol Education Program

NGT	normal glucose tolerance
NHLBI	National Heart, Lung, and Blood Institute
NO	nitric oxide
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor-1
PP	pulse pressure
PWV	pulse wave velocity
SAD	systolic arterial diameter
SBP	systolic blood pressure
SI	beta stiffness index
SNP	single nucleotide polymorphism
T2DM	type 2 diabetes
TNF-α	tumour necrosis factor alpha
VLDL	very-low-density lipoprotein
WHO	World Health Organization
YEM	Young's elastic modulus

1 INTRODUCTION

A sedentary lifestyle and obesity have become an epidemic in modern Western society. Striking evidence suggests that even childhood obesity is a strong predictor for mortality (Franks et al. 2010). There is a clear relationship between atherosclerosis, cardiovascular disease (CVD) and obesity (Poirier et al. 2006). Abdominal obesity is strongly related to insulin resistance, type 2 diabetes (T2DM) and a cluster of risk factors known as metabolic syndrome (MetS) (Eckel et al. 2010). These are major mediators of increased CVD risk in overweight subjects (Poirier et al. 2006). Atherosclerosis is now considered an inflammatory disease, and insulin resistance and MetS have been shown to be proinflammatory states (Eckel et al. 2005). It is known that T2DM is a greater CVD risk factor in women than in men (Beckman et al. 2002), and it has been suggested that this would also be the case with MetS (Iglseder et al. 2005). The latter has not, however, been consistently proven. MetS predisposes to T2DM (Lorenzo et al. 2003), which is one of the major CVD risk factors (Ridger and Libby 2008), as well as coronary heart disease (CHD) (Bonora et al. 2003) and mortality (Isomaa et al. 2001, Lakka et al. 2002, Malik et al. 2004). However, its independent role in the pathogenesis of atherosclerosis has raised controversy. It has been debated whether the syndrome as an entity offers additive information apart from its components and traditional CVD risk factors (Kahn et al. 2005).

In addition to the modifiable risk factors, such as obesity, genetic factors play a key role in the pathogenesis of atherosclerosis and CVD. It has been estimated that roughly 50% of the CVD risk is based on inheritance (Zdravkovic et al. 2002). However, the genetic basis of atherosclerosis has not been clearly characterized to date. Major genome-wide association studies have identified several genetic loci associated with CHD. A common variant located on chromosome 9p21.3 is the one with the strongest association with CHD in these studies (Burton et al. 2007, Helgadottir et al. 2007, McPherson et al. 2007, Samani et al. 2007, Schunkert et al. 2008). However, the exact role of this genetic locus in the disease process is not clear. Another approach in genetic studies investigating CVD risk is to study a gene associated with a known or suspected risk factor – i.e., a candidate gene study. As an inflammatory disease, atherosclerosis has been associated with inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6). Genes that are associated with the production of such markers have been studied as possible risk factors for atherosclerosis. A single G>C base exchange polymorphism in the promoter region of the IL-6 gene has been associated with IL-6 production (Fishman et al. 1998, Burzotta et al. 2001, Hulkkonen et al. 2001)

and atherosclerosis (Georges et al. 2001, Humphries et al. 2001, Chiappelli et al. 2005), but the results have been inconsistent. Some studies have suggested that allele G is the risk allele (Fernandez-Real et al. 2000, Huth et al. 2006), while other studies have associated allele C with increased CVD risk (Georges et al. 2001, Humphries et al. 2001, Chiappelli et al. 2005).

Atherosclerotic changes are usually present years or even decades before the onset of the clinical disease (Enos et al. 1953, McNamara et al. 1971, Tuzcu et al. 2001). Subclinical atherosclerotic lesions can be studied with several techniques. Arterial endothelial function is usually impaired very early in the disease process (Ross 1999) and it can be evaluated with ultrasound (Corretti et al. 2002). Thickening and stiffening of the arterial walls is another typical feature of early atherosclerosis (Pignoli et al. 1986). Wall structure and elasticity can be evaluated with ultrasonic methods (Pignoli et al. 1986), and arterial stiffness may also be estimated by measuring the movement of the pulse wave along the arterial tree (Oliver and Webb 2003).

The specific aims of this thesis were to study the independent associations of MetS and glucose intolerance with early atherosclerosis, in addition to possible sex differences in these associations. Another major study aim was to evaluate the associations of contemporary candidate genes with cardiovascular risk factors and early atherosclerosis. Two approaches were used in the selection of the genetic variants of interest: a polymorphism with the strongest association with CHD in genome-wide association studies, and a polymorphism strongly associating with cardiovascular risk factors and CHD in candidate gene studies. Therefore, the roles of a single nucleotide polymorphism on chromosome 9p21.3 (rs1333049) and IL6–174 G>C genotype in the early stages of atherosclerosis were evaluated. These aims were investigated in a sub-population of a large Finnish health examination survey, the Health 2000 Survey. The polymorphism on chromosome 9p21.3 was also studied in the population of the Cardiovascular Risk in Young Finns Study, a multi-centre study of atherosclerotic risk factors in children and young adults.

2 REVIEW OF THE LITERATURE

2.1 Pathophysiology of atherosclerosis

Atherosclerosis is a progressive disease. It typically leads to arterial wall thickening by the accumulation of lipids, leukocytes, smooth muscle cells and fibrous material (Lusis 2000). Symptomatic CHD, stroke and peripheral arterial disease appear in the late stages of this process. Asymptomatic lesions in the arterial wall are present years or even decades before the onset of clinical disease (Enos et al. 1953, McNamara et al. 1971, Tuzcu et al. 2001). Atherosclerosis usually begins in the abdominal aorta and then progresses to coronary and carotid arteries (McGill 1968).

A normal arterial wall consists of three layers. The innermost layer facing the lumen is called the intima. It has a monolayer of endothelial cells on the luminal side and a sheet of elastic fibres, the internal elastic lamina, on the peripheral side. Normally, the intima is a very thin region consisting mainly of extracellular connective tissue matrix, primarily proteoglycans and collagen. The middle layer, media, consists of smooth muscle cells. The outermost layer is called the adventitia, consisting primarily of smooth muscle cells, fibroblasts and connective tissue (Lusis 2000). Morphology of a normal arterial wall is illustrated in Figure 2.1.

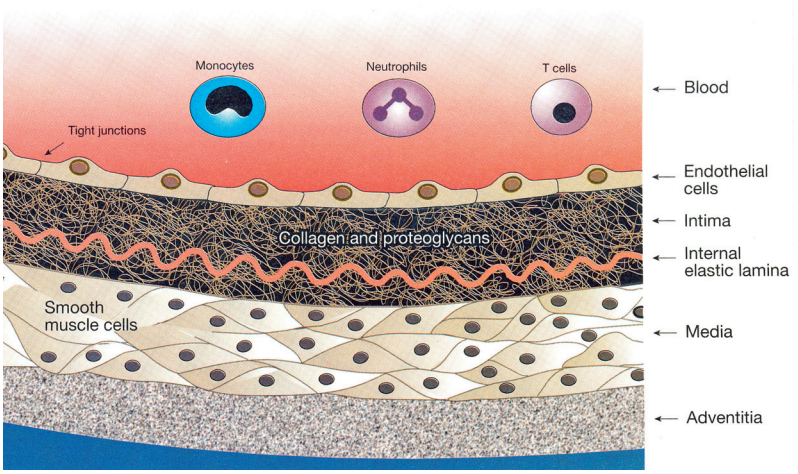

FIGURE 2.1 *Morphology of a normal arterial wall. Reprinted by permission from Macmillan Publishers Ltd: Nature, copyright (2000) (Lusis 2000).*

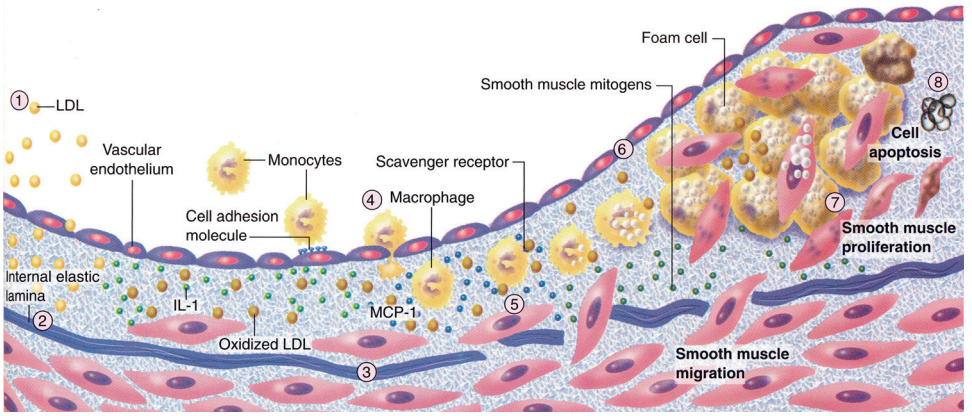

FIGURE 2.2 *Schematic of the evolution of the atherosclerotic plaque. 1, Accumulation of lipoprotein particles in the intima. The modification of these lipoproteins is depicted by the darker colour. The modifications include oxidation and glycation. 2, Oxidative stress including products found in modified lipoproteins can induce local cytokine elaboration. 3, The cytokines thus induce increased expression of adhesion molecules for leukocytes that cause their attachment and chemoattractant molecules that direct their migration into the intima. 4, Blood monocytes, on entering the artery wall in response to chemoattractant cytokines such as monocyte chemoattractant protein 1 (MCP-1), encounter stimuli that can augment their expression of scavenger receptors. 5, Scavenger receptors mediate the uptake of modified lipoprotein particles and promote the development of foam cells. Macrophage foam cells are a source of mediators such as more cytokines and matrix metalloproteinases. 6, SMCs in the intima divide, and other SMCs migrate into the intima from the media. 7, SMCs can then divide and elaborate extracellular matrix, promoting extracellular matrix accumulation in the growing atherosclerotic plaque. In this manner, the fatty streak can evolve into a fibro-fatty lesion. 8, In later stages, calcification can occur (not depicted), and fibrosis continues, sometimes accompanied by SMC death (including programmed cell death, or apoptosis), yielding a relatively acellular fibrous capsule surrounding a lipid-rich core that may also contain dying or dead cells and their detritus. IL-1 = interleukin-1; LDL = low-density lipoprotein, SMCs = smooth muscle cells. Reprinted from Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine, 8, Libby P, The vascular Biology of Atherosclerosis, 989, Copyright Elsevier (2008) (Libby 2008).*

Atherosclerosis is now widely considered to be an inflammatory process. This is based on a hypothesis known as the response-to-injury theory. A dysfunctional endothelium is believed to play an important role in the initiation of the process (Ross and Glomset 1973, Ross 1999). In very early stages of atherosclerosis, low-density lipoprotein (LDL) particles are deposited in the intima. These particles are subsequently modified to induce cytokine production. This, in turn, causes leukocytes (macrophages and lymphocytes) to enter the intima. Macrophages then uptake the modified lipoprotein particles and begin to transform into so called foam cells. These cells further promote the inflammatory process and the production of cytokines. The inflammatory process continues with an increase in the amount of smooth muscle cells in the intimal layer. Smooth muscle cells produce extracellular

material, and the fibro-fatty lesion entitled an atherosclerotic plaque begins to form. Cellular death and apoptosis also take place in these lesions. Atherosclerotic plaques may produce symptoms by reducing blood flow. However, acute myocardial infarction is often caused by thrombosis after superficial erosion or rupture of the plaque (Libby 2008). The pathogenesis of atherosclerosis is depicted in Figure 2.2.

2.2 Markers of early atherosclerosis

2.2.1 Endothelial function

The endothelium and its function have been recognized as key factors in the pathogenesis of atherosclerosis. Endothelial cells have a wide variety of functions maintaining homeostasis in the vessel wall. It is believed that endothelial dysfunction is one of the earliest steps in the initiation of atherosclerosis (Ross 1999). The dilatation of blood vessels in response to increased flow and shear stress is known as flow-mediated dilatation (FMD). This phenomenon is mainly mediated by endothelium-derived nitric oxide (NO). Non-invasive measurement of brachial FMD by ultrasound has been used to evaluate endothelial function (Corretti et al. 2002). It has been shown to correlate well with coronary FMD (Takase et al. 1998), and reduction in brachial FMD has been related to an increased risk of cardiovascular events (Gokce et al. 2002, Yeboah et al. 2007).

2.2.2 Arterial intima-media thickness

Thickening of the arterial intima is an early sign of atherosclerosis. Atherosclerotic lesions often evolve progressively into plaques that protrude into the vessel lumen (Libby 2008). This process can be evaluated by measuring the intimal + medial thickness (intima-media thickness) with ultrasound (Figure 2.3). The measurement of carotid artery intima-media thickness (CIMT) has become a widely utilized non-invasive marker for subclinical atherosclerosis. It has been shown to relate closely to microscopically measured intima-media thickness (Pignoli et al. 1986). Increased CIMT has been reported to associate with prevalent CVD (Salonen et al. 1994, Burke et al. 1995), and CIMT also correlates with the angiographically measured extent of CHD (Lekakis et al. 2000, Kablak-Ziembicka et al. 2004). Several trials have found an increased risk of myocardial infarction or stroke in subjects with increased CIMT (Bots et al. 1997, Chambless et al. 1997, O'Leary et al. 1999, Chambless et al. 2000). CIMT is also commonly used as a surrogate marker in studies evaluating the effect of drugs on myocardial infarction risk,

and in a recent meta-analysis a decrease in CIMT was reported to associate with reduced event risk. However, in some studies CIMT has not appeared to be a useful surrogate marker for the risk of myocardial infarction. The authors of the meta-analysis concluded that statin therapy, for example, might lower the risk via mechanisms that are not reflected in changes in CIMT (Goldberger et al. 2010).

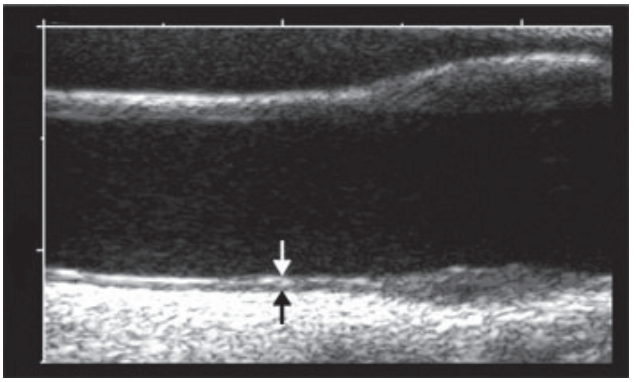

FIGURE 2.3 *Intima-media layers (arrows) of the common carotid artery in an ultrasound image.*

2.2.3 Arterial stiffness and elasticity

Thickening of the arterial wall tends to reduce elasticity and increase stiffness in the vessel wall. Age and systolic blood pressure (SBP) are major determinants of arterial stiffness. It may also be the result of increased intima-media thickness associated with atherosclerosis (Cohn 2006). Therefore, arterial stiffening is not considered merely a marker of age and elevated blood pressure but rather a product of multiple pathological mechanisms affecting the arterial tree (Cruickshank et al. 2002). Measurements of arterial stiffness and elasticity have been used as surrogates in evaluating atherosclerosis.

The ejection of blood from the heart initiates an arterial pressure wave travelling towards the periphery. The velocity of this pressure wave reflects the elastic properties and stiffness of the arteries (Oliver and Webb 2003). Arterial stiffness can therefore be evaluated by measuring pulse wave velocity (PWV) along the arterial tree. PWV has been shown to be an independent predictor of coronary events (Boutouyrie et al. 2002) and cardiovascular mortality in several patient groups (Blacher et al. 1999, Laurent et al. 2001, Cruickshank et al. 2002). Previously, PWV measurements have been performed mostly with methods utilizing Doppler ultrasound or mechanoelectrical pulse transducers (Lehmann et al. 1992, Wilkinson et al. 1998). PWV can also be measured by means of whole-

body impedance cardiography (ICG_{WB}). This method is highly repeatable and reproducible (Kööbi et al. 2003).

Ultrasonically measured carotid artery elasticity has also been used as a marker of arterial stiffness. On the basis of ultrasound measurements and blood pressure, several different elasticity indices have been derived. Carotid artery compliance (CAC) measures the ability of the carotid artery to expand as a response to pulse pressure (PP) caused by cardiac contraction and relaxation (Juonala et al. 2005). The beta stiffness index (SI) is a marker of arterial elasticity that is considered to be relatively independent of blood pressure (Hirai et al. 1989). Young's elastic modulus (YEM), on the other hand, is a measure of arterial wall stiffness that is independent of intima-media thickness (Salomaa et al. 1995). Carotid artery stiffness (CAS) has been related to CVD mortality (Blacher et al. 1998, Stork et al. 2004).

Other methods that have been used to estimate arterial stiffness include the ambulatory arterial stiffness index (AASI) and indices derived from pulse wave analysis. AASI is a recently proposed marker of arterial stiffness (Dolan et al. 2006) that is based on 24-h ambulatory blood pressure measurements. This is a rather simple and inexpensive method, but it has been reported to be only moderately reproducible (Stergiou et al. 2010). Pulse wave analysis is a method that employs aplanation tonometry to record arterial waveforms. These can be measured from, for example, the carotid and brachial arteries. It has been suggested that the measurements are relatively easily assessed (Wilkinson et al. 1998). Several indices used as surrogates for arterial stiffness have been derived from pulse wave analysis. These include the augmentation index (AIx), carotid-brachial pressure amplification and central pulse pressure. A recent analysis with the population of the Framingham Heart Study compared PWV, AIx, carotid-brachial pressure amplification and central pulse pressure in their ability to predict CVD events, and PWV was the only method significantly associating with increased risk for a first CVD event (Mitchell et al. 2010). The sensitivity of AIx in assessing arterial stiffness has also been questioned (Cheng et al. 2007).

2.3 Risk factors for atherosclerosis

2.3.1 Lipid risk factors

2.3.1.1 Low-density lipoprotein cholesterol

LDL particles are the main carriers of cholesterol in humans. Their accumulation and modification in the intima play an important role in the initiation of endothelial dysfunction and atherosclerosis (Lusis 2000). LDL modification

includes oxidation, and oxidized LDL, in turn, promotes foam cell formation as well as inflammation (Steinberg 1997). These deleterious processes are enhanced by raised LDL levels in plasma (Lusis 2000). Subjects suffering from familial hypercholesterolaemia have a markedly elevated LDL cholesterol (LDL-C) level, and they are at high risk of developing CHD early in life (by the third to fourth decade of life for men and 8 to 10 years later for women) (Genest and Libby 2008). It has been convincingly shown that lowering the LDL-C level reduces the CHD risk (Grundy et al. 2004a). These facts underline the importance of LDL as a risk factor for atherosclerosis. LDL-C level has been associated with increased CIMT (Salonen et al. 1988, Raitakari et al. 2003) and arterial stiffness (Smilde et al. 1998, Juonala et al. 2005).

2.3.1.2 High-density lipoprotein cholesterol

It has been shown in epidemiological studies that high-density lipoprotein cholesterol (HDL-C) level has an inverse relationship with CVD. A process known as reverse cholesterol transport has been suggested to be at least partly responsible for this protective effect of HDL-C. According to this theory, high-density lipoprotein (HDL) moves cholesterol away from the vessel wall and from the peripheral tissues (Brewer 2004). HDL also has the ability to protect LDL from oxidation (Navab et al. 2002). Another protective mechanism is probably the ability of HDL to decrease the amount of endothelial-cell adhesion molecules that promote leukocyte migration into the vessel wall (Barter et al. 2002). A low HDL-C level has been linked to increased CIMT (Salonen et al. 1988) and arterial stiffness (Havlik et al. 2001).

2.3.1.3 Triglycerides

The major proportion of ingested fat consists of triglycerides, which are used for the transportation and storage of energy (Genest and Libby 2008). The triglyceride concentration in the blood is highly diet dependent and also has an inverse relationship with the HDL-C concentration. The role of the triglyceride level as an independent risk factor for atherosclerosis has, therefore, been controversial (Ridger and Libby 2008). One recent meta-analysis, however, concluded that an elevated triglyceride level is associated with CHD independently of HDL-C and this association is similar in fasting and non-fasting participants (Sarwar et al. 2007). Another meta-analysis in subjects from the Asia-Pacific region also suggested that the serum triglyceride level is an independent risk factor for CHD and stroke (Asia Pacific Cohort Studies Collaboration 2004). One possible mechanism explaining these associations is that the exposure of the arterial wall to triglyceride-rich lipoproteins, such as very-low-density lipoprotein (VLDL),

may promote atherosclerosis (Ridger and Libby 2008). However, the mechanisms are not fully understood. An increased triglyceride level has been linked to early atherosclerosis (Li et al. 2004).

2.3.1.4 Other lipid risk factors

Smaller size and increased density in LDL particles has been connected with a high atherosclerosis risk in particular (Gardner et al. 1996), although this concept has been questioned (Sacks and Campos 2003). The high atherogenity of these particles may be a result of low binding affinity for the LDL receptor, prolonged plasma half-life and low resistance to oxidative stress (Tribble et al. 1992, Chapman et al. 1998). Small dense LDL particles have been linked to increased CIMT (Skoglund-Andersson et al. 1999).

Apolipoproteins (apo) are structural components of lipoprotein molecules. Different apolipoproteins are associated with different lipoprotein molecules. For example, apoA-I and apoA-II are associated with HDL, and apoB is a component of atherogenic lipoproteins such as VLDL and LDL (Genest and Libby 2008). It has been suggested that measurements of apoA-I and apoB levels may be better in predicting CVD risk than the LDL-C level (Walldius et al. 2001). In a study by Pischon et al. apoB turned out to be a stronger predictor for CHD in men than did any of the cholesterol measurements (Pischon et al. 2005). In women, however, apoB was not superior in predicting CHD when compared to non-HDL cholesterol (total cholesterol-HDL cholesterol) (Ridker et al. 2005). ApoB has been related to increased CIMT (Sharrett et al. 1994).

Lipoprotein (a) is a particle resembling LDL in which apoB is connected with a certain glycoprotein, apoprotein(a) (Ridger and Libby 2008). High levels of lipoprotein (a) have been linked to an increased risk of coronary events and stroke (Danesh et al. 2000, Suk Danik et al. 2006, Kiechl et al. 2007). The mechanisms by which lipoprotein (a) promotes atherosclerosis may include its local actions in the atherosclerotic lesions, and it may also have prothrombotic features (Ridger and Libby 2008). In studies by Raitakari et al. and Grebe et al., increased CIMT and lipoprotein (a) were not significantly associated (Raitakari et al. 1999, Grebe et al. 2007), although some authors have suggested that such an association exists (Schreiner et al. 1996).

2.3.2 Smoking

Smoking is one of the most important risk factors for CHD (Ridger and Libby 2008). It has also been shown to increase mortality from cerebrovascular disease, although not to the same extent as in the case of CHD (Ezzati et al. 2005). It is a

well-known risk factor for peripheral arterial disease as well (Ambrose and Barua 2004). Smoking promotes atherosclerosis by several different mechanisms. It may cause endothelial dysfunction by reducing nitric oxide synthesis in the endothelial cells (Barua et al. 2003) and by increasing LDL oxidation (Heitzer et al. 1996). Smoking has also been linked to increased inflammation (Bazzano et al. 2003). Smokers have an adverse lipid profile compared to non-smokers, and it has been suggested that insulin resistance, promoted by smoking, is the key factor behind this phenomenon (Reaven and Tsao 2003). Both active and passive smoking have been associated with increased CIMT (Howard et al. 1994b) and decreased arterial compliance (Li et al. 2005).

2.3.3 Hypertension

Hypertension is one of the most significant risk factors for CVD (Stamler et al. 1993), especially for stroke (Lawes et al. 2004). It has an important and complex role in the pathogenesis of atherosclerosis. Sustained elevation in blood pressure causes endothelial changes such as increased permeability, decreased endothelium-dependent vasodilatation, increased leukocyte adherence to the endothelial surface and subsequent macrophage accumulation in the intima. Hypertension also increases smooth muscle cell proliferation and enhances inflammation in the arterial wall (Chobanian 1990, Chobanian and Alexander 1996). On the other hand, inflammation and endothelial dysfunction may play an important role in the development of hypertension (Savoia and Schiffrin 2006). Isolated systolic hypertension and elevated pulse pressure are signs of increased vascular wall stiffness and can thus be considered markers of existing atherosclerosis. It is therefore not surprising that hypertension has been linked to increased CIMT and arterial stiffness (Riley et al. 1986, Gariepy et al. 1993, Zanchetti et al. 2001, Czernichow et al. 2005).

2.3.4 Diabetes, insulin resistance, and metabolic syndrome

2.3.4.1 Diabetes

Diabetes (DM) is a powerful risk factor for CVD morbidity and mortality. CVD event rates are roughly 2- to 4-fold in diabetic subjects in relation to their age-matched non-diabetic counterparts (Ridger and Libby 2008). In a large Canadian population-based cohort study, DM was found to increase CVD risk as much as ageing of 15 years (Booth et al. 2006). Interestingly, T2DM increases the risk for CVD events more in women than in men (Beckman et al. 2002).

As many as 90 percent of diabetic patients suffer from T2DM (Beckman, et al 2008). It has been proposed that atherosclerosis and T2DM have a similar inflammatory basis (Eckel et al. 2005). There have been several trials investigating the effect of glycaemic control on CVD events and mortality. These studies have yielded some support for the hypothesis that strict glycaemic control reduces CVD-related morbidity and mortality (Beckman, et al 2008). The findings, however, suggest that hyperglycaemia per se is not the only factor contributing to the CVD risk in diabetics.

Excess adipose tissue is usually present in T2DM. It is believed that adipose tissue in obese subjects secretes an increased amount of proinflammatory agents such as tumour necrosis factor-alpha (TNF-α) and IL-6 (Berg and Scherer 2005), which play an important role in initiating inflammatory processes. Visceral fat that is associated with abdominal obesity has been shown to be more active than subcutaneous fat in this regard (Shoelson et al. 2006). Excess adipose tissue also secretes an increased amount of free fatty acids (FFAs). This inhibits insulin function, especially in muscle tissue, promoting so-called insulin resistance (Belfort et al. 2005). This is an important mechanism linking obesity and hyperglycaemia.

Elevated blood glucose and increased FFA concentrations may induce a decrease in endothelial nitric oxide (NO) production, leading to vasoconstriction, hypertension and smooth muscle cell proliferation. They also enhance inflammation and increase the production of cytokines contributing to endothelial dysfunction. Hyperglycaemia promotes thrombosis by platelet activation and increases the production of potentially atherogenic advanced glycation end products (AGEs). Part of the proinflammatory action of hyperglycaemia is caused by the increased AGE production (Creager et al. 2003).

The American Diabetes Association (ADA) (Genuth et al. 2003) and World Health Organization (WHO) (Alberti and Zimmet 1998) diagnostic criteria for DM recognize two intermediate metabolic states between normal glucose tolerance (NGT) and DM. These pre-diabetic states, known as impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), represent two distinct subgroups of abnormal glucose metabolism. It has been shown recently that impaired insulin release is a predominant feature in IFG, whereas peripheral insulin resistance is characteristic of IGT (O'Rahilly et al. 1994, Stancakova et al. 2009). There is also evidence that even these milder abnormalities in glucose metabolism increase the risk for all cause and CVD mortality (Barr et al. 2007). Hu et al. reported that women who eventually developed diabetes had a threefold relative risk of myocardial infarction before the actual diagnosis of DM (Hu et al. 2002).

A deteriorating trend in CIMT (Wagenknecht et al. 1998, Bonora et al. 2000, Henry et al. 2004, Sigurdardottir et al. 2004, Mohan et al. 2006) and CAS (Henry et al. 2003) according to the worsening of glucose tolerance has been reported.

It is also well known that subjects with T2DM have increased CIMT (Kawamori et al. 1992, Folsom et al. 1994, Pujia et al. 1994, Niskanen et al. 1996, Bonora et al. 1997) in comparison to subjects without impairment in glucose metabolism. Some (Hanefeld et al. 1999a, Mohan et al. 2006, Zhang et al. 2006, Faeh et al. 2007), but not all (Niskanen et al. 1996, Tuomilehto et al. 1998, Wagenknecht et al. 1998), previous studies have found significantly higher CIMT also in subjects with IGT when compared to those with NGT. In a recent meta-analysis Brohall et al. concluded that subjects with IGT have slightly increased CIMT compared to subjects with normal glucose metabolism (Brohall et al. 2009). Non-diabetic glucose intolerance and increased stiffness of the carotid arteries have also been related previously (Salomaa et al. 1995, van Popele et al. 2000). Most of the previous studies have not reported a difference in carotid atherosclerosis between subjects with IFG and NGT (Bonora et al. 1999, Hanefeld et al. 1999b, Tropeano et al. 2004, Faeh et al. 2007), although some studies have suggested increased CIMT (Zhang et al. 2006) or CAS (van Popele et al. 2006) in IFG when compared to normal glucose metabolism. Some reports have suggested that the effect of glucose metabolism impairment on early carotid atherosclerosis would be greater in women than in men (Salomaa et al. 1995, Kawamoto et al. 2007b). The data regarding sex-related differences is limited, however, and other studies have not confirmed this hypothesis (Folsom et al. 1994, Bonora et al. 2000).

2.3.4.2 Insulin resistance and metabolic syndrome

Obesity-induced insulin resistance leading to hyperglycaemia and, subsequently, evident diabetes is believed to be part of the entity known as MetS. The term metabolic syndrome means the clustering of cardiovascular risk factors such as central obesity, hypertension, dyslipidemia and glucose intolerance. Insulin resistance is considered to be a key factor between these abnormalities. Insulin resistance by itself can directly reduce endothelium-dependent vasodilatation (Laakso et al. 1990). It has been reported to be an independent predictor for myocardial infarction and death (Hedblad et al. 2002). Insulin resistance has also been associated with the risk of stroke (Kernan et al. 2002) and congestive heart failure (Ingelsson et al. 2005).

In insulin resistance there is an excess FFA flux to the liver, and the production of triglycerides in the liver is high. The liver secretes excess amount of VLDL, and a high level of this triglyceride-rich lipoprotein in the blood causes hypertriglyceridemia. Subsequently, cholesteryl ester exchange between HDL and VLDL becomes prominent, leading to a decreased concentration of HDL-C. MetS and T2DM are typically accompanied with dyslipidemia where hypertriglyceridemia and low HDL-C predominate (Eckel et al. 2005, Cornier et al. 2008). Although the LDL-C concentration does not markedly differ in

subjects with or without MetS, an increased triglyceride concentration results in the synthesis of smaller and denser LDL particles (Kwiterovich 2002). Insulin resistance is also typically associated with hypertension (Ferrannini et al. 1987). Several mechanisms behind this have been proposed. Insulin is a vasodilator, and this effect may be impaired in insulin resistance. An elevated circulating glucose concentration increases pancreatic insulin secretion, resulting in hyperinsulinaemia. This may cause sodium retention and sympathetic stimulation. FFAs themselves may also promote vasoconstriction (Eckel et al. 2005). Adipose-tissue-derived angiotensinogen, leptin and resistin may also have a hypertensive effect in insulin-resistant subjects (Cornier et al. 2008). Endothelial inflammation has been recognized as an important part of MetS (Sjöholm and Nyström 2005), and it has been shown that elevation in an inflammatory marker, (namely) high sensitivity C-reactive protein (hsCRP), is associated with increased CVD risk in women with MetS (Ridker et al. 2003). An illustration of the suggested pathophysiology of MetS is depicted in Figure 2.4.

There are various definitions for MetS. The first international definition for MetS was introduced in 1998 by the WHO (Alberti and Zimmet 1998). Impaired glucose metabolism, insulin resistance, raised arterial pressure, raised triglyceride concentration and/or low HDL-C as well as central obesity and microalbuminuria were the components included in this definition. Insulin resistance and microalbuminuria are somewhat laboured to measure, which has restricted the use of this criterion in practice. The European Group for the Study of Insulin Resistance (EGIR) proposed their definition for MetS in 1999 (Balkau and Charles 1999). Their goal was to create a more practical criterion than the one introduced by the WHO. Microalbuminuria was not included in the EGIR definition, and fasting insulin measurement, as a marker for insulin resistance, was suggested instead of a clamp study insisted by the WHO. In 2001 the National Cholesterol Education Program (NCEP) Adult Treatment Panel III proposed their widely used clinical definition for MetS (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001). The American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI) suggested only minor adjustments to the NCEP definition in 2005. They added specific medications for the components of the syndrome to be taken into account (Grundy et al. 2005). The International Diabetes Federation (IDF) also published a worldwide definition of MetS in 2005 (Alberti et al. 2005). Both the NCEP and the IDF criteria include increased waist circumference, elevated blood pressure, and elevated concentrations of fasting blood glucose and trigylerides as well as low HDL-C as components of the syndrome. These definitions have two basic differences. First of all, the IDF definition has a significantly lower cut-off point for waist circumference than the NCEP definition. Secondly, the IDF definition

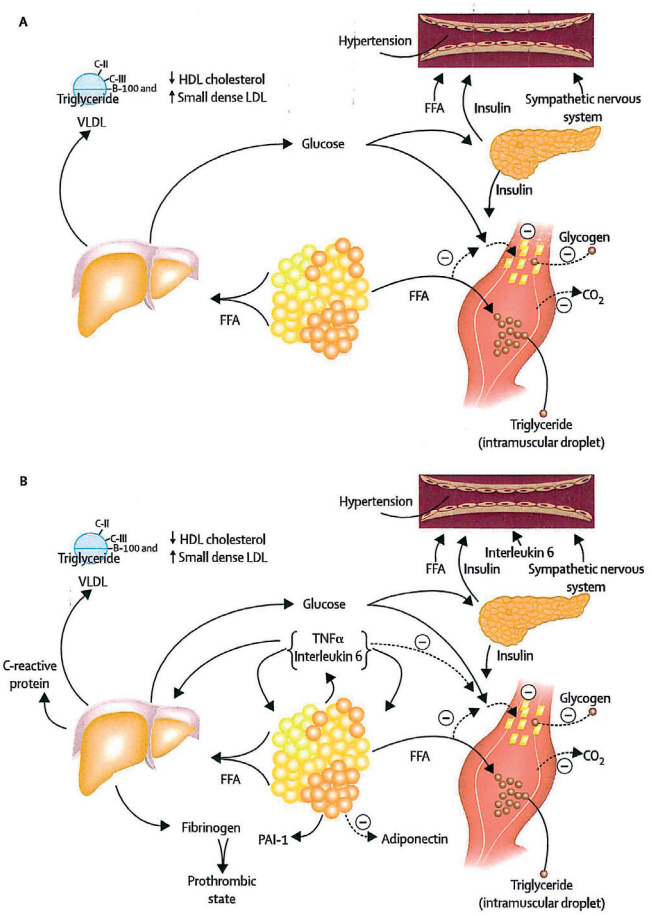

FIGURE 2.4 *The pathophysiology of metabolic syndrome (insulin resistance). A: Free fatty acids (FFAs) are released in abundance from an expanded adipose tissue mass. In the liver, FFAs induce an increased production of glucose, triglycerides and the secretion of very low-density lipoproteins (VLDL). Associated lipid/lipoprotein abnormalities include a reduction in the high-density lipoprotein (HDL) cholesterol level and an increased density of low-density lipoproteins (LDL). FFAs also reduce insulin sensitivity in muscle by inhibiting insulin-mediated glucose uptake. Associated defects include a reduction in glucose partitioning to glycogen and increased lipid accumulation in triglyceride (TG). Increases in circulating glucose and, to some extent, FFAs increase pancreatic insulin secretion, resulting in hyperinsulinaemia. Hyperinsulinaemia may result in enhanced sodium reabsorption and increased sympathetic nervous system (SNS) activity and contribute to the hypertension, as might the increased level of circulating FFAs. B: Superimposed and contributory to the insulin resistance produced by excessive FFAs is the paracrine and endocrine effect of the proinflammatory state. Produced by a variety of cells in adipose tissue, including adipocytes and monocyte-derived macrophages, the enhanced secretion of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α), among others, results in more insulin resistance and lipolysis of adipose tissue triglyceride stores to circulating FFAs. IL-6 and other cytokines also are increased in the circulation and may enhance hepatic glucose production, the production of VLDL by the liver and insulin resistance in muscle tissue. Cytokines and FFAs also increase the production of fibrinogen and plasminogen activator inhibitor-1 (PAI-1) by the liver that complements the overproduction of PAI-1 by adipose tissue. This results in a pro-thrombotic state. Reductions in the production of the anti-inflammatory and insulin sensitizing cytokine adiponectin are also associated with metabolic syndrome and may contribute to the pathophysiology of the syndrome. Reprinted from The Lancet, 365, Eckel RH, Grundy SM, Zimmet PZ, The metabolic syndrome, 1415–1428, Copyright (2005), with permission from Elsevier (Eckel et al. 2005).*

makes the presence of increased waist circumference mandatory for the diagnosis, while the NCEP definition considers waist circumference to be equally important as the other components. In 2009 the IDF and AHA/NHLBI published new unified criteria for MetS attempting to resolve the differences between the (various) definitions (Alberti et al. 2009). In these new criteria, central obesity is not an obligatory component for the syndrome, but rather one of five equally important components. However, no simple cut off value for waist circumference was suggested at this point. Population- and country-specific cut-off values are recommended by this new definition.

MetS has been shown to be a predictor of T2DM (Lorenzo et al. 2003), CHD (Bonora et al. 2003) and mortality (Isomaa et al. 2001, Lakka et al. 2002, Malik et al. 2004). The presence of MetS has been related to increased CIMT in several studies including diabetic and non-diabetic subjects (Anand et al. 2003, McNeill et al. 2004, Scuteri et al. 2004, Ahluwalia et al. 2006, Skilton et al. 2007). It has been associated with increased CIMT in subjects free of diabetes as well (Hulthe et al. 2000, Leoncini et al. 2005, Tzou et al. 2005, Mohan et al. 2006, Ishizaka et al. 2009). Arterial stiffness and MetS have also been related with one another (Scuteri et al. 2004, Ferreira et al. 2005, Li et al. 2005, Schillaci et al. 2005, Ahluwalia et al. 2006). As mentioned earlier, T2DM has been shown to increase CVD events in women to a larger extent than in men. It has also been suggested that MetS would be a stronger risk factor for early atherosclerosis in women than in men (Iglseder et al. 2005, Kawamoto et al. 2007a, Lee et al. 2010). The data regarding sex differences is, however, limited and controversial (Ishizaka et al. 2009). The various definitions and the whole concept of MetS have received much criticism (Kahn et al. 2005). There are doubts as to whether the syndrome is more than the sum of its parts as a CVD risk factor. Some investigators have reported that MetS would be a predictor of atherosclerosis independently of its components (Scuteri et al. 2004, Kawamoto et al. 2005). This issue, however, is highly controversial (Bayturan et al. 2010, Mente et al. 2010).

2.3.5 Obesity

Obesity is clearly associated with increased CVD risk (Grundy 2002). In the U.S. Diabetes Prevention Program, diabetes incidence was reduced by 58% and an average of 5.6 kg weight loss was observed with dietary changes and increased physical activity (Knowler et al. 2002). It is not quite clear how much of the association between obesity and CVD risk is mediated by co-existing deterioration in other known risk factors, such as insulin resistance, the components of MetS, physical inactivity and proinflammatory effects of obesity. It is possible, however, that obesity affects CVD risk via some as yet undiscovered mechanisms (Poirier et al. 2006).

There is strong evidence suggesting that obesity decreases life expectancy (Fontaine et al. 2003). However, some investigators have reported a less convincing effect on all cause mortality (Flegal et al. 2005), especially in regard to minor overweight (body mass index ≤30). Smoking and pre-existing disease are major confounders in the subject matter, since both tend to decrease body weight and, on the other hand, increase mortality. Indeed, obesity has been strongly associated with mortality after these factors have been eliminated (Adams et al. 2006). Body mass index (BMI) seems to have a J-shaped association with mortality, which also adds to the complexity of this topic.

2.3.6 Markers of inflammation

As discussed earlier, inflammation and proinflammatory cytokines have a crucial role in the pathogenesis of atherosclerosis (Ross 1999, Libby et al. 2010). The measurement of inflammatory markers such as hsCRP has raised more and more attention in CVD risk evaluation.

2.3.6.1 High-sensitivity C-reactive protein

CRP is the main downstream mediator of the acute phase and it plays an important role in the human innate immunity response (Pradhan et al. 2001). There is comprehensive evidence that CRP, measured with high-sensitivity assays, is an independent predictor for CVD. It predicts risk independently of the traditional risk factors included in global risk assessment algorithms, such as the Framingham Risk Score (FRS), and also independently of MetS. hsCRP has been shown to be an important risk predictor at all levels of LDL-C (Libby and Ridker 2006). It also predicts the incidence of T2DM, supporting the hypothesis that diabetes would have an inflammatory basis (Pradhan et al. 2001). The JUPITER trial investigators reported that statin therapy significantly reduced major CVD events in subjects without hyperlipidemia, which led the authors to conclude that part of the risk reduction might be caused by the anti-inflammatory effects of statins (Ridker et al. 2008). The causal role of CRP in the pathogenesis of atherosclerosis has been studied in large genetic trials. Recent reports are arguing against such a role (Zacho et al. 2008, Elliott et al. 2009).

The relationship between CRP and CIMT is controversial. There are some studies reporting a modest but independent relationship between elevated CRP and increased CIMT (Sitzer et al. 2002). Most studies have not, however, found significant correlations independently of other risk factors (Blackburn et al. 2001, Folsom et al. 2001, Makita et al. 2005, Lorenz et al. 2007). Wang et al. found an independent relationship between CRP and CIMT in women but not

in men (Wang et al. 2002). Kivimäki et al. used CRP genetics with a Mendelian randomization method, concluding that there is no causal association between CRP and CIMT (Kivimäki et al. 2007). CRP has also been related to arterial stiffness (Mattace-Raso et al. 2004, Yasmin et al. 2004, Kullo et al. 2005). Some of the studies found this relation to be independent of other CVD risk factors (Mattace-Raso et al. 2004, Yasmin et al. 2004), while other investigators concluded this to be mediated by confounding risk factors (Kullo et al. 2005).

2.3.6.2 Other markers of inflammation

Adipose tissue is a major source of inflammatory cytokine IL-6 (Allen and Febbraio 2009), which promotes CRP synthesis in the liver (Pradhan et al. 2001). It has been proposed to induce insulin resistance (Lazar 2005). The circulating IL-6 level has been reported to be elevated in obesity (Bastard et al. 2000), and IL-6, like CRP, has been shown to be a predictor for T2DM (Pradhan et al. 2001). The possible role of IL-6 in the pathogenesis of insulin resistance and diabetes is, however, controversial. It is possible that IL-6 level is a marker of obesity-induced inflammation rather than an active mediator in this process (Pradhan et al. 2001). There are also reports that IL-6 might have favourable effects in controlling obesity-associated pathology, such as opposing weight gain (Wernstedt et al. 2004, Berg and Scherer 2005). In some experimental studies IL-6 has enhanced insulin sensitivity in humans and mice (Carey et al. 2006, Sadagurski et al. 2010), raising even more controversy. It is possible that IL-6 acts as a promoter of inflammation in some situations, while depressing inflammatory process in other circumstances (Berg and Scherer 2005, Allen and Febbraio 2009). An increased IL-6 level has been suggested to predict increase in CIMT (Lee et al. 2007), and it has been related to arterial stiffness (Roman et al. 2005).

Adipose tissue produces various inflammatory modulators, such as leptin, adiponectin and resistin, which have been suggested to associate with atherosclerosis (Berg and Scherer 2005). Leptin enhances insulin sensitivity and reduces appetite. However, hyperleptinaemia has been related to insulin resistance and obesity that might be caused by leptin resistance in tissues, and it may act as a pathophysiological trigger in CVD (Ren 2004). The adiponectin level is decreased in obese subjects (Berg and Scherer 2005), and subjects with the highest adiponectin levels have a markedly reduced CVD risk (Pischon et al. 2004). It has been suggested that adiponectin acts directly on endothelial and vascular smooth muscle cells. It also has anti-inflammatory and insulin-sensitizing effects, which makes it an interesting substance regarding the pathogenesis of insulin resistance and MetS (Goldstein and Scalia 2004, Cornier et al. 2008). Resistin is another adipocyte-derived substance that has been linked to obesity, MetS and diabetes. It has been hypothesized that resistin may play a pathogenic role in insulin resistance-related abnormalities (Cornier et al. 2008).

2.3.7 Genetic risk factors

Atherosclerosis has a genetic basis, and its heritability has been estimated to be nearly 50% (Zdravkovic et al. 2002). However, there are no genetic screening tests in a substantive role in clinical practice yet (Ridger and Libby 2008). Part of the difficulty in creating such a test is caused by the fact that atherotrombotic disease is a complex process with multiple genetic factors contributing to the overall risk (Watkins and Farrall 2006). An example of a genetic risk factor for CHD that has been well characterized is a polymorphism in the apoE gene. In a large meta-analysis carriers of the epsilon 4 allele had a 42% higher risk for CHD when compared to subjects with the epsilon 3/3 genotype (Song et al. 2004).

Common variations in the genetic code are called polymorphisms. This means that they are present in more than 1 percent of the population. A single nucleotide polymorphism (SNP) is a common variation in single nucleotide that does not necessarily alter protein structure. Lately, it has become possible to use powerful genome-wide scan techniques (Kennedy et al. 2003) in genetic studies. Hundreds of thousands of SNPs and alleles can now be investigated simultaneously.

There are several methods in regard to genetic studies that can be utilized in different situations. So-called linkage analysis can be used to identify genomic regions that contain a possible disease gene. This method is usually applied to study Mendelian traits, which are controlled by a single gene locus, and it is performed in families (Dawn Teare and Barrett 2005). In genetic association studies polymorphisms are associated with traits, which can be, for example, some quantitative characteristics or diseases (Cordell and Clayton 2005). Association studies can be used to investigate common disorders with no clear Mendelian inheritance (Lohmueller et al. 2003). The difference between linkage and association studies is that in the latter the same allele (or alleles) is associated with a trait in a similar manner across the whole population. Linkage, on the other hand, allows different alleles to be associated with a trait in different families (Cordell and Clayton 2005). Mendelian randomization is a method used in genetic epidemiology based on Mendel's second law according to which inheritance of one trait is independent of the inheritance of other traits. In CVD epidemiology, for example, a common genetic polymorphism known to have an effect on a certain risk factor can be used. In this method the association between a genetic variant and the investigated outcome is not generally confounded by other risk factors. This means that an epidemiologic study of genetic variants has similar properties as the intention to treat analyses in randomized controlled trials (Davey Smith and Ebrahim 2005).

2.3.7.1 Genome-wide association approach: the coronary heart disease risk variant on chromosome 9p21.3 (rs1333049)

Recent genome-wide association studies have identified several novel loci that are strongly associated with CHD. Specifically, a common variant located in a region adjacent to the cyclin-dependent kinase inhibitors CDKN2A (encoding p16INK4a) and CDKN2B (p15INK4b) on chromosome 9p21.3 has been associated with increased risk in several separate genome-wide association and follow-up studies (Burton et al. 2007, Helgadottir et al. 2007, McPherson et al. 2007, Samani et al. 2007, Schunkert et al. 2008). Of the SNPs in this locus, rs1333049 is the one with the strongest association. The risk-associated alleles, defined by the C allele of rs1333049, or alleles of other SNPs in strong linkage disequilibrium with it, have consistently shown an increased risk of 25% to 40% per copy of allele. The region associated with CHD on chromosome 9p21.3 is located adjacent to the genes that play a central role in the regulation of the cell cycle and may be implicated in the pathogenesis of atherosclerosis through their role in transforming growth-factor-β-induced growth inhibition (Hannon and Beach 1994, Kalinina et al. 2004). Interestingly, rs1333049 is located on a region of 9p21.3 that does not contain a protein-coding gene. Recent studies have shown that this region contains a gene for a large noncoding RNA, ANRIL, which is expressed in atherosclerotic tissue (Pasmant et al. 2007, Broadbent et al. 2008). It has been suggested that ANRIL may regulate the expression of adjacent genes (Pasmant et al. 2007). The association of locus 9p21.3 with early atherosclerosis has not been studied extensively.

2.3.7.2 Candidate gene approach: IL6–174 G>C genotype

A single G>C base exchange polymorphism in the promoter region of the IL-6 gene (IL6–174 G>C, rs1800795) on chromosome 7 has been associated with IL-6 production. In some studies, allele G homozygous and G/C heterozygous subjects have shown a higher expression of the IL-6 protein, higher transcriptional activity and higher inducible IL-6 responses than those homozygous for allele C (Fishman et al. 1998, Burzotta et al. 2001, Hulkkonen et al. 2001). In some populations higher plasma levels of IL-6 have been associated with allele C (Chiappelli et al. 2005, Boiardi et al. 2006). The effect of this polymorphism on circulating IL-6 level is therefore complex, and a recent joint analysis of participants in 17 studies did not find any significant association between the IL6–174 G>C polymorphism and circulating IL-6 level (Huth et al. 2009). The authors of this joint analysis concluded that it is possible that the effect on IL-6 level may be seen only in certain risk populations such as diabetics.

The relationship of this IL-6-related polymorphism with cardiovascular risk factors and the risk for cardiovascular events has also been controversial. Some studies have related allele C to an increased risk of myocardial infarction (Georges et al. 2001), while others have not found this association (Bennet et al. 2003). A recent meta-analysis did not observe a significant association between this polymorphism and the risk for CHD (Sie et al. 2006). Results have been controversial also in regard to the association of this polymorphism and stroke. In a systematic review by Tso et al., the authors concluded that there seems to be an association between IL6–174 G>C polymorphism and stroke, but the risk allele cannot be clearly assigned (Tso et al. 2007). Allele G has been related to peripheral arterial disease (Flex et al. 2002, Libra et al. 2006). On the other hand some reports suggest that allele C would be associated with increased mortality (Hurme et al. 2005).

Allele C has also been linked to obesity in some studies (Klipstein-Grobusch et al. 2006), but in the joint analysis by Huth et al. no association between BMI and the IL6–174 G>C polymorphism was found (Huth et al. 2009). Higher CRP level (Sie et al. 2006) and insulin resistance (Kubaszek et al. 2003) have also been linked to allele C. Some studies, however, have suggested that G allele carriers would be more insulin resistant and thus at greater risk regarding this matter (Fernandez-Real et al. 2000). Indeed, allele C was reported to be protective against T2DM in a joint analysis of 21 different studies (Huth et al. 2006). Furhtermore, in a more recent joint analysis, carriers of this allele had lower fasting glucose levels independently of BMI (Huth et al. 2009).

Allele C of this polymorphism has been previously associated with increased CIMT in British subjects (Mayosi et al. 2005). The same report also included a meta-analysis of previous studies with similar results. A recent study by Hulkkonen et al. found an association between allele C and increased arterial stiffness among young men in the Cardiovascular Risk in Young Finns Study cohort (Hulkkonen et al. 2009).

2.3.8 Other risk factors

There are numerous other proposed risk factors for atherosclerosis. Homocysteine is an amino acid produced by methionine metabolism. Hyperhomocysteinaemia, usually due to an insufficient dietary intake of folic acid, has been suggested to increase the risk of CHD (Boushey et al. 1995). In a study using Mendelian randomization, a causal role of hyperhomocysteinaemia and stroke was suggested (Casas et al. 2005). In a large systematic review of 57 studies, however, homocysteine concentration was only weakly related to CHD (Ford et al. 2002).

More importantly, clinical trials of homocysteine reduction have failed to show considerable benefits (Lonn et al. 2006). The role of homocysteine as a CVD risk factor remains to be clarified.

Fibrinogen is an acute-phase reactant and major determinant of blood viscosity and platelet aggregation. It has been strongly related to CVD risk (Danesh et al. 2005), but studies investigating the effect of fibrinogen reduction have not yielded convincing benefits (Meade et al. 2002). Markers of fibrinolysis, such as plasminogen activator inhibitor-1 (PAI-1), have also been investigated as possible risk factors. PAI-1 is an endogenous inhibitor of fibrinolysis. There are reports relating PAI-1 to CHD, but it has not been demonstrated to add considerably to the risk as defined by traditional risk factors (Ridger and Libby 2008).

3 AIMS OF THE STUDY

Obesity has become a major risk factor for cardiovascular disease. It predisposes to insulin resistance, MetS and T2DM. The independent roles of these metabolic abnormalities in the atherosclerotic process, however, have not been clarified. On the other hand, atherosclerosis has a strong genetic basis that is not well characterized. These questions were investigated in the present study, with (particular) focus on the early stages of atherosclerosis. Two approaches were used in the selection of the contemporary genetic variants of interest: the polymorphism most strongly associating with CHD in genome-wide association studies, and a polymorphism strongly associating with cardiovascular risk factors and CHD in candidate gene studies were selected to be investigated in detail. Therefore, the roles of two single nucleotide polymorphisms, rs1333049 (on chromosome 9p21.3) and IL6–174 G>C, in the early stages of atherosclerosis were evaluated. A sub-population of a large Finnish health examination survey, the Health 2000 Survey, and the population of the Cardiovascular Risk in Young Finns Study, a multi-centre study of atherosclerosis risk factors in children and young adults, were used in the investigations. The specific aims of the present thesis are as follows:

1. To investigate the independent associations of different stages of glucose intolerance with early carotid atherosclerosis (original publication V).
2. To evaluate the independent association of metabolic syndrome with early atherosclerosis (original publications I and III).
3. To study possible sex-related differences in the associations of metabolic syndrome, glucose intolerance and early atherosclerosis (original publications I and V).
4. To study the relationship of the CHD-associated variant on chromosome 9p21.3 and subclinical atherosclerosis (original publication II).
5. To evaluate whether IL6–174 G>C genotype is related to the risk factors and markers of early atherosclerosis (original publication IV).

4 SUBJECTS AND METHODS

4.1 Subjects

4.1.1 The Health 2000 Survey

A subpopulation of a large Finnish cross-sectional health examination survey (the Health 2000 Survey) carried out in 2000–2001 (Aromaa and Koskinen 2004) was studied. The overall study cohort was a two-stage stratified cluster sample (8,028 persons) representing the entire Finnish population aged 30 years and older. To study CVD and diabetes more thoroughly, a supplemental study was carried out (sample size 1,867; participation rate 82%). Subjects in the supplemental study, a subpopulation of the Health 2000 Survey, were 45 years and older, and the study was executed in the areas located within 150 km from the five Finnish University Hospitals, because specialized equipment was required. In addition to detailed risk factor assessments, a carotid ultrasound examination and an oral glucose tolerance test (OGTT) were included in the supplemental study. There were 1,353 subjects (607 men and 746 women; mean age, 58 years; range, 46–76 years) with available carotid ultrasound data. Subjects with type 1 diabetes were excluded from the study investigating the effect of glucose tolerance on early atherosclerosis. After this exclusion there were 1,304 subjects (579 men and 725 women; mean age, 58 years; range, 46–76 years) with available carotid ultrasound and glucose tolerance status data. For the genetic studies, there were 1,334 and 1,295 subjects with available carotid ultrasound and genotyping data for IL6–174 G>C and rs133049, respectively.

In the catchment areas of Tampere and Turku University Hospitals, 401 individuals (176 men and 225 women; mean age, 58 years; range, 46–76 years) of the supplemental study underwent the whole-body impedance cardiography measurements. The study areas from the original Health 2000 Survey are shown in Figure 4.1.

4.1.2 The Cardiovascular Risk in Young Finns Study

The Cardiovascular Risk in Young Finns Study is a multi-centre study of atherosclerosis risk factors in children and young adults. The first cross-sectional study was conducted in 1980 and included 3,596 healthy children and adolescents

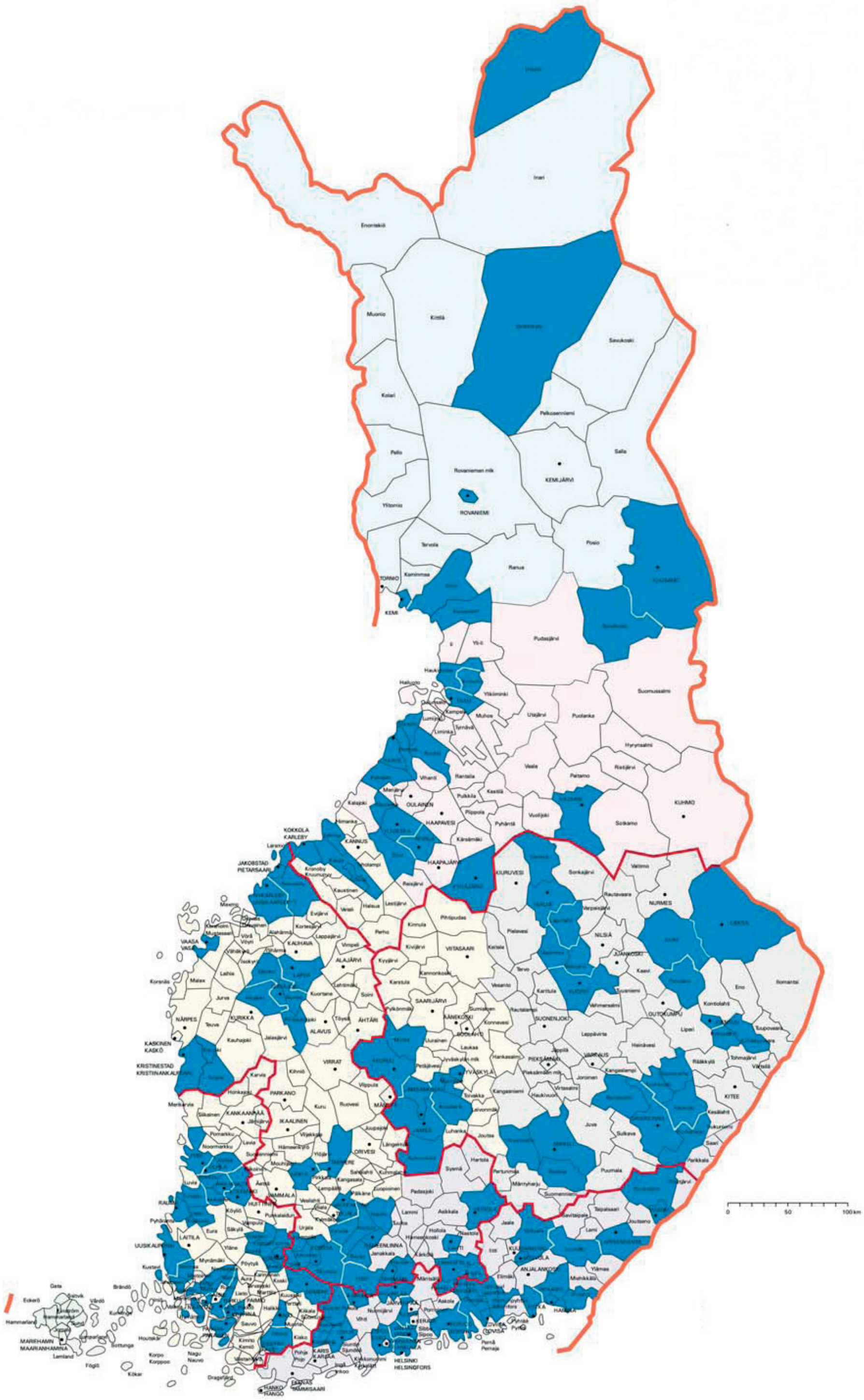

FIGURE 4.1 *Study areas of the original Health 2000 Survey.*

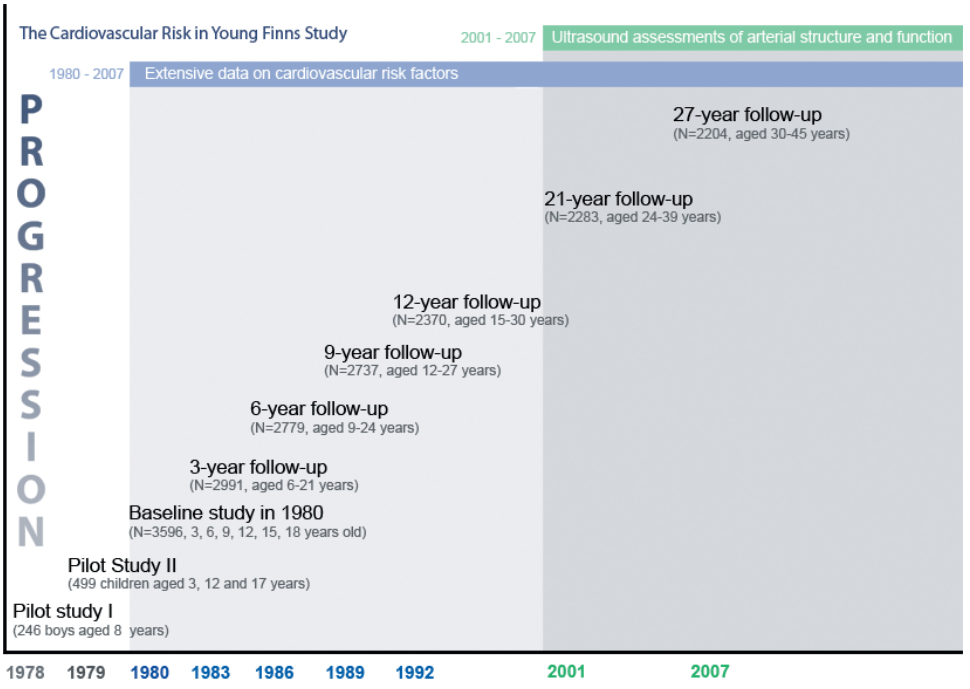

FIGURE 4.2 *Progression of the Cardiovascular Risk in Young Finns Study.*

aged 3, 6, 9, 12, 15 and 18 years. Thereafter, these subjects have been followed with periodic examinations (Figure 4.2.). In 2001, 2,620 individuals, who had then reached the age of 24–39 years, were studied (Juonala et al. 2004a). In addition to detailed risk factor assessments, ultrasound measurements of CIMT and FMD were carried out (Raitakari et al. 2003, Juonala et al. 2004b) as well as genotyping for the coronary artery disease risk variant on chromosome 9p21.3. Genotyping was available for 2,277 individuals and CIMT and FMD data for 2,251 and 2,095 subjects, respectively.

4.2 Methods

4.2.1 Physical examination and questionnaires

4.2.1.1 The Health 2000 Survey

Waist circumference was not measured in the supplemental study; therefore, the Health 2000 Survey parameters were used to define the presence of MetS. The mean

time interval between the Health 2000 Survey and the supplemental study was 1 year and 4 months (range 10–23 months). The mean (±SD) change in body weight during this time was 0.52 (±3.43) kg. This was not considered clinically significant, and it is likely that the change in waist circumference was not clinically significant either.

Waist circumference was measured with the subjects in the standing position by using the standards created for population health studies (Seidell et al. 2001). In the Health 2000 Survey, blood pressure was measured from the right arm with a mercury sphygmomanometer (Mercuro 300, Speidel & Keller, Juningen, Germany). The first measurement was taken after the subjects had rested at least 5 minutes in sitting position. Korotkoff's first phase was used as the sign of SBP and the fifth phase as the sign of diastolic blood pressure (DBP). The measurement was repeated 2 minutes after the first measurement and the average of the 2 measurements was used to define the presence of MetS. Current smoking was evaluated with a questionnaire and this data was also collected from the Health 2000 Survey. Those who were currently smoking were defined as smokers and the rest of the subjects as non-smokers.

All the other measurements were collected from the data of the supplemental study. In the supplemental study, blood pressure was measured from the right arm after at least 10 minutes' rest. The measurement was taken 3 times with 1- to 2-minute intervals. The automatic Omron M4 manometer (Omron Matsusaka, Japan, and Netherlands) was used in these measurements. The average of the 3 measurements was used in the analysis. Pulse pressure was calculated as the remainder of the average systolic and the average diastolic blood pressure. Height and weight were measured and BMI was calculated.

4.2.1.2 The Cardiovascular Risk in Young Finns Study

Blood pressure was measured using a random-zero sphygmomanometer. Values for SBP and DBP were defined by Korotkoff phases I and V, respectively. The averages of 3 measurements obtained after 5 minutes of sitting with 1 to 2 minutes between readings were used in the analyses. Height and weight were measured and BMI was calculated. Smoking habits were ascertained with a questionnaire.

4.2.2 Blood collection and analyses

In both study populations, venous blood samples were drawn from the antecubital vein after an overnight fast. HDL-C, total cholesterol and triglyceride concentrations were determined enzymatically (Roche Diagnostics, GmbH, Mannheim, Germany for HDL-C; Olympus System Reagent, Hamburg, Germany for total cholesterol and triglycerides) with a clinical chemistry

analyzer (Olympus, AU400, Hamburg, Germany). LDL-C was calculated with the Friedewald formula. Concentrations of hsCRP were determined using a chemiluminescent immunometric assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA). OGTT (in the Health 2000 Study only) was carried out after 10–12 hours of fasting. Subjects were given 75 g of glucose in a 10% solution. Venous blood samples for glucose determination were taken before and 2 h after the glucose load. Plasma glucose was determined by the glucose dehydrokinase method (Diagnostica Merck, Darmstadt, Germany) in a clinical chemistry analyzer (Konelab, Vantaa, Finland).

4.2.3 Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit (Qiagen Inc., Hilden, Germany). rs1333049 was genotyped by means of allelic discrimination using a standard TaqMan assay. Fluorescence was detected post polymerase chain reaction using the ABI Prism 7900HT Sequence Detection System, and genotypes were called with ABI Prism SDS software version 2.1 (Applied Biosystems, Foster City, CA, USA). IL6–174 G>C genotyping was performed by employing the 5' - nuclease assay and fluorogenic allele-specific TaqMan probes and primers (Livak 1999) as well as the ABI Prism 7900HT Sequence Detection System.

4.2.4 Metabolic syndrome, glucose tolerance and Framingham risk scoring

Two different criteria were used to define MetS. According to the NCEP definition (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001), MetS is present if at least three of the following criteria are met: waist circumference > 102 cm in men and > 88 cm in women, triglycerides ≥ 1.7 mmol/L, HDL cholesterol < 1.03 mmol/L in men and < 1.29 mmol/L in women, blood pressure ≥ 130/≥ 85 mmHg, and fasting glucose ≥ 5.6 mmol/L. The fasting glucose threshold of the NCEP criterion was modified in 2004 (Grundy et al. 2004b). According to the IDF definition (Alberti et al. 2005), MetS is present if waist circumference is increased (≥ 94 cm for men and ≥ 80 cm for women) and at least two of the following four factors are present: 1) triglycerides ≥ 1.7 mmol/L, or specific treatment for this lipid abnormality; 2) HDL cholesterol < 1.03 mmol/L in men and < 1.29 mmol/L in women, or specific treatment; 3) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, or

treatment for previously diagnosed hypertension; and 4) fasting plasma glucose ≥ 5.6 mmol/L, or previously diagnosed type 2 diabetes.

Subjects using insulin were considered to have T2DM and did not participate in OGTT. Subjects with oral hypoglycaemic medication or a previous T2DM diagnosis were considered to have T2DM, regardless of their OGTT results. The ADA criteria for diabetes (Genuth et al. 2003) were used in the classification of subjects with no previously diagnosed diabetes as follows: 1) subjects with fasting venous plasma glucose of >7.0 mmol/l or 2-h venous plasma glucose of >11.1 mmol/l in an OGTT were considered to have T2DM; 2) subjects with fasting venous plasma glucose of <7.0 mmol/l and 2-h venous plasma glucose of 7.8–11.0 mmol/l in an OGTT were considered to have IGT; 3) subjects with fasting venous plasma glucose of 5.6–6.9 mmol/l and 2-h venous plasma glucose of <7.8 mmol/l in an OGTT were considered to have IFG; and, finally, 4) subjects with fasting venous plasma glucose of <5.6 mmol/l and 2-h venous plasma glucose of <7.8 mmol/l in an OGTT were considered to have NGT. In this classification, subjects with isolated IGT (normal fasting glucose) and those with both IFG and IGT are categorized into the same group (IGT group). IFG and IGT are considered as distinct entities, and it would have been logical to report the results for isolated IFG and isolated IGT separately. However, the number of subjects with isolated IGT was very small (n=15 in a smallest subgroup), and for this reason the results are presented with four glucose tolerance categories, as described above.

The Framingham risk score was defined using previously published CHD score sheets (Wilson et al. 1998). It gives an estimation of a subject's risk for CHD over a period of 10 years. The risk score is calculated using the information of the subject's sex, age, diabetes status, LDL-C and HDL-C levels, blood pressure and smoking status.

4.2.5 Ultrasound examinations

4.2.5.1 The Health 2000 Survey

After a 5-minute rest in the supine position, blood pressure was measured from the right arm with a digital blood pressure monitor three times at 1.5-minute intervals. Pulse pressure was calculated as the remainder of mean SBP and DBP. High-resolution B-mode carotid ultrasound examination of the right carotid artery was performed immediately after the blood pressure measurement according to a standardized protocol. A 7.5 MHz or 10 MHz linear array transducer was used. The jugular vein was used as an acoustic window whenever possible as described by Selzer (Selzer et al. 1994). The sonographer first focused on the distal 1 cm of the common carotid artery (CCA) using the beginning of the carotid artery

bulb (the site where the two parallel walls of the CCA diverge) as an anatomical landmark. The transducer was positioned to visualize both the far and near wall lumen-intima and media-adventitia interfaces at a single angle. The sonographer then focused on the carotid artery bulb whose distal boundary was the flow divider and proximal boundary the site where the two parallel walls of the CCA diverge. The transducer was positioned to visualize the far wall interfaces at three interrogation angles (lateral, anterior and posterior).

One reader was responsible for reading all the ultrasound images. The computer software PROSOUND (Prosound, University of California (Selzer et al. 1994, Selzer et al. 2001)) and its Windows version PROWIN 23.1 were used to track the far wall lumen-intima and media-adventitia echoes to determine CIMT over the distal 1 cm segment of the CCA and the carotid bulb. Three summary measures were calculated: 1) the mean of the three average intima-media thickness (IMT) measurements of the CCA (mean CCA IMT), 2) the mean of the three average IMT measurements of the carotid bulb (mean bulb IMT), and 3) the mean of these two means (mean IMT). The latter was used as a summary measure in this study. The maximum CCA IMT value was also used in the present study.

Carotid artery elasticity indices were used to evaluate arterial stiffness. For the elasticity calculations, the computer software PROWIN 23.1 was used to determine the arterial diameter over the distal 1 cm length of the CCA in three images at peak systole and end diastole. Systolic and diastolic arterial diameters were calculated as the mean of the three systolic and diastolic arterial diameters, respectively.

Elasticity measurements

On the basis of the ultrasound measurements and supine blood pressure measurements taken immediately before the ultrasound examination, the following indices of arterial elasticity were calculated:

$$Ep\ (mmHg) = (PP \times DAD)/ADC$$
$$YEM\ (mmHg) = [Ep \times DAD/(2 \times IMT)]$$
$$SI = Ln\ (SBP/DBP)/(ADC/DAD)$$
$$CAC\ (\%/10\ mmHg) = 100 \times 10 \times [(ADC/DAD)/PP]$$

where, Ep = Peterson's elastic modulus; PP (pulse pressure) = systolic blood pressure - diastolic blood pressure; ADC (arterial diameter change) = systolic arterial diameter (SAD) - diastolic arterial diameter (DAD); YEM (Young's elastic modulus); IMT = intima-media thickness (of the far wall of CCA at end diastole); SI = beta stiffness index; SBP = systolic blood pressure; DBP = diastolic blood pressure; CAC = carotid artery compliance.

4.2.5.2 The Cardiovascular Risk in Young Finns Study

In the Cardiovascular Risk in Young Finns Study, carotid ultrasound examinations were performed using a high-resolution ultrasound system (Sequoia 512, Acuson) with a 13.0 MHz linear array transducer. CIMT was measured at roughly 10 mm from the bifurcation on the left CCA, 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 (Raitakari et al. 2003). At least four measurements were taken at each scan of the CCA incident with the R-wave of the continuously monitored electrocardiogram to derive mean and maximum CIMT. One reader blinded to subjects' details analyzed the scans. To assess brachial artery FMD, the left brachial artery diameter was measured both at rest and during reactive hyperaemia. Increased flow was induced by the inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 minutes, followed by release. Three measurements of arterial diameter were performed at end-diastole at a fixed distance from an anatomic marker at rest and at 40, 60 and 80 seconds after cuff release. The vessel diameter after reactive hyperaemia was expressed as the percentage relative to the resting scan. The average of the three measurements at each time point was used in the calculation of the maximum FMD (the greatest value between 40 and 80 seconds) (Juonala et al. 2004b).

4.2.6 Pulse wave velocity measurements

PWV was measured with ICG_{WB} using a commercially available circulation monitor device CircMon B202 (JR Medical Ltd, Tallinn, Estonia) (Figure 4.3.). Subjects were first interviewed and then electrodes (Blue Sensor type R-00-S; Medicotest A/S, Ölstykke, Denmark) were applied in a supine position for at least 15 minutes prior to the 10-minute PWV measurements. A pair of electrically connected current electrodes was placed on the distal part of the extremities just proximal to the wrists and ankles. Voltage sensing electrodes were placed proximally to the current electrodes, with a distance of 5 cm between the centres of the electrodes. With this electrode configuration, the recorded heart-synchronous changes in impedance reflect the weighted sum of the pulsatile plethysmograms of the vessels between the electrodes, i.e. almost the whole vascular system. The foot of the whole-body impedance cardiogram coincides with pulse transmission in the aortic arch, making it possible to estimate the beginning of pulse wave transmission in the arterial system. Identically, with voltage-sensing electrodes applied to any distal region between the current electrodes, pulse-related impedance changes can be recorded. In this population the distal impedance plethysmogram was recorded

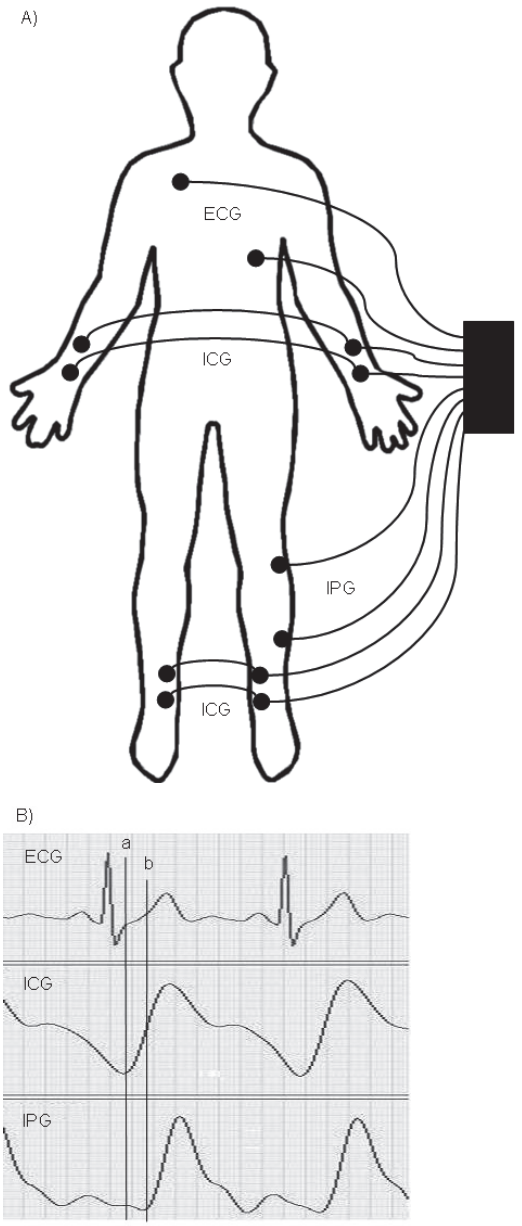

FIGURE 4.3 *A, Placement of electrodes in whole-body impedance cardiography with an additional voltage-sensing channel on the left calf for PWV measurement. B, Synchronous recording of ECG, whole-body ICG, and IPG. The time difference between the feet of the ICG (a) and IPG (b) indicates the pulse transit time from aortic arch to popliteal artery. PWV = pulse wave velocity, ECG = electrocardiogram, ICG =impedance cardiography, IPG =impedance plethysmogram. Reprinted from Aatola H, Hutri-Kähönen N, Juonala M, Viikari JS, Hulkkonen J, Laitinen T, Taittonen L, Lehtimäki T, Raitakari OT, Kähönen M. Lifetime risk factors and arterial pulse wave velocity in adulthood: the cardiovascular risk in young Finns study, Hypertension, 55, 3, 807, with permission from Wolters Kluwer Health (Aatola et al. 2010).*

from a popliteal artery at knee joint level. The active electrode was placed on the lateral side of the knee joint and the reference electrode on the calf, the distance between the electrodes being approximately 20 cm. The time difference between the feet of these impedance plethysmograms, recorded from the aortic arch and popliteal artery, was measured. The time resolution of the CircMon recordings was 5 ms. The evaluation of the ICG_{WB} method and PWV measurement using ICG_{WB} has been described in detail previously (Kööbi et al. 1997, Kööbi et al. 2003). The reproducibility values of the PWV measurements by whole-body impedance cardiography (2.42 m/s) and Doppler ultrasound (2.17 m/s) are similar (Kööbi et al. 2003).

4.2.7 Statistical methods

Statistical analyses were performed using SPSS for Windows (versions 12.0, 13.0, 14.0, 15.0, 16.0.1; SPSS Inc., Chicago, IL, USA). In the analysis of the genetic studies, the Statistical Analysis System, SAS (version 8.1; SAS Institute Inc., Cary, NC, USA), Statistica for Windows 6.0 (StatSoft Inc., Tulsa, OK, USA) and Arlequin (Excoffier et al. 2005) were also employed. The skewed distributions of triglycerides and hsCRP were corrected logarithmically before statistical analyses. Sufficiently normal distributions were achieved with these corrections.

Possible interactions were tested using the SPSS General Linear Model. Student's t-test (in case there were two groups) or analysis of variance (ANOVA - in case there were more than two groups) were applied to compare the means of continuous variables. Tukey's or Dunnett's T3- test was used for multiple comparisons in ANOVA. The Chi-square test was employed in the assessment of the differences in prevalence rates. Pearson's correlation coefficient was used to examine univariate associations of risk factors and markers of early atherosclerosis. The SPSS general linear model (ANCOVA) was used in the calculation of adjusted means and in testing the significance in differences between them. In case the ANCOVA p value for the variable of interest was significant, Fisher's least significant difference test was used in pair-wise group comparisons. Linear regression analysis was performed to examine independent relationships between CVD risk factors and the markers of early atherosclerosis.

Univariate data comparisons between rs1333049 genotype groups (and between subjects in the two studies) were based on ANOVA for continuous variables and Chi-square test for categorical variables. In the analyses regarding the rs1333049 genotype, linear regression analysis was used to identify clinical and laboratory variables that were independently associated with subclinical atherosclerosis and to assess the effect of this genotype on early atherosclerosis taking these variables into account. 95% CI for allele frequencies were calculated using the confidence

interval calculation programme CIA (version 2.1.2).

Kruskall-Wallis ANOVA was used to evaluate the association of IL6–174 G>C with the risk factors and early markers of atherosclerosis. The extent of the genetic effect of IL6–174 G>C on selected risk factors was studied with linear regression analysis. Before the analysis the regression models were assessed for multicollinearity. Variance inflation factors for covariates in the presented models were at acceptable levels, ranging from 1.023 to 1.735 (tolerances ranging from 0.576 to 0.988).

5 RESULTS

5.1 Characteristics of the study populations

The main study population of this thesis consisted of the subjects in the supplemental study of the Health 2000 Survey. There were 1,353 subjects with available carotid ultrasound data in this population. The clinical characteristics of these subjects are presented in Table 5.1. The relationship of the CHD-associated variant on chromosome 9p21.3 (rs1333049) and early carotid atherosclerosis was investigated in the population of the Cardiovascular Risk in Young Finns Study (studied in 2001) as well. In this population, there were 2,277 individuals with available genotyping data for rs1333049. Their clinical characteristics are shown in Table 5.2.

5.2 Association of glucose tolerance and early atherosclerosis (original publication V)

The effect of glucose tolerance status on CIMT and carotid artery elasticity (YEM, SI, and CAC) was studied in the Health 2000 Survey supplemental study population. The interaction between sex and glucose tolerance, in determining CIMT and the elasticity indices, was tested. A statistically significant interaction ($p < 0.05$) was observed regarding CIMT and CAC, and therefore the results are reported separately for men and women. The clinical characteristics and the unadjusted means for the markers of carotid atherosclerosis according to glucose tolerance status are presented in Tables 5.3 and 5.4 for men and women, respectively. Of the 125 (66 men and 59 women) diabetic subjects, 51 (28 men and 23 women) had previously diagnosed T2DM. Of these 51, 11 (6 men and 5 women) subjects used insulin and 31 (20 men and 11 women) were on oral hypoglycaemic medication. There was an overall tendency for both sexes towards a deteriorating trend in various CVD risk factors with the worsening of glucose tolerance.

Unadjusted means of CIMT and elasticity indices had a worsening trend in both sexes across the glucose tolerance continuum, with the exception that the lowest mean CIMT in men was seen in the IFG group. Significant differences between the groups were seen in all variables and in both sexes (ANOVA $p<0.01$ for all). In pair-wise group comparisons, means for all variables in the T2DM group were significantly worse as compared to the NGT group in men and

TABLE 5.1 *Clinical and laboratory parameters of the Health 2000 Survey supplemental study population with available carotid ultrasound data (n = 1353).*

	Male n=572-607#	Female n=717-746#	*p*
Age (years)	58.0±7.8	58.4±8.2	0.431
Current smoking (%)*	27.5	18.9	<0.001
Body mass index (kg/m^2)	27.4±3.9	27.0±4.8	0.077
Waist circumference (cm)*	99.2±11.3	88.8±12.7	<0.001
HDL cholesterol (mmol/l)	1.4±0.4	1.7±0.4	<0.001
LDL cholesterol (mmol/l)	3.4±0.9	3.4±0.9	0.269
Total cholesterol (mmol/l)	5.5±1.0	5.7±0.9	0.005
Triglycerides (mmol/l)	1.5±1.2	1.3±0.6	<0.001
Fasting glucose (mmol/l)	6.1±1.3	5.6±1.0	<0.001
2 h glucose (mmol/l)	7.1±3.2	6.9±2.8	0.402
hsCRP (mg/l)	2.8±4.0	3.0±4.5	0.586
SBP (mmHg)	141.4±20.4	136.4±22.5	<0.001
DBP (mmHg)	87.3±10.8	82.0±9.7	<0.001
PP (mmHg)	54.1±13.8	54.4±16.2	0.771
Medication for hypertension	28.3	29.6	0.604
Statin medication	13.0	10.7	0.193
MetS defined by NCEP (%)*	39.7	35.8	0.139
MetS defined by IDF (%)*	48.6	41.0	0.005
IFG	41.1	24.8	<0.001
IGT	18.2	16.3	0.366
T2DM	10.3	7.4	0.062

Values are means ± SD except for the prevalences of current smoking, medications, MetS, IFG, IGT and DM2, which are expressed as %. HDL= high-density lipoprotein, LDL= low-density lipoprotein, 2 h glucose = plasma glucose concentration 2 hours after glucose ingestion in the oral glucose tolerance test, hsCRP = high sensitivity C-reactive protein, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure, MetS = metabolic syndrome, NCEP = National Cholesterol Education Program, IDF = International Diabetes Federation, IFG = impaired fasting glucose, IGT = impaired glucose tolerance, T2DM = type 2 diabetes.

* Data from the Health 2000 Survey. # Some variables have missing data.

women ($p<0.05$ for all). In women, the IGT group also differed significantly from the NGT group in regard to all dependent variables and the IFG group in regard to CIMT and CAC ($p<0.01$ for all). In women there seemed to be a larger change in the elasticity indices across the glucose tolerance continuum than in men.

TABLE 5.2 *Clinical and laboratory parameters in the population of the Cardiovascular Risk in Young Finns Study (studied in 2001) with available genotyping data for rs1333049 (n=2277).*

	Male	Female	
	n=992-1022#	n=1220-1255#	*p*
Age (years)	31.7±5.0	31.7±5.0	0.994
Current smoking (%)	30.0	19.6	<0.001
Body mass index (kg/m²)	25.7±4.1	24.5±4.6	<0.001
Waist circumference (cm)	89.8±10.8	79.3±11.4	<0.001
HDL cholesterol (mmol/l)	1.2±0.3	1.4±0.3	<0.001
LDL cholesterol (mmol/l)	3.4±0.9	3.2±0.8	<0.001
Total cholesterol (mmol/l)	5.3±1.0	5.1±0.9	<0.001
Triglycerides (mmol/l)	1.5±1.0	1.2±0.7	<0.001
Fasting glucose (mmol/l)	5.2±0.9	4.9±0.7	<0.001
hsCRP (mg/l)	1.5±3.3	2.3±4.4	<0.001
SBP (mmHg)	121.6±12.3	112.7±12.3	<0.001
DBP (mmHg)	73.2±11.2	68.8±10.0	<0.001
Medication for hypertension	2.7	2.5	0.807
Cholesterol medication	0.1	0.6	0.030

Values are means ± SD except for the prevalences of current smoking and medications, which are expressed as %. HDL= high-density lipoprotein, LDL= low-density lipoprotein, hsCRP = high sensitivity C-reactive protein, SBP = systolic blood pressure, DBP = diastolic blood pressure.

Some variables have missing data.

Age-adjusted means in the markers of early atherosclerosis according to the glucose tolerance status are shown in Table 5.5. In 56 men and 75 women, carotid artery elasticity indices could not be determined. The number of subjects in the statistical analysis was therefore smaller in regard to these indices as compared to CIMT. Significant differences between the groups were seen in all variables and in both sexes (ANCOVA $p<0.05$ for all). In men, CIMT was significantly increased in subjects with T2DM as compared to those without glucose metabolism impairment. In women, however, mean CIMT increased with the worsening of glucose tolerance, and all the other groups had a significantly higher CIMT than the NGT group. Mean CAC was significantly lower in all glucose intolerance groups and mean YEM significantly higher in the IGT and T2DM groups when compared to the NGT group in both sexes. In women, the change across the groups appeared to be larger in both YEM and CAC than in men. In women, the T2DM and IGT groups had a significantly higher mean SI than the NGT group, while in men only the T2DM group differed significantly from the NGT group in regard to SI.

TABLE 5.3 *Clinical characteristics of the male study subjects according to the glucose tolerance status.*

	Glucose tolerance				
	NGT	IFG	IGT	T2DM	ANOVA
	n = 152-174*	n = 215-235*	n = 97-104*	n =58-66*	p-value
Age (years)	57.8±8.1	56.7±7.0	59.5±8.2	61.3±7.8#	<0.001
Smoking (%)	30.5	29.4	20.2	24.2	0.230
SBP (mmHg)	135.8±19.2	140.4±18.5	146.2±21.9§	150.8±23.2¶	<0.001
DBP (mmHg)	84.8±10.0	88.3±9.4§	88.1±12.0	88.6±13.5	0.004
BMI (kg/m^2)	26.0±3.0	27.5±3.9¶	28.4±3.9¶	30.1±4.5¶	<0.001
Total cholesterol (mmol/l)	5.5±0.9	5.6±0.9	5.7±1.0	5.3±1.1	0.075
LDL cholesterol (mmol/l)	3.5±0.8	3.5±0.9	3.5±0.9	3.0±1.0#	0.001
HDL cholesterol (mmol/l)	1.5±0.4	1.4±0.4	1.4±0.4	1.3±0.4§	0.001
Triglycerides (mmol/l)	1.2±0.6	1.4±0.6	1.6±0.9§	2.3±1.8¶	<0.001
Fasting glucose (mmol/l)	5.2±0.2	6.0±0.3¶	6.0±0.4¶	8.3±2.2¶	<0.001
2 h glucose (mmol/l)	5.3±1.2	5.7±1.1#	8.9±0.9¶	14.3±3.8¶	<0.001
hsCRP	2.5±2.9	2.4±2.9	3.4±5.9	4.2±5.9§	0.001
Antihypertensive med (%)	21.8	20.4	34.6	62.1	<0.001
Statins (%)	12.1	9.4	15.4	25.8	0.005
Previous CVD (%)	11.5	9.8	16.3	27.3	0.002
CIMT (mm)	0.950±0.197	0.934±0.209	0.989±0.261	1.127±0.351§	<0.001
YEM (mmHg)	6591±4146	7008±3951	8575±8681	9402±6207#	0.001
SI	3.59±0.49	3.63±0.43	3.69±0.51	3.84±0.52#	0.005
CAC (%/10 mmHg)	0.98±0.45	0.90±0.40	0.83±0.41	0.71±0.37¶	<0.001

Values are unadjusted means + SD except for the prevalence rates which are expressed as %.

*= Variation of n is caused by some missing data. NGT= normal glucose tolerance; IFG= impaired fasting glucose; IGT= impaired glucose tolerance; T2DM= type 2 diabetes; SBP= systolic blood pressure; DBP= diastolic blood pressure; BMI= body mass index; 2 h glucose= plasma glucose 2 h after glucose load in oral glucose tolerance test; hsCRP= high sensitivity C-reactive protein; antihypertensive med= % of subjects using antihypertensive medication; statins= % of subjects using statins; previous CVD= % of subjects with previous knowledge of coronary heart disease, stroke, or arterial stenosis, or thrombosis in a lower limb; YEM = Young's elastic modulus; SI = beta stiffness index; CAC = carotid artery compliance.

#p <0.05, §p < 0.01, ¶p <0.001, pairwise comparison with the NGT group; Dunnett's T3 correction for multiple comparisons in ANOVA.

The results of the ANCOVA model with adjustments for age and other CVD risk factors are presented in Table 5.6. Among men, none of the parameters differed significantly between the groups (ANCOVA p>0.1 for all) after these further adjustments. In women, adjusted means in CIMT did not differ significantly between the groups in this model (p=0.341). In the elasticity indices, however, significant differences were observed (ANCOVA p<0.05 for all) even after these adjustments. In this model, the T2DM group had significantly higher YEM and SI when compared to the NGT group. The T2DM and IFG groups had significantly lower CAC compared to the NGT group. Along with glucose tolerance (p<0.001),

TABLE 5.4 *Clinical characteristics of the female study subjects according to glucose tolerance status.*

	Glucose tolerance				
	NGT	IFG	IGT	T2DM	ANOVA
	n = 336-371*	n =160-176*	n =105-119*	n =49-59*	p-value
Age (years)	56.5±7.6	58.7±8.3#	60.4±8.4¶	63.8±7.6¶	<0.001
Smoking (%)	20.8	19.9	15.1	13.6	0.373
SBP (mmHg)	129.0±20.8	138.1±19.7¶	146.6±21.5¶	155.6±22.2¶	<0.001
DBP (mmHg)	79.7±9.8	83.3±8.4¶	85.7±9.4¶	85.6±8.2¶	<0.001
BMI (kg/m^2)	25.6±4.3	27.4±4.7¶	28.9±4.9¶	30.6±4.5¶	<0.001
Total cholesterol (mmol/l)	5.6±0.9	5.8±0.9	5.8±0.9	5.5±1.0	0.053
LDL cholesterol (mmol/l)	3.3±0.8	3.5±0.9	3.5±0.8	3.3±0.9	0.078
HDL cholesterol (mmol/l)	1.8±0.4	1.7±0.4	1.6±0.4¶	1.4±0.3¶	<0.001
Triglycerides (mmol/l)	1.1±0.5	1.3±0.6¶	1.5±0.7¶	1.8±0.7¶	<0.001
Fasting glucose (mmol/l)	5.1±0.3	5.9±0.3¶	5.7±0.5¶	7.3±2.2¶	<0.001
2 h glucose (mmol/l)	5.6±1.1	6.1±1.0¶	9.0±0.8¶	14.1±4.0¶	<0.001
hsCRP	2.4±4.2	2.3±2.4	5.0±6.8¶	4.5±4.5¶	<0.001
Antihypertensive med (%)	17.5	29.5	40.3	69.5	<0.001
Statins (%)	6.7	9.7	13.4	23.7	<0.001
Previous CVD (%)	5.4	11.9	12.6	15.3	0.006
CIMT (mm)	0.852±0.175	0.923±0.226§	0.957±0.227¶	0.998±0.255¶	<0.001
YEM (mmHg)	5824±3757	6487±3772	7577±4144§	10424±7142¶	<0.001
SI	3.56±0.46	3.65±0.44	3.75±0.47§	3.92±0.47¶	<0.001
CAC (%/10 mmHg)	1.10±0.58	0.89±0.39¶	0.78±0.43¶	0.61±0.32¶	<0.001

Values are unadjusted means + SD except the prevalence rates which are expressed as %.

*= Variation of n is caused by some missing data. NGT= normal glucose tolerance; IFG= impaired fasting glucose; IGT= impaired glucose tolerance; T2DM= type 2 diabetes; SBP= systolic blood pressure; DBP= diastolic blood pressure; BMI= body mass index; 2 h glucose= plasma glucose 2 h after glucose load in oral glucose tolerance test; hsCRP= high sensitivity C-reactive protein; antihypertensive med= % of subjects using antihypertensive medication; statins= % of subjects using statins; previous CVD= % of subjects with previous knowledge of coronary heart disease, stroke, or arterial stenosis, or thrombosis in a lower limb; YEM = Young's elastic modulus, SI = beta stiffness index, CAC = carotid artery compliance.

#p <0.05, §p < 0.01, ¶p <0.001, pair-wise comparison with the NGT group; Dunnett's T3 correction for multiple comparisons in ANOVA.

SBP (p<0.001) and age (p=0.012) remained as significant predictors in women for YEM in this model. In regard to SI the significant predictors were SBP (p<0.001), age (p<0.001) and glucose tolerance (p=0.038). In the model investigating CAC, the significant predictors were SBP (p<0.001), age (p<0.001), LDL-C (p=0.030) and glucose tolerance (p=0.031).

TABLE 5.5 *Carotid artery intima-media thickness (CIMT) and elasticity indices according to glucose tolerance status in men and women, adjusted for age.*

	Glucose tolerance				
	NGT	IFG	IGT	T2DM	ANCOVA
Men	n =152-174*	n =215-235*	n =97-104*	n =59-66*	p-value
CIMT (mm)	0.953±0.016	0.953±0.014	0.969±0.021	1.083±0.026¶	<0.001
YEM (mmHg)	6589±437	7191±371	8397±549#	9032±709§	0.007
SI	3.59±0.04	3.65±0.03	3.66±0.05	3.79±0.06§	0.045
CAC (%/10 mmHg)	0.98±0.03	0.88±0.03#	0.86±0.04#	0.76±0.05¶	0.002
Women	n=336-371*	n=160-176*	n=105-119*	n=49-59*	
CIMT (mm)	0.875±0.009	0.918±0.014§	0.930±0.017§	0.928±0.024#	0.005
YEM (mmHg)	6043±223	6433±320	7257±398§	9782±586¶	<0.001
SI	3.59±0.02	3.64±0.03	3.70±0.04#	3.83±0.06¶	0.001
CAC (%/10 mmHg)	1.06±0.03	0.90±0.04¶	0.84±0.05¶	0.73±0.07¶	<0.001

Values are age-adjusted means ± SE. *= variation of n is caused by missing elasticity data for some subjects. NGT= normal glucose tolerance, IFG= impaired fasting glucose, IGT= impaired glucose tolerance, T2DM= type 2 diabetes, YEM = Young's elastic modulus, SI = beta stiffness index, CAC = carotid artery compliance.

#p <0.05, §p < 0.01, ¶p <0.001, pair-wise comparison with the NGT group; Fisher's least significant difference test was used if a significant ANCOVA p value for glucose tolerance was observed.

TABLE 5.6 *Carotid artery intima-media thickness (CIMT) and elasticity indices according to glucose tolerance status in men and women, adjusted for age, smoking, LDL cholesterol, BMI, SBP, triglycerides and HDL cholesterol.*

	Glucose tolerance				
	NGT	IFG	IGT	T2DM	ANCOVA
Men	n =149-171*	n =213-233*	n =94-100*	n =51-57*	p-value
CIMT (mm)	0.972±0.017	0.965±0.014	0.978±0.022	1.046±0.031	0.101
YEM (mmHg)	6969±457	7062±381	7969±567	8222±783	0.317
SI	3.59±0.04	3.64±0.03	3.64±0.05	3.72±0.07	0.461
CAC (%/10 mmHg)	0.95±0.03	0.89±0.03	0.90±0.04	0.88±0.06	0.587
Women	n=335-369*	n=157-173*	n=103-117*	n=48-58*	
CIMT (mm)	0.899±0.011	0.927±0.014	0.923±0.018	0.905±0.026	0.341
YEM (mmHg)	6260±247	6172±321	6448±404	9042±593¶	<0.001
SI	3.59±0.03	3.62±0.04	3.66±0.05	3.80±0.07§	0.038
CAC (%/10 mmHg)	1.03±0.03	0.93±0.04#	0.94±0.05	0.84±0.07#	0.031

Values are adjusted means ± SE. Adjustments for age, smoking, LDL cholesterol, BMI, SBP, triglycerides and HDL cholesterol. *= variation of n is caused by missing elasticity data in some subjects. There were also a few missing covariate values. BMI= body mass index, SBP= systolic blood pressure, NGT= normal glucose tolerance, IFG= impaired fasting glucose, IGT= impaired glucose tolerance, T2DM= type 2 diabetes, YEM = Young's elastic modulus, SI = beta stiffness index, CAC = carotid artery compliance.

#p <0.05, §p < 0.01, ¶p <0.001, pair-wise comparison with the NGT group; Fisher's least significant difference test was used if a significant ANCOVA p value for glucose tolerance was observed.

When the second ANCOVA model was further adjusted with the subjects' status in regard to antihypertensive and statin medications as well previous CVD (previous knowledge of CHD, stroke, or arterial stenosis or thrombosis in a lower limb) the main findings remained essentially similar (data not shown). The only exception was that in regard to SI, the ANCOVA p value was borderline ($p=0.067$ vs. $p=0.038$ in the previous model) significant in women.

5.3 Association of metabolic syndrome and early atherosclerosis (original publications I and III)

In these studies MetS was defined by the NCEP definition unless otherwise stated. The prevalence of MetS in the Health 2000 Survey supplemental study was 40% and 49% in men and 36% and 41% in women using the NCEP and IDF definitions, respectively. Subjects with MetS were more frequently on antihypertensive medication and had higher BMI, waist circumference, blood pressure and higher concentrations of triglycerides, fasting plasma glucose and hsCRP as well as lower HDL-C concentration ($p<0.05$ for all) than the subjects without MetS in both sexes. Women with metabolic MetS were older, used statins more often and had higher LDL-C and total cholesterol concentrations than women without MetS. In men, these variables did not differ significantly according to the presence of MetS. Current smoking status did not differ significantly according to the presence of MetS ($p>0.1$) in either of the sexes. Clinical characteristics of the Health 2000 Survey supplemental study population are presented in Table 5.7 according to the presence of MetS.

5.3.1 Metabolic syndrome, its components and carotid intima-media thickness (original publication III)

The effects of MetS and its components on CIMT (mean IMT) were studied in the Health 2000 Survey supplemental study population. A borderline statistically significant interaction was found between sex and the presence of metabolic syndrome in determining CIMT ($p=0.058$), and the sexes were therefore analyzed separately. For both sexes, CIMT was significantly ($p<0.001$ for both) higher in subjects with MetS (1.02 and 0.98 mm in men and women, respectively) than in those without MetS (0.94 and 0.85 mm in men and women, respectively). Age, SBP, pulse pressure, HDL-C, triglycerides, fasting plasma glucose, hsCRP and FRS correlated statistically significantly with CIMT in both sexes (Table 5.8).

TABLE 5.7 *Clinical characteristics of the Health 2000 Survey supplemental study population (with available carotid ultrasound data) according to the presence of MetS (n = 1353).*

	Male			Female		
	MetS			MetS		
	Yes (n=225-241)*	No (359-366)*	p	Yes (n=257-267)*	No (469-479)*	p
Age (years)	58.7 ± 7.6	57.6 ± 8.0	0.075	61.3 ± 8.3	56.7 ± 7.7	<0.001
Smoking (%)	24.9	29.2	0.242	18.4	19.2	0.775
Body mass index (kg/m^2)	29.7 ± 3.9	26.0 ± 3.2	<0.001	30.1 ± 4.9	25.3 ± 3.8	<0.001
Waist circumference (cm)	106.8 ± 10.1	94.2 ± 9.1	<0.001	97.5 ± 11.9	83.9 ± 10.4	<0.001
Heart rate (beats/min)	67.6 ± 12.9	65.3 ± 11.9	0.023	68.6 ± 11.6	67.9 ± 10.0	0.421
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.6 ± 0.4	<0.001	1.4 ± 0.3	1.9 ± 0.4	<0.001
LDL cholesterol (mmol/l)	3.4 ± 0.9	3.5 ± 0.9	0.208	3.6 ± 0.9	3.2 ± 0.8	<0.001
Total cholesterol (mmol/l)	5.4 ± 1.0	5.6 ± 1.0	0.147	5.8 ± 1.0	5.6 ± 0.8	0.002
Triglycerides (mmol/l)	2.0 ± 1.6	1.2 ± 0.6	<0.001	1.6 ± 0.8	1.0 ± 0.4	<0.001
Fasting glucose (mmol/l)	6.6 ± 1.6	5.8 ± 1.0	<0.001	6.1 ± 1.4	5.3 ± 0.5	<0.001
hsCRP (mg/l)	3.6 ± 5.2	2.3 ± 2.9	<0.001	4.2 ± 4.7	2.3 ± 4.3	<0.001
SBP (mmHg)	148.9 ± 20.7	136.4 ± 18.6	<0.001	147.3 ± 21.7	130.3 ± 20.7	<0.001
DBP (mmHg)	90.1 ± 11.0	85.4 ± 10.2	<0.001	85.3 ± 9.0	80.2 ± 9.6	<0.001
PP (mmHg)	58.7 ± 15.0	51.1 ± 12.1	<0.001	62.0 ± 17.2	50.1 ± 13.9	<0.001
Medication for hypertension	41.5	19.7	<0.001	46.1	20.5	<0.001
Statin medication	15.4	11.5	0.165	14.6	8.6	0.010

Values are means ± SD except for smoking and medications, which are expressed as%.

MetS = metabolic syndrome defined by the NCEP definition, NCEP = National Cholesterol Education Program, HDL= high-density lipoprotein, LDL= low-density lipoprotein, hsCRP = high sensitivity C-reactive protein, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure.

* Variation of n is due to missing data for some subjects.

Adapted from Atherosclerosis, 204, Sipilä K, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kukkonen-Harjula K, Lehtimäki T, Kesäniemi YA, Koivistoinen T, Nieminen MS, Tuomilehto J, Kähönen M, Metabolic syndrome and carotid intima media thickness in the Health 2000 Survey, 276-281, Copyright (2009), with Permission from Elsevier.

TABLE 5.8 *Correlations between cardiovascular risk factors and CIMT in men (n=496-607*) and women (n =628-746*).*

	Men		Women	
	r	p	r	p
Age (years)	0.464	<0.001	0.515	<0.001
Body mass index (kg/m²)	0.045	0.267	0.171	<0.001
Waist circumference (cm)	0.077	0.057	0.189	<0.001
HDL cholesterol (mmol/l)	-0.131	0.001	-0.157	<0.001
LDL cholesterol (mmol/l)	-0.069	0.094	0.131	<0.001
Total cholesterol (mmol/l)	-0.055	0.174	0.113	0.002
Triglycerides (mmol/l)	0.161	<0.001	0.231	<0.001
Fasting glucose (mmol/l)	0.116	0.004	0.134	<0.001
hsCRP (mg/l)	0.122	0.003	0.111	0.003
SBP (mmHg)	0.137	0.001	0.352	<0.001
DBP (mmHg)	-0.008	0.849	0.148	<0.001
PP (mmHg)	0.208	<0.001	0.402	<0.001
FRS	0.370	<0.001	0.428	<0.001

CIMT = carotid artery intima-media thickness, r = Pearson's correlation coefficient, HDL= high-density lipoprotein, LDL= low-density lipoprotein, hsCRP = high sensitivity C-reactive protein, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure, FRS = Framingham risk score.

* Variation of n is due to missing data for some subjects.

Adapted from Atherosclerosis, 204, Sipilä K, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kukkonen-Harjula K, Lehtimäki T, Kesäniemi YA, Koivistoinen T, Nieminen MS, Tuomilehto J, Kähönen M, Metabolic syndrome and carotid intima media thickness in the Health 2000 Survey, 276-281, Copyright (2009), with Permission from Elsevier.

BMI, waist circumference, LDL-C, total cholesterol and DBP had significant correlations with CIMT in women but not in men.

A stepwise linear regression model was used to investigate the independent effects of MetS components and other CVD risk factors on CIMT (Table 5.9, model A). The initial model included variables significantly correlating with CIMT in the univariate model. In men, age and triglycerides were independent predictors explaining 24% of the variation in CIMT. In women, age, pulse pressure and triglycerides were independent predictors explaining 29% of the variation in CIMT. The same regression analyses were performed with MetS included in the model as an independent variable (Table 5.9, model B). In men this did not change the results, and MetS did not appear in the final model as an independent predictor. In women, however, MetS remained in the final model as an independent predictor ($p<0.001$) for CIMT together with age and pulse pressure. The change in the explained variance of the model was 1% (29%–

TABLE 5.9. *Linear regression models of the relationships between cardiovascular risk factors and CIMT.*

MODEL A	Men (n =594)			Women (n=720)			
	CIMT			CIMT			
Risk variable	β ± SE	p	R^2 change (%)	Risk variable	β ± SE	p	R^2 change (%)
Age (years)	0.014 ± 0.001	<0.001	21	Age (years)	0.010 ± 0.001	<0.001	26
Triglycerides (mmol/l)	0.205 ± 0.041	<0.001	3	PP (mmHg)	0.002 ± <0.001	<0.001	3
				Triglycerides (mmol/l)	0.107 ± 0.037	0.004	1
R^2	24 %			R^2	29 %		

MODEL B	Men (n =594)			Women (n=720)			
	CIMT			CIMT			
Risk variable	β ± SE	p	R^2 change (%)	Risk variable	β ± SE	p	R^2 change (%)
Age (years)	0.014 ± 0.001	<0.001	21	Age (years)	0.010 ± 0.001	<0.001	26
Triglyserides (mmol/l)	0.205 ± 0.041	<0.001	3	PP (mmHg)	0.002 ± <0.001	<0.001	3
				Metabolic syndrome	0.059 ± 0.015	<0.001	2
R^2	24%			R^2	30%		

CIMT = carotid artery intima-media thickness, β = regression coefficient, SE = standard error, PP = pulse pressure, R^2 = adjusted R square value of the whole model, R^2 change = change in the adjusted R square value after the addition of the respective variable into the model. The initial stepwise regression model for men included age, fasting plasma glucose, high-density lipoprotein cholesterol, pulse pressure, high-sensitivity CRP, triglycerides (models A and B) and metabolic syndrome (model B only) as independent variables. The initial stepwise regression model for women included age, waist circumference, fasting plasma glucose, high-density lipoprotein cholesterol, pulse pressure, high-sensitivity CRP, triglycerides, low-density lipoprotein cholesterol (models A and B) and metabolic syndrome (model B only) as independent variables.

Adapted from Atherosclerosis, 204, Sipilä K, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kukkonen-Harjula K, Lehtimäki T, Kesäniemi YA, Koivistoinen T, Nieminen MS, Tuomilehto J, Kähönen M, Metabolic syndrome and carotid intima media thickness in the Health 2000 Survey, 276-281, Copyright (2009), with Permission from Elsevier.

30%) in women after the inclusion of MetS, in addition to its components. Another linear regression model was performed using MetS (instead of its components) and other CVD risk factors (significantly correlating with CIMT in univariate model) as independent variables (Table 5.10). In this model, age and MetS were independent determinants for CIMT in both sexes. All the above-mentioned regression models were also performed using the presence of antihypertensive medication and statin

TABLE 5.10 *Linear regression models of the relationships between metabolic syndrome, other cardiovascular risk factors and CIMT.*

Men (n = 597)				Women (n = 722)			
	CIMT				CIMT		
Risk variable	β ± SE	p	R^2 change (%)	Risk variable	β ± SE	p	R^2 change (%)
Age (years)	0.014 ± 0.001	<0.001	21	Age (years)	0.012 ± 0.001	<0.001	26
Metabolic syndrome	0.064 ± 0.018	<0.001	2	Metabolic syndrome	0.074 ± 0.014	<0.001	3
R^2	22 %			R^2	29 %		

CIMT =carotid artery intima-media thickness, β = regression coefficient, SE = standard error, R^2 = adjusted R square value of the whole model, R^2 change = change in the adjusted R square value after the addition of the respective variable into the model. The initial stepwise regression model for men included age, high-sensitivity CRP and metabolic syndrome as independent variables. The initial stepwise regression model for women included age, high-sensitivity CRP, low-density lipoprotein cholesterol and metabolic syndrome as independent variables.

Adapted from Atherosclerosis, 204, Sipilä K, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kukkonen-Harjula K, Lehtimäki T, Kesäniemi YA, Koivistoinen T, Nieminen MS, Tuomilehto J, Kähönen M, Metabolic syndrome and carotid intima media thickness in the Health 2000 Survey, 276-281, Copyright (2009), with Permission from Elsevier.

medication as independent variables. The main findings remained similar (data not shown). The results remained essentially similar also when the enter method was used instead of a stepwise method in the regression models.

To further evaluate the sex-related differences in the effect of MetS on CIMT, adjusted means (with different adjustments) for CIMT were calculated with ANCOVA (unpublished data). As mentioned earlier, CIMT was higher in subjects with MetS in comparison to those who did not suffer from it in both sexes. This finding remained statistically significant in both sexes after the adjustment with age and other CVD risk factors ($p=0.006$ and $p<0.001$ for men and women respectively). When further adjusted with the components of MetS, women, but not men, with MetS still had significantly higher CIMT compared to the ones without the syndrome ($p=0.186$ and $p=0.008$ for men and women respectively). The patterns of unadjusted and adjusted means for CIMT in men and women according to the presence of MetS are shown in Figure 5.1.

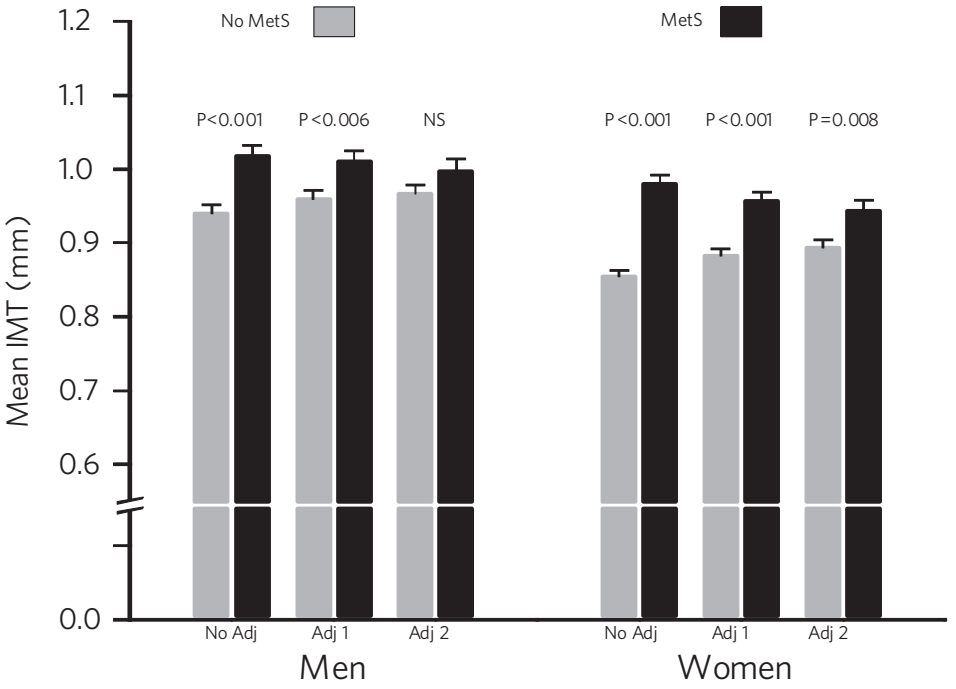

FIGURE 5.1 *Unadjusted and adjusted means for CIMT in men and women. MetS = metabolic syndrome; NS = non significant; LDL-C = low-density lipoprotein cholesterol; hsCRP = high sensitivity C-reactive protein; No Adj = no adjustment; Adj 1= adjusted for age, LDL-C, hsCRP and smoking; Adj 2 = adjusted for risk factors in the Adj 1 model and the components of MetS.*

In order to examine the combined effects of MetS and the Framingham Risk Score on CIMT, the population was divided into four risk profile categories. In the first category, subjects had low FRS (indicating a 10-year CHD risk less than 10%) and no MetS. The second category consisted of subjects with low FRS and MetS. In the third category, subjects had high FRS (indicating a 10-year CHD risk greater than 10%) and no MetS. Finally, the fourth category consisted of subjects with MetS and high FRS. In both sexes, there were significant differences in CIMT between the risk categories (ANOVA p value <0.001). In men CIMT was significantly higher in subjects with high FRS compared to subjects with low FRS, regardless of the presence or absence of MetS ($p<0.01$) (Figure 5.2). In women who had low FRS and MetS, CIMT was significantly higher compared to women with low FRS without MetS ($p=0.012$). A similar pattern was observed in women with high FRS ($p=0.007$) (Figure 5.2).

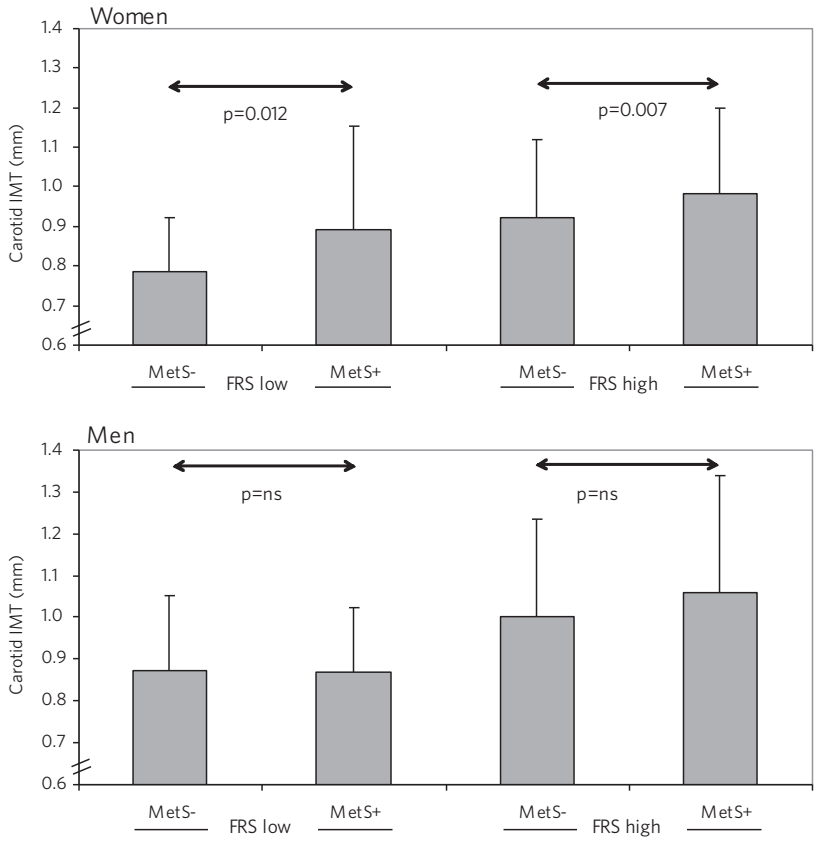

FIGURE 5.2 *Carotid artery intima-media thickness (mean and SD) according to the risk profile category. IMT = intima-media thickness, FRS = Framingham risk score, MetS = metabolic syndrome, ns = non significant. Reprinted from Atherosclerosis, 204, Sipilä K, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kukkonen-Harjula K, Lehtimäki T, Kesäniemi YA, Koivistoinen T, Nieminen MS, Tuomilehto J, Kähönen M, Metabolic syndrome and carotid intima media thickness in the Health 2000 Survey, 276-281, Copyright (2009), with Permission from Elsevier.*

5.3.2 Metabolic syndrome, its components and arterial pulse wave velocity (original publication I)

The relationships of MetS, its components and PWV were evaluated in a smaller subpopulation of the Health 2000 Survey supplemental study. There were 401 individuals (176 men and 225 women) included in these analyses. The NCEP and IDF definitions for MetS were compared as predictors of PWV. Due to the smaller sample size, men and women were analyzed together. Moreover, the interaction between sex and MetS status in determining PWV was not significant (p=0.304). Analyses were

TABLE 5.11 *Clinical characteristics of the subjects with available PWV data (n = 400) according to the MetS status.*

	MetS by NCEP definition			MetS by IDF definition		
	Yes (n=156-162)*	No (n=227-238)*	p	Yes (n=175-182)*	No (n=208-218)*	p
Age (years)	59.5 ± 7.7	57.6 ± 7.9	0.017	59.5 ± 7.6	57.4 ± 8.0	0.009
Sex (men, %)	44	44	0.883	46	43	0.555
Current smoking (%)	24	23	0.748	24	23	0.689
Body mass index (kg/m^2)	29.3 ± 3.9	25.6 ± 3.7	< 0.001	29.0 ± 3.8	25.5 ± 3.9	< 0.001
Waist circumference (cm)	101.5 ± 11.2	89.2 ± 10.8	< 0.001	100.9 ± 10.8	88.5 ± 11.0	< 0.001
Heart rate (beat/min)	64.9 ± 10.1	63.3 ± 10.7	0.133	64.6 ± 9.7	63.4 ± 11.1	0.260
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.7 ± 0.5	< 0.001	1.4 ± 0.4	1.7 ± 0.5	< 0.001
LDL cholesterol (mmol/l)	3.6 ± 0.9	3.3 ± 0.9	0.008	3.5 ± 0.9	3.3 ± 0.9	0.028
Total cholesterol (mmol/l)	5.7 ± 1.0	5.5 ± 0.9	0.213	5.6 ± 1.0	5.6 ± 0.9	0.429
Triglycerides (mmol/l)	1.7 ± 0.8	1.2 ± 0.5	< 0.001	1.7 ± 0.8	1.1 ± 0.5	< 0.001
Fasting glucose (mmol/l)	6.3 ± 1.7	5.5 ± 0.7	< 0.001	6.2 ± 1.6	5.5 ± 0.7	< 0.001
hsCRP (mg/l)	4.1 ± 5.6	2.2 ± 3.0	< 0.001	4.0 ± 5.4	2.1 ± 2.8	< 0.001
SBP (mmHg)	142.3 ± 19.5	129.1 ± 18.4	< 0.001	140.2 ± 19.3	129.6 ± 19.1	< 0.001
DBP (mmHg)	85.1 ± 9.2	81.0 ± 9.7	< 0.001	85.0 ± 8.7	80.1 ± 10.0	< 0.001
PP (mmHg)	57.2 ± 15.4	48.2 ± 11.8	< 0.001	55.3 ± 15.4	48.9 ± 12.1	< 0.001

Values are means ± SD except for sex and smoking, which are expressed as %.

PWV = pulse wave velocity, MetS = metabolic syndrome, NCEP = National Cholesterol Education Program, IDF = International Diabetes Federation, HDL= high-density lipoprotein, LDL= low-density lipoprotein, hsCRP = high sensitivity C-reactive protein, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure.

* Variation of n is caused by some missing data.

Adapted from Metabolism, 56, Sipilä K, Koivistoinen T, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kööbi T, Kukkonen-Harjula K, Majahalme S, Kähönen M, Metabolic syndrome and arterial stiffness: the Health 2000 Survey, 320-326, Copyright (2007), with permission from Elsevier.

also made separately for subjects without known CVD or diabetes, and the results for men and women separately are presented as well (unpublished data).

The prevalence of MetS was 41% and 47% in men and 40% and 44% in women by using the NCEP and IDF definitions, respectively. Subjects with MetS were older and had higher BMI, waist circumference, blood pressure and higher

concentrations of LDL-C, triglycerides, fasting plasma glucose and hsCRP as well as lower HDL-C concentrations ($p<0.05$ for all) than the subjects without MetS, regardless of the definition used. Resting heart rate, total cholesterol level, or current smoking status did not significantly differ ($p>0.1$ for all) according to the MetS status. Twenty-eight percent of the study population used antihypertensive medication, and 11% were on statins. Selected clinical and demographic data is shown in Table 5.11. In both sexes, PWV was significantly higher in subjects with MetS (for both definitions, $p<0.01$) than in those without it (Figure 5.3). Correlations between CVD risk factors and PWV are given in Table 5.12. When the sexes were analysed together, all risk factors correlated significantly with PWV, with the exceptions of total cholesterol and LDL-C. In women, there were significant correlations for total cholesterol and LDL-C as well.

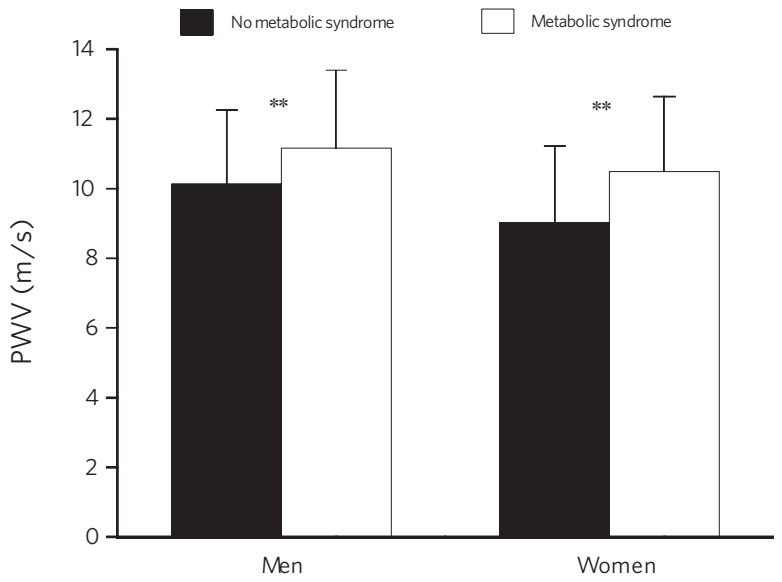

FIGURE 5.3 *Pulse wave velocity (mean and SD) in men and women with or without metabolic syndrome, according to the NCEP definition. **P<0.01. The pattern was essentially the same using the IDF definition. NCEP = National Cholesterol Education Program, IDF = International Diabetes Federation. Reprinted from Metabolism, 56, Sipilä K, Koivistoinen T, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kööbi T, Kukkonen-Harjula K, Majahalme S, Kähönen M, Metabolic syndrome and arterial stiffness: the Health 2000 Survey, 320-326, Copyright (2007), with permission from Elsevier.*

TABLE 5.12 *Correlations between cardiovascular risk factors and PWV in all subjects (n = 393-401*), in subjects free of CVD and DM (n = 197-200*), in men (n=171-176*), and in women (n =222-225*).*

	All subjects		Subjects free of CVD and diabetes		Men		Women	
	r	p	r	p	r	p	r	p
Age (years)	0.510	< 0.001	0.518	< 0.001	0.521	<0.001	0.506	<0.001
Body mass index (kg/m^2)	0.238	< 0.001	0.274	< 0.001	0.283	<0.001	0.212	0.001
Waist circumference (cm)	0.343	< 0.001	0.353	< 0.001	0.308	<0.001	0.277	<0.001
HDL cholesterol (mmol/l)	-0.161	0.001	-0.255	<0.001	-0.083	0.277	-0.111	0.098
LDL cholesterol (mmol/l)	0.062	0.217	0.208	0.003	-0.105	0.169	0.185	0.006
Total cholesterol (mmol/l)	0.028	0.577	0.120	0.090	-0.113	0.135	0.183	0.006
Triglycerides (mmol/l)	0.199	< 0.001	0.174	0.014	0.130	0.086	0.216	0.001
Fasting glucose (mmol/l)	0.341	< 0.001	0.252	< 0.001	0.392	<0.001	0.257	<0.001
CRP (mg/l)	0.226	< 0.001	0.214	0.003	0.346	<0.001	0.149	0.026
SBP (mmHg)	0.627	< 0.001	0.694	< 0.001	0.572	<0.001	0.656	<0.001
DBP (mmHg)	0.392	< 0.001	0.496	< 0.001	0.253	<0.001	0.448	<0.001
PP (mmHg)	0.619	< 0.001	0.642	< 0.001	0.588	<0.001	0.662	<0.001

PWV = pulse wave velocity, CVD = cardiovascular disease, DM = diabetes mellitus, r = Pearson correlation coefficient, HDL= high-density lipoprotein, LDL= low-density lipoprotein, CRP = C-reactive protein, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, PP = pulse pressure.

* Variation of n is caused by some missing data.

A stepwise linear regression model was used to investigate the independent effects of risk factors in determining PWV. The initial regression model included risk factors significantly correlating with PWV in the univariate model. The independent predictors for PWV were SBP, age, fasting blood glucose and waist circumference in the whole population and SBP, age and waist circumference in subjects without CVD and DM, explaining 55% and 61% of the variation in PWV, respectively (Table 5.13). In men SBP, age, fasting glucose and hsCRP, and in women SBP, age and fasting glucose prevailed as independent predictors, explaining 60% and 51% of the variation in PWV, respectively (Table 5.14). The inclusion of MetS (in addition to its components) into the regression models did not change the results.

TABLE 5.13 *Linear regression models of the relationships between cardiovascular risk factors and PWV in all subjects (n = 390) and in subjects free of CVD and DM (n = 196).*

All subjects				Subjects free of CVD and diabetes			
Risk variable	β ± SE	p	R^2 change	Risk variable	B ± SE	p	R^2 change
SBP (mmHg)	0.05±0.00	< 0.001	40%	SBP (mmHg)	0.06±0.01	<0.001	48%
Age (years)	0.10±0.01	< 0.001	11%	Age (years)	0.10±0.01	<0.001	12%
Fasting glucose (mmol/l)	0.27±0.07	< 0.001	3%	Waist circumference (cm)	0.02±0.01	0.042	1%
Waist circumference (cm)	0.03±0.01	< 0.001	1%				
R^2	55 %				61%		

PWV = pulse wave velocity, CVD = cardiovascular disease, DM = diabetes mellitus, β = regression coefficient, SE = standard error, R^2 change = change in the adjusted R^2 value after the addition of the respective variable into the model, SBP = systolic blood pressure, R^2 = adjusted R square value of the whole model. The initial stepwise regression models included age, sex, waist circumference, fasting plasma glucose, high-density lipoprotein cholesterol, SBP, high-sensitivity C-reactive protein, triglycerides, and low-density lipoprotein cholesterol (only in subjects free of CVD and diabetes) as independent variables.

Adapted from Metabolism, 56, Sipilä K, Koivistoinen T, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kööbi T, Kukkonen-Harjula K, Majahalme S, Kähönen M, Metabolic syndrome and arterial stiffness: the Health 2000 Survey, 320-326, Copyright (2007), with permission from Elsevier.

TABLE 5.14 *Linear regression models of the relationships between cardiovascular risk factors and PWV in men and women*

Men (n =170)				Women (n=218)			
Risk variable	β ± SE	p	R^2 change	Risk variable	β ± SE	p	R^2 change
SBP (mmHg)	0.056±0.007	<0.001	33%	SBP (mmHg)	0.056±0.006	<0.001	43%
Age (years)	0.121±0.014	<0.001	21%	Age (years)	0.084±0.015	<0.001	7%
Fasting glucose (mmol/l)	0.358±0.082	<0.001	5%	Fasting glucose (mmol/l)	0.206±0.101	0.042	1%
hsCRP (mg/ml)	0.894±0.261	0.001	3%				
R^2	60%			R^2	51%		

PWV = pulse wave velocity, β = regression coefficient, SE = standard error, R^2 change = change in the adjusted R^2 value after the addition of the respective variable in to the model, SBP = systolic blood pressure, hsCRP = high-sensitivity C-reactive protein, R^2 = adjusted R square value of the whole model. The initial stepwise regression model for men included SBP, age, fasting plasma glucose, hsCRP and waist circumference as independent variables. The initial stepwise regression model for women included SBP, age, fasting plasma glucose, hsCRP, waist circumference, triglycerides, low-density lipoprotein-cholesterol and smoking as independent variables.

Another regression model was calculated, including MetS (instead of its components) and other CVD risk factors in the model. The independent predictors for PWV were age, MetS, sex and hsCRP in the whole population and age, MetS and sex in subjects without CVD and DM. These models explained 33%–34% of the variation in PWV (depending on the definition of MetS used) (Table 5.15). In men age, hsCRP and MetS (defined by the NCEP definition) and in women age and MetS (defined by the NCEP definition) were independent predictors for PWV, explaining 35% and 30% of the variation in PWV, respectively. The regression coefficient for MetS was higher in women (β= 1.047) than in men (β= 0.703) (Table 5.16). All the main findings remained essentially similar when the enter method was used instead of the stepwise method in the regression models (data not shown).

TABLE 5.15 *Linear regression models for the relationships between MetS (defined by NCEP or IDF), other cardiovascular risk factors and PWV in all subjects (n= 392) and in subjects free of CVD and DM (n= 197).*

All subjects				Subjects free of CVD and diabetes			
Risk variable	β ± SE	p	R^2 change	Risk variable	β ± SE	p	R^2 change
Age (years)	0.14±0.01	< 0.001	26%	Age (years)	0.13±0.02	<0.001	26
MetS using the	0.92±0.20	< 0.001	4%	MetS using the	0.84±0.25	<0.001	5
NCEP definition				NCEP definition			
Sex	-0.82±0.19	< 0.001	3%	Sex	-0.72±0.23	0.002	2
hsCRP (mg/l)	0.52±0.21	0.016	0.8%				
R^2	34%				33%		
Age (years)	0.14±0.01	<0.001	26%	Age (years)	0.13±0.02	<0.001	26%
Sex	-0.80±0.19	<0.001	3%	MetS using the IDF	0.78±0.24	0.001	4%
				definition			
MetS using the IDF	0.67±0.20	0.001	3%	Sex	-0.69±0.24	0.004	3%
definition							
hsCRP (mg/l)	0.60±0.21	0.005	1%				
R^2	33%				33%		

MetS = metabolic syndrome, PWV=pulse wave velocity, CVD = cardiovascular disease, DM = diabetes mellitus, β = regression coefficient, SE = standard error, R^2 change = change in the adjusted R^2 value after the addition of the respective variable in to the model, NCEP = National Cholesterol Education Program, IDF = International Diabetes Federation, hsCRP = high-sensitivity C-reactive protein, R^2 = adjusted R square value of the whole model.

Initial stepwise regression models included age, sex, hsCRP, MetS (defined by NCEP or IDF) and low-density lipoprotein cholesterol (only in subjects free of CVD and diabetes) as independent variables.

Adapted from Metabolism, 56, Sipilä K, Koivistoinen T, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kööbi T, Kukkonen-Harjula K, Majahalme S, Kähönen M, Metabolic syndrome and arterial stiffness: the Health 2000 Survey, 320-326, Copyright (2007), with permission from Elsevier.

TABLE 5.16 *Linear regression models for the relationships between MetS (defined by NCEP), other cardiovascular risk factors and PWV in men and women.*

	Men (n =171)				Women (n =219)		
Risk variable	β ± SE	p	R^2 change	Risk variable	β ± SE	p	R^2 change
Age (years)	0.136±0.018	<0.001	26%	Age (years)	0.136±0.17	<0.001	26%
hsCRP (mg/ml)	1.189±0.348	0.001	8%	MetS	1.047±0.272	<0.001	5%
MetS	0.703±0.297	0.019	2%				
R^2	35%			R^2	30%		

MetS = metabolic syndrome, PWV = pulse wave velocity, NCEP = National Cholesterol Education Program, β = regression coefficient, SE = standard error, R^2 = adjusted R square value of the whole model, R^2 change = change in the adjusted R square value after the addition of the respective variable in to the model, hsCRP= high-sensitivity C-reactive protein. Initial stepwise regression model for men included age, hsCRP and MetS syndrome as independent variables. Initial stepwise regression model for women included age, hsCRP, low-density lipoprotein-cholesterol, smoking and MetS syndrome as independent variables.

5.4 Coronary heart disease-associated locus on chromosome 9p21.3 and subclinical atherosclerosis (original publication II)

The association between the CHD-associated locus on chromosome 9p21.3 (rs1333049) and subclinical atherosclerosis was studied in the Health 2000 Survey supplemental study and the Cardiovascular Risk in the Young Finns Study (studied in 2001) populations. The age range of the subjects was 24 to 39 years (55% female) in the Young Finns Study and 46 to 76 years (55% female) in the Health 2000 cohort. The frequency of the C allele for rs1333049 was 0.41 (95% CI: 0.40 to 0.43) in the Young Finns Study and 0.42 (95% CI: 0.40 to 0.44) among the Health 2000 subjects, and the genotypes were in Hardy Weinberg equilibrium in both cohorts. The characteristics of the subjects in each study as partitioned by genotype for rs1333049 are shown in Table 5.17. Overall, the older subjects from the Health 2000 survey had higher BMI, LDL-C and HDL-C levels as well as higher average systolic and diastolic blood pressures when compared with the subjects in the Young Finns Study ($p<0.001$). However, there was no significant difference in any of these traits according to rs1333049 genotype in either age group (Table 5.17). Both mean and maximum CIMT were higher in the Health 2000 cohort when compared to the Young Finns subjects (Table 5.17). However, the rs1333049 genotype yielded no effect on either phenotype in either the Young Finns Study ($p=0.959$ and $p=0.977$, respectively) or in the Health 2000 cohort ($p=0.714$ and $p=0.729$). Specifically, a higher CIMT was not observed in subjects

TABLE 5.17 *Demographic and phenotypic characteristics of subjects in the Cardiovascular Risk in Young Finns Study and in the Health 2000 cohort as partitioned by genotype for the coronary heart disease risk variant on chromosome 9p21.3.*

	Cardiovascular Risk in Young Finns				Health 2000 Cohort			
Phenotype	GG	GC	CC	p	GG	GC	CC	p
Age (years)	31.6 (5.0) 790	31.8 (5.0) 1093	31.8 (5.0) 394	0.522	58.0 (8.1) 454	58.6 (8.2) 599	57.8 (7.5) 242	0.292
Men (%)	44 790	45 1093	46 394	0.506	48 454	44 599	599 242	0.394
BMI (kg/m2)	25.1 (4.3) 784	25.0 (4.4) 1084	25.2 (4.5) 391	0.742	27.2 (4.6) 450	27.1 (4.3) 597	27.4 (4.5) 241	0.618
LDL-C (mmol/l)	3.27 (0.83) 782	3.30 (0.86) 1074	3.24 (0.82) 389	0.470	3.39 (0.87) 450	3.40 (0.86) 586	3.43 (0.91) 237	0.839
HDL-C (mmol/l)	1.30 (0.32) 790	1.30 (0.32) 1091	1.26 (0.30) 394	0.084	1.59 (0.43) 453	1.57 (0.42) 598	1.57 (0.45) 242	0.798
Triglycerides (mmol/l)	1.31 (0.73) 790	1.34 (0.92) 1093	1.38 (0.89) 394	0.643	1.32 (0.66) 453	1.43 (1.14) 598	1.42 (0.82) 242	0.382
SBP (mm Hg)	116.7 (12.8) 785	116.8 (13.4) 1075	116.0 (12.7) 393	0.788	138.0 (22.2) 454	139.0 (21.3) 598	137.6 (21.3) 241	0.599
DBP (mm Hg)	70.7 (10.3) 785	70.8 (11.1) 1075	70.9 (10.8) 393	0.980	84.5 (11.2) 454	84.3 (10.0) 598	84.3 (10.2) 241	0.932
Smoking (%)	25 785	23 1093	22 394	0.506	24 454	22 599	22 242	0.485
Mean CIMT (mm)	0.58 (0.09) 779	0.58 (0.09) 1082	0.58 (0.10) 390	0.959	0.93 (0.23) 454	0.93 (0.23) 599	0.94 (0.22) 242	0.714
Max CIMT (mm)	0.62 (0.10) 779	0.62 (0.10) 1082	0.62 (0.10) 390	0.977	0.99 (0.21) 454	1.00 (0.22) 599	1.00 (0.21) 242	0.729
FMD (%)	7.84 (4.34) 718	8.04 (4.47) 1015	8.12 (4.39) 362	0.521	NA	NA	NA	NA

Values are means (SD) or prevalences in %. The figure after or below each value is the number of subjects for whom the data was available. BMI= body mass index, LDL-C= low-density lipoprotein cholesterol, HDL-C= high-density lipoprotein cholesterol, SBP = systolic blood pressure, DBP = diastolic blood pressure, CIMT = carotid artery intima media thickness, FMD = brachial artery flow-mediated dilatation, NA = not available. The p values are unadjusted values based on analysis of variance for continuous variables and on Chi-square test for smoking and sex. The CHD-risk-associated allele is the C allele.

Adapted from Samani NJ, Raitakari OT, Sipilä K, Tobin MD, Schunkert H, Juonala M, Braund PS, Erdmann J, Viikari J, Moilanen L, Taittonen L, Jula A, Jokinen E, Laitinen T, Hutri-Kähönen N, Nieminen MS, Kesäniemi YA, Hall AS, Hulkkonen J, Kähönen M, Lehtimäki T. Coronary artery disease-associated locus on chromosome 9p21 and early markers of atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 2008;28(9):1679-1683.

carrying the CHD-risk-associated allele (C) in either cohort. Likewise, brachial FMD responses (available in the Young Finns Study) were similar across the genotypes (Table 5.17, p=0.521).

The results of the multivariable linear regression analysis of mean CIMT in the two studies are shown in Table 5.18. In the Young Finns Study, there were highly significant independent associations of age, sex, BMI and SBP with CIMT and a borderline significant association of smoking. In the Health 2000 cohort, there were similarly significant independent associations of age, sex and SBP with CIMT. HDL-C and smoking, but not BMI, were also independently associated with CIMT in this cohort. Taking these factors into account, the rs1333049 genotype had no independent association with mean CIMT (Table 5.18). The results for maximum CIMT were similar (data not shown).

TABLE 5.18 *Determinants of mean CIMT in the Cardiovascular Risk in Young Finns Study and the Health 2000 Cohort: Results from multivariable regression analysis*

	Young Finns Study		Health 2000 Cohort	
	β (SE)	p	β (SE)	p
Sex	0.0101 (0.0043)	0.017	0.0247 (0.0094)	0.009
Age	0.0050 (0.0004)	<0.001	0.0097 (0.0006	<0.001
BMI	0.0024 (0.0005)	<0.001	0.0015 (0.0011)	0.190
SBP	0.0054 (0.0020)	0.010	0.0096 (0.0024)	<0.001
DBP	0.0060 (0.0025)	0.015	0.0036 (0.0050)	0.472
LDL-C	0.0036 (0.0023)	0.121	0.0009 (0.0051)	0.863
HDL-C	0.0004 (0.0066)	0.947	-0.0264 (0.0131)	0.045
Triglycerides	-0.0062 (0.0045)	0.161	0.0337 (0.0296)	0.255
Smoking	0.0086 (0.0048)	0.050	0.0242 (0.0107	0.024
rs1333049 GG/GC vs. CC	-0.0009 (0.0048)	0.845	0.0107 (0.0112)	0.337

CIMT = carotid artery intima media thickness, β = regression coefficient (beta), SE = standard error, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, LDL-C= low-density lipoprotein cholesterol, HDL-C= high-density lipoprotein cholesterol. The beta values are based on the following: age, per year increase; BMI, per 1 kg/m2 increase; for SBP and DBP, per 10 mmHg increase; for LDL-C, HDL-C and triglycerides, per 1 mmol/l increase. The beta for triglycerides is for log-transformed values. The beta coefficients for the comparison of GG/GC vs. CC genotypes are shown. The results were similar when modelling GG vs. GC vs. CC or GG vs. CC. The CHD-risk-associated allele is the C allele.

Adapted from Samani NJ, Raitakari OT, Sipilä K, Tobin MD, Schunkert H, Juonala M, Braund PS, Erdmann J, Viikari J, Moilanen L, Taittonen L, Jula A, Jokinen E, Laitinen T, Hutri-Kähönen N, Nieminen MS, Kesäniemi YA, Hall AS, Hulkkonen J, Kähönen M, Lehtimäki T. Coronary artery disease-associated locus on chromosome 9p21 and early markers of atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 2008;28(9):1679-1683.

5.5 Association of IL6-174 G>C genotype with the risk factors and markers of early atherosclerosis (original publication IV)

The association of IL6-174 G>C genotype (rs1800795) with the risk factors and markers of early atherosclerosis (CIMT and CAC) was studied in the Health 2000 Survey supplemental study population. The characteristics of this population according to the IL6-174 G>C genotype are shown in Table 5.19. This genotype was determined in 1,334 subjects (n = 727 women, n = 607 men). The distribution (prevalence given in parenthesis) of IL6–174 G>C genotypes in our population was as follows: GG, n = 258 (19.3%); GC, n = 685 (51.3%); and CC, n = 391 (29.3%). The allele frequency for allele G was 0.450 and for allele C 0.550. These figures are similar with previously published figures in the Finnish population (Hulkkonen et al. 2001, Hulkkonen et al. 2009) and other European populations (Huth et al. 2009). As expected, the distributions of individual IL-6 alleles followed the Hardy–Weinberg equation (p=0.21).

In men, several significant associations between IL6–174 G>C genotype and cardiovascular risk factors were found. Serum total cholesterol levels and LDL-C levels increased according to IL6–174 G>C genotype in the order GG>GC>CC (Table 5.19). Opposite associations (GG<GC<CC) were observed with serum triglyceride, fasting insulin and fasting glucose concentrations as well as BMI (Table 5.19). IL6–174 allele G homozygocity seemed to be associated with a beneficial effect on HDL-C, SBP and DBP, but these findings were only borderline significant (Table 5.19). No significant associations between IL6–174 G>C genotype and CAC, CIMT or hsCRP were found in this cohort.

In order to evaluate the independent associations of IL6–174 G>C genotype and CVD risk factors, linear regression analyses were carried out. In the multivariable models IL6–174 G>C genotype remained an independent predictor for serum total cholesterol and LDL-C concentrations when adjusted for age, BMI, current smoking, use of statins and concentrations of fasting glucose and fasting insulin (Table 5.20). Moreover, the trend in the association of IL6–174 G>C genotype and SBP increased in significance (p=0.028) in the multivariable model when adjusted by age, BMI, current smoking, antihypertensive treatment and concentrations of fasting glucose, fasting insulin and total cholesterol (Table 5.20). IL6–174 G>C genotype was also associated with fasting glucose concentration when adjusted for age, BMI and current smoking (Table 5.20), and with BMI when adjusted for age, current smoking and concentrations of fasting glucose and total cholesterol (p=0.043, β=0.470, SE=0.232, whole model R^2=7%). The trends in the associations between IL6–174 G>C genotype and HDL-C concentration, triglyceride level and DBP could not be reassured (p=0.139–0.652). When linear

TABLE 5.19 *The associations of IL6–174 G>C genotype with cardiovascular risk factors and markers of early atherosclerosis (n = 727 female, n = 607 male).*

Parameter	Men, mean±SD				Women, mean±SD			
	IL-6 genotype				IL-6 genotype			
	GG (n=127)	GC (n=310)	CC (n=170)	p*	GG (n=131)	GC (n=375)	CC (n=221)	p*
Age (years)	57.8±7.70	57.8±7.54	58.5±8.26	0.77	58.9±8.14	58.9±8.51	58.2±7.99	0.61
Total cholesterol (mmol/l)	5.70 ± 0.88	5.51 ± 0.98	5.38 ± 0.97	<0.01	5.75 ± 0.95	5.66 ± 0.93	5.60 ± 0.89	0.33
HDL-C (mmol/l)	1.47 ± 0.34	1.43 ± 0.40	1.38 ± 0.38	0.06	1.70 ± 0.40	1.70 ± 0.45	1.72 ± 0.43	0.76
LDL-C (mmol/l)	3.64 ± 0.83	3.41 ± 0.88	3.30 ± 0.91	<0.01	3.49 ± 0.91	3.38 ± 0.88	3.32 ± 0.83	0.28
Triglycerides (mmol/l)	1.34 ± 0.75	1.56 ± 1.41	1.58 ± 0.87	0.05	1.29 ± 0.70	1.31 ± 0.66	1.24 ± 0.59	0.45
SBP (mm Hg)	137 ± 17.2	142 ± 20.2	144 ± 23.2	0.08	135 ± 21.3	136 ± 23.1	137 ± 22.3	0.65
DBP (mm Hg)	85.4 ± 9.6	88.0 ± 10.6	87.7 ± 11.5	0.09	81.5 ± 9.6	81.3 ± 9.7	82.9 ± 9.5	0.13
Mean CIMT (mm)	0.96 ± 0.23	0.97 ± 0.24	0.97 ± 0.23	0.88	0.89 ± 0.21	0.91 ± 0.22	0.89 ± 0.19	0.51
CAC (%/10mmHg)	0.95 ± 0.46	0.85 ± 0.38	0.91 ± 0.43	0.30	0.95 ± 0.57	0.98 ± 0.52	0.90 ± 0.51	0.15
hsCRP (mg/l)	3.03 ± 5.59	2.66 ± 2.93	2.84 ± 4.39	0.49	3.34 ± 5.78	3.01 ± 4.77	2.83 ± 3.54	0.90
Fasting insulin (mmol/l)	9.06 ± 5.24	10.8 ± 8.41	11.6 ± 8.25	0.02	8.63 ± 4.88	9.01 ± 5.71	9.17 ± 7.05	0.70
Fasting glucose (mmol/l)	5.93 ± 0.97	6.11 ± 1.34	6.34 ± 1.59	0.04	5.51 ± 0.73	5.64 ± 1.06	5.69 ± 1.00	0.39
BMI (kg/m^2)	26.8 ± 3.42	27.5 ± 4.32	28.0 ± 3.81	0.03	26.9 ± 4.27	27.2 ± 4.60	27.2 ± 5.48	0.82

BMI= body mass index, CIMT = carotid artery intima media thickness, CAC = carotid artery compliance, SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL-C= high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, hsCRP = high-sensitivity C-reactive protein.

* Kruskall–Wallis ANOVA test for a trend (2 degrees of freedom).

Adapted from Atherosclerosis, 207, Riikola A, Sipilä K, Kähönen M, Jula A, Nieminen MS, Moilanen L, Kesäniemi YA, Lehtimäki T, Hulkkonen J, Interleukin-6 promoter polymorphism and cardiovascular risk factors: the Health 2000 Survey, 466-470, Copyright (2009), with permission from Elsevier.

regression model R^2 values were calculated with and without the IL-6 genotype, the IL-6 genotype explained 0.7%–1.3% of the variation observed in total cholesterol, LDL-C, fasting glucose, BMI and SBP levels. Among women there were no significant associations between IL6–174 G>C genotype and cardiovascular risk factors or the above-mentioned early markers of atherosclerosis (Table 5.20).

TABLE 5.20 *Multivariable linear regression model of the relationships between IL-6 genotype and total cholesterol, LDL-C, SBP as well as fasting glucose levels adjusted with common cardiovascular risk factors in men (n = 727).*

Parameter	Total cholesterol, β±SE	LDL-C, β ±SE	SBP, β ±SE	Fasting glucose level, β ±SE
IL-6 genotype	−0.123±0.056*	−0.133±0.051**	2.567±1.167*	0.176±0.077*
Age	−0.011±0.005*	−0.007±0.005	0.407±0.110***	0.014±0.007*
BMI	0.012±0.011	0.017±0.011	0.945±0.238***	0.074±0.014***
Smoking	0.021±0.087	0.016±0.080	−3.344±1.814	0.069±0.121
Fasting glucose level	−0.010±0.034	−0.067±0.033*	2.404±0.699***	
Fasting insulin level	−0.014±0.006*	−0.011±0.006	0.054±0.133	
Total cholesterol			3.529±0.861***	
Statin medication	−0.594±0.116***	−0.643±0.107***		
Antihypertensive medication			−0.652±1.986	
R^2	9.1%	11.8%	13.7%	6.7%

β= regression coefficient, SE = standard error, R^2 = adjusted R square value of the whole model, LDL-C= low-density lipoprotein cholesterol, SBP = systolic blood pressure, BMI = body mass index.

For IL-6 genotype, the regression coefficient is for a 1-unit change in allele G content (null or 1 or 2 copies of allele G). For smoking, the regression coefficient is for categorical change from non-smoker to smoker, and for statin and antihypertensive treatments the regression coefficient is for categorical change of being with or without medication.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Adapted from Atherosclerosis, 207, Riikola A, Sipilä K, Kähönen M, Jula A, Nieminen MS, Moilanen L, Kesäniemi YA, Lehtimäki T, Hulkkonen J, Interleukin-6 promoter polymorphism and cardiovascular risk factors: the Health 2000 Survey, 466-470, Copyright (2009), with permission from Elsevier.

6 DISCUSSION

6.1 Study populations

6.1.1 The Health 2000 Survey

The overall study cohort of the Health 2000 Survey (8,028 subjects) represents the entire Finnish population aged 30 years and older. This survey was designed to evaluate the risk factor levels, morbidity and functional capacity of the Finnish population. The results presented in this thesis are based mainly on the data from the supplemental study (sample size 1,867), which was carried out to perform a detailed evaluation in regard to CVD and diabetes. The sample size in the additional study is based on power calculations to ensure that the relationships of interest could be efficiently studied. The subjects in the supplemental study were, due to logistical reasons, living in the area located within 150 km from one of the five Finnish University Hospitals. The sample is thus not representative of the whole Finnish population. Nevertheless, it is a comprehensive cross-section of the middle-aged and elderly Finnish population living in urban areas.

There are some study limitations concerning this population. The results are based on cross-sectional data and the causality between the observed relationships cannot be assessed. Furthermore, the subjects are Caucasians and the results may not apply to other ethnicities. Although a comprehensive evaluation of the risk factors for atherosclerosis was made in the supplemental study, waist circumference was not measured. For this reason, the presence or absence of MetS was defined using the data of the main study. The mean time interval between the Health 2000 Survey and the supplemental study was 1 year and 4 months (range 10–23 months), and the mean (SD) change in body weight during this time was 0.52 (3.43) kg. This time interval was considered not to have a significant effect on the results.

6.1.2 The Cardiovascular Risk in Young Finns Study

The Cardiovascular Risk in Young Finns Study population (3,596 subjects in 1980) was randomly chosen from the national population register from the areas of all five Finnish University Hospitals (Juonala et al. 2004a). Therefore, the original population was quite representative of the young Finnish population living in urban areas. This population, studied in 2001 (2,620 subjects), formed the second

cohort in the study evaluating the relationships between the CHD-risk-associated variant on chromosome 9p21.3 and early carotid atherosclerosis. In 2001, 63.5% of the original population participated in the follow-up study. Although the amount of dropouts is substantial, the risk profile among the remaining subjects is well representative of the original population (Juonala et al. 2004a).

There are some study limitations concerning this population. The present results are based on cross-sectional data and causality between the observed relationships cannot be assessed. Moreover, the subjects are Caucasians and the results may not apply to other ethnicities.

6.2 Methodological considerations

6.2.1 Ultrasound measurements

6.2.1.1 Carotid artery intima-media thickness

Atherosclerotic changes measured by ultrasound can be considered as indirect subclinical markers for systemic atherosclerosis. Previous studies have shown that CIMT is increased in subjects with CHD (Craven et al. 1990, Salonen et al. 1994, Khoury et al. 1997) and it also associates with the extent of atherosclerosis in coronary arteries (Khoury et al. 1997, Lekakis et al. 2000, Kablak-Ziembicka et al. 2004). Several prospective studies have also demonstrated that increased CIMT is related to an elevated risk of myocardial infarction and stroke (Belcaro et al. 1996, Bots et al. 1997, Chambless et al. 1997, O'Leary et al. 1999, Chambless et al. 2000). Subjects with increased CIMT had a 1.3–5.1-fold risk of myocardial infarction and a 1.3–8.5-fold risk of stroke in these studies (Bots et al. 1997, Chambless et al. 1997, O'Leary et al. 1999, Chambless et al. 2000). Increased CIMT has also been associated with increased mortality (Staub et al. 2006).

CIMT has been mostly measured from CCA because this is relatively easy. Atherosclerotic plaques, however, are usually seen first in the carotid bulb, suggesting that measuring this segment of the arterial wall might be the most sensitive marker for early atherosclerosis. Measuring several segments simultaneously may decrease errors in the measurements and capture the systemic nature of atherosclerosis better (Howard et al. 1994a, Espeland et al. 1999). Although CIMT has been validated well, it is only an estimation of systemic atherosclerosis, and this must be noted when interpreting the results.

In the Health 2000 Survey a high-resolution B-mode carotid ultrasound examination of the right carotid artery was performed. One reader was responsible for reading all ultrasound images. CIMT was measured from three digitized end

diastole images of the CCA and the carotid bulb. The means of the CCA and carotid bulb IMT measurements, as well as the maximum CCA IMT value, were used in the present study. The intra-reader reproducibility of the measurements was assessed. The reader made the measurement twice from 571 randomly selected images from 108 study subjects several weeks apart. The mean difference of these two measurements was 0.001 mm (SD 0.123). The intraclass correlation and the coefficient of variation were 0.934 ($p<0.001$) and 9.2%, respectively.

In the Cardiovascular Risk in Young Finns Study, carotid ultrasound examinations were performed using a high-resolution ultrasound system, and CIMT was measured from the left CCA. One reader blinded to the subjects' details analyzed the scans. The between-visit (2 visits 3 months apart) coefficient of variation in mean CIMT values for a subset of the subjects was 6.4% (Raitakari et al. 2003). This method was thus reproducible and valid in both study populations.

6.2.1.2 Carotid artery elasticity

Arterial stiffness has been shown to be a predictor for coronary events and cardiovascular mortality (Blacher et al. 1999, Laurent et al. 2001, Boutouyrie et al. 2002, Cruickshank et al. 2002). Stiffness of the carotid arteries, specifically, has been related to CHD (Hirai et al. 1989), stroke (Tsivgoulis et al. 2006) and all-cause as well as cardiovascular mortality (Blacher et al. 1998). In the present study, it was evaluated using indices that measure different aspects of carotid artery elasticity. CAC measures the ability of an artery to expand in response to pulse pressure caused by cardiac contraction and relaxation (Juonala et al. 2005). On the other hand, YEM is an estimate of arterial stiffness controlling for intima-media thickness, and SI has been considered to be relatively independent of blood pressure (Salomaa et al. 1995).

For the elasticity calculations the arterial diameter of the CCA was measured. The indices of arterial elasticity were calculated on the bases of ultrasound measurements and supine blood pressure. A possible limitation of this method is the fact that blood pressure was not measured from the same artery as the arterial diameters. However, the validity of this method in estimating central arterial stiffness has been demonstrated (Imura et al. 1986, Stefanadis et al. 1990). The intra-reader reproducibility of the arterial diameter measurements was assessed. The reader measured the arterial diameter twice from 594 randomly selected images from 99 study subjects several weeks apart. The mean difference of the two measurements was 0.014 mm (SD 0.145). The intraclass correlation and coefficient of variation were 0.990 ($p<0.001$) and 1.3%, respectively. This method was therefore reproducible and valid.

6.2.1.3 Brachial flow-mediated dilatation

FMD of the brachial artery has been shown to correlate with coronary endothelial function (Anderson et al. 1995). It has also been reported to predict cardiovascular events (Bonetti et al. 2003). Brachial FMD was measured in the Cardiovascular Risk in Young Finns Study. To assess this parameter, the left brachial artery diameter was measured both at rest and during reactive hyperaemia (Juonala et al. 2004b). The vessel diameter after reactive hyperaemia was expressed as the percentage relative to the resting scan. This method has been originally described by Celermajer et al. (Celermajer et al. 1992). The greatest weakness of this method is the variability of the measurements. FMD responses are affected by numerous physical and environmental factors (Corretti et al. 2002). The 3-month between-visit coefficient of variation was 3.2% for brachial artery diameter measurements and 26.0% for FMD measurements (Juonala et al. 2004b). The fluctuation in FMD measurements was substantial while the reproducibility of the brachial artery diameter measurements was very good. This suggests that much of the variation in FMD was caused by physiological factors and not by measurement error.

6.2.2 Pulse wave velocity measurement

Arterial stiffness can be evaluated by measuring the velocity of the pulse wave along the arterial tree. Previously, PWV measurements have been taken mostly by methods using Doppler ultrasound or mechanoelectrical pulse transducers (Lehmann et al. 1992, Wilkinson et al. 1998). It can also be measured with the ICG_{WB} method that was used in the present study. The evaluation of the PWV measurement using this method has been described in detail previously (Kööbi et al. 1997, Kööbi et al. 2003). ICG_{WB} has not been utilized in scientific studies as much as other techniques, but the reproducibility values of PWV measurements by ICG_{WB} (2.42 m/s) and Doppler ultrasound (2.17 m/s) are similar (Kööbi et al. 2003), suggesting that ICG_{WB} can be used in epidemiological studies as well. Indeed, this method has recently been successfully applied in an epidemiological setting in the Cardiovascular Risk in Young Finns Study (Aatola et al. 2010).

6.3 Glucose tolerance and early atherosclerosis

The results of the present thesis show that there are sex-related differences in the extent of early carotid atherosclerosis according to the glucose tolerance status. These findings suggest that glucose intolerance might have a stronger association with early atherosclerosis in women than in men.

It is known that subjects with T2DM have significantly increased early carotid atherosclerosis when compared to those without glucose tolerance impairment (Kawamori et al. 1992, Folsom et al. 1994, Pujia et al. 1994, Niskanen et al. 1996, Bonora et al. 1997, Emoto et al. 1998, Tropeano et al. 2004, Sharrett et al. 2006, Martens et al. 2008). Some (Hanefeld et al. 1999a, Mohan et al. 2006, Zhang et al. 2006, Faeh et al. 2007), but not all (Niskanen et al. 1996, Tuomilehto et al. 1998, Wagenknecht et al. 1998) of the previous studies have found similar results in regard to IGT. In a recent meta-analysis, Brohall et al. concluded that CIMT in subjects with IGT is slightly increased in comparison to those with normal glucose metabolism (Brohall et al. 2009). Non-diabetic glucose intolerance has also been associated with increased stiffness of the carotid arteries (Salomaa et al. 1995, van Popele et al. 2000). The present results, after the adjustment for age, are in concert with previous reports in regard to diabetic subjects. With reference to IGT, however, some sex-related differences were observed. In the current population, women with IGT had a significantly worse profile in terms of carotid atherosclerosis than women with normal glucose metabolism after age had been taken into account. Among men the IGT group differed significantly from the control group only in regard to YEM and CAC. In the age-adjusted model even IFG was related to decreased CAC in both sexes and to increased CIMT in women. Some authors have reported similar findings (van Popele et al. 2006, Zhang et al. 2006), but most of the previous studies have not observed significant changes in early carotid atherosclerosis in subjects with IFG (Bonora et al. 1999, Hanefeld et al. 1999b, Tropeano et al. 2004, Faeh et al. 2007). The present finding of the deteriorating trend in the atherosclerosis of the carotid arteries across the glucose tolerance continuum is in concert with earlier literature (Henry et al. 2003). In the current population, subjects with IGT tended to have increased CIMT and decreased carotid artery elasticity when compared to those with IFG. This observation is in line with previous data (Hanefeld et al. 2000).

The fact that the observed associations weakened substantially after the adjustments for age and other cardiovascular risk factors leads to the conclusion that these associations are partly mediated by the overall risk factor profile of the subjects with glucose metabolism disorders. Nonetheless, there were sex-related differences even after these adjustments. No significant differences between the groups were seen in men, but a trend of worsening carotid artery elasticity across the glucose tolerance categories was observed in women even after the further adjustments. In pair-wise comparison the diabetes group had significantly less elastic carotid arteries than the group of subjects with normal glucose metabolism. Furthermore, even the IFG group had significantly lower carotid artery compliance than the control group. All in all, there were different patterns between the sexes in the relationships between glucose tolerance and early atherosclerosis.

The number of studies with sex-specific results has been limited concerning the association of glucose tolerance and early carotid atherosclerosis. In a study by Kawamoto et al., T2DM had a stronger association with elevated CIMT in women than in men (Kawamoto et al. 2007b). On the other hand, other studies have not confirmed this finding (Folsom et al. 1994, Wei et al. 1996, Temelkova-Kurktschiev et al. 1999). In these studies, only subjects with normal glucose metabolism or diabetes were involved. Two studies investigated the associations between glucose tolerance and CIMT in subjects with normal glucose tolerance, IGT or T2DM and did not find significant sex-related differences (Niskanen et al. 1996, Bonora et al. 2000). Salomaa et al. observed a stronger association between blood glucose and carotid artery elasticity in women than in men (Salomaa et al. 1995) – in this study, however, glucose tolerance was categorized according to the fasting glucose values only. In a study by van Popele et al., the stiffness of the carotid arteries was evaluated in subjects with normal glucose tolerance, IFG or T2DM, and no sex-related differences were seen (van Popele et al. 2006). Henry et al. did not find a significant interaction between glucose tolerance and sex regarding arterial stiffness (Henry et al. 2003). Their study included subjects with normal glucose tolerance, T2DM or impaired glucose metabolism – i.e., subjects with IFG or IGT. None of the above-mentioned studies have included both IFG and IGT groups. All in all, previous results regarding sex-related differences are limited and controversial. The variation in the findings might be partly explained by population differences between the studies and by variation in the classification for glucose intolerance. Moreover, most of the studies have not specifically investigated sex-related differences. An overview of the studies evaluating sex-related differences regarding glucose tolerance as a risk factor for early atherosclerosis is provided in Table 6.1.

To conclude, the present results suggest that women with glucose intolerance may be at a greater risk for early atherosclerotic changes than men. In both sexes, carotid atherosclerosis tends to increase with the worsening of glucose tolerance, but the changes are at least partly mediated by the overall deterioration in the cardiovascular risk factor profile of the subjects with impaired glucose metabolism.

TABLE 6.1 *Studies investigating the association of glucose tolerance and early carotid atherosclerosis in men and women separately.*

Reference	n	n male/ female	Age	Origin of the subjects	Categories of glucose tolerance	Marker of atherosclerosis	Findings
Kawamoto et al. 2007b	918	394/524	Elderly (mean age in men 66 and in women 72 years)	Japanese	NGT and DM	CIMT	DM had a stronger association with CIMT in women than in men
Salomaa et al. 1995	4701	2068/2633	45–64 years	Caucasian and African Americans	Normal, borderline or diabetic fasting glucose value	YEM, SI, CAC, and Ep	Blood glucose had a stronger association with CAS in women than in men
Folsom et al. 1994	14430	6474/7956	45–64 years	Caucasian and African Americans	NGT and DM	CIMT	DM associated similarly with CIMT in both sexes
Wei et al. 1996	867	343/524	35–64 years	Mexicans and Mexican Americans	NGT and DM	CIMT	DM associated similarly with CIMT in both sexes
Temelkova-Kurktschiev et al. 1999	142 (71 cases and controls)	96/46	40–70 years	German	NGT and DM	CIMT	DM associated similarly with CIMT in both sexes
Niskanen et al. 1996	203	99/104	45–64	Finnish	NGT, IGT, and DM	CIMT	DM and IGT associated similarly with CIMT in both sexes
Bonora et al. 2000	826	412/414	40–79	Italian	NGT, IGT, and DM	CIMT	DM and IGT associated similarly with CIMT in both sexes
van Popele et al. 2006	2987	1254/1733	>60 years	Dutch	NGT, IFG, and DM	Carotid artery distensibility	IFG and DM associated similarly with CAS in both sexes
Henry et al. 2003	747	375/372	Mean age 68.5 years	Dutch	NGT, IFG or IGT, and DM	Distensibility and compliance of the carotid artery	Glucose tolerance groups associated similarly with CAS in both sexes

NGT= normal glucose tolerance, DM= diabetes mellitus, CIMT= carotid artery intima-media thickness, YEM= Young's elastic modulus, SI= beta stiffness index, CAC= carotid artery compliance, Ep= Peterson's elastic modulus, CAS= carotid artery stiffness, IGT= impaired glucose tolerance, IFG= impaired fasting glucose.

6.4 Metabolic syndrome and early atherosclerosis

The findings of the present thesis show that MetS is associated in both sexes with increased early atherosclerosis. The results suggest, however, that the association may be stronger in women than in men.

6.4.1 Metabolic syndrome as an independent risk factor

MetS has been shown to be a risk factor for T2DM (Lorenzo et al. 2003), CHD (Bonora et al. 2003) and mortality (Isomaa et al. 2001, Lakka et al. 2002, Malik et al. 2004). It has also been associated with increased CIMT (Hulthe et al. 2000, Anand et al. 2003, McNeill et al. 2004, Scuteri et al. 2004, Ahluwalia et al. 2006, Skilton et al. 2007) as well as increased arterial stiffness (PWV as a surrogate) in several studies (Czernichow et al. 2005, Ferreira et al. 2005, Li et al. 2005, Schillaci et al. 2005, Ahluwalia et al. 2006). The results of the current study are well in line with previous data, and the associations were seen in both sexes (regarding PWV and CIMT) and with two different MetS definitions (regarding PWV).

There were no significant differences between the NCEP and IDF definitions in their ability to determine PWV in the present study. The main difference between the two definitions is that the latter makes central obesity mandatory for diagnosis. The IDF definition also has a lower waist circumference threshold. Because of the latter, the IDF definition produces a higher prevalence for MetS than the NCEP definition. In the current population, the prevalence figures were remarkably high, 45.4% and 40.4%, for the IDF and NCEP definitions, respectively. This is probably related to the age structure of the study population (45 years and above). Nevertheless, this increases our understanding of the magnitude of MetS as an important cardiovascular risk factor among the Finnish middle-aged population.

Type 2 diabetes is a stronger risk factor for cardiovascular events in women than in men (Beckman et al. 2002). Because T2DM and MetS are closely related, it is reasonable to hypothesize that there might be similar sex-specific differences in the relationship between MetS and early atherosclerosis. Indeed, some reports have suggested that the association between MetS and CIMT is more pronounced in women than in men (Iglseder et al. 2005). Furthermore, in four different studies, MetS has been independently associated with CIMT only in women (Kawamoto et al. 2007a, Chen et al. 2008, Lee et al. 2010, Lin et al. 2010). Nishida et al. suggested that the increase in the number of MetS components is associated more strongly with CIMT in women than in men (Nishida et al. 2007). Skilton et al. studied the effect of this syndrome, defined by three different definitions (NCEP, IDF or AHA), on CIMT. They concluded that the IDF, but not the other definitions,

was independently associated with CIMT in women (Skilton et al. 2007). In men, none of the definitions were found to associate with CIMT independently. Several studies have reported that MetS is an independent predictor of PWV only in women (Choi et al. 2004, Ferreira et al. 2007, Protogerou et al. 2007). In a recent investigation with Chinese subjects, Lin et al. found a stronger association between arterial stiffness (PWV and carotid artery elasticity) and MetS in women than in men (Lin et al. 2010). On the other hand, Scuteri et al. suggested that MetS is associated with a similar deterioration in CIMT, aortic PWV and stiffness of the carotid arteries in both sexes (Scuteri et al. 2010). Empana et al. did not find sex-related differences in the effect of MetS on CIMT either (Empana et al. 2007). Interestingly, Ishizaka et al. found a significant association between MetS and increased CIMT in men but not in women when studying subjects without diabetes (Ishizaka et al. 2009). In summary, some of the previous evidence suggests that MetS would have a more pronounced influence on early atherosclerosis in women than in men. The matter is, however, somewhat controversial. An overview of the studies evaluating sex-related differences regarding MetS as a risk factor for early atherosclerosis is shown in Table 6.2.

TABLE 6.2 *Studies investigating the associations of metabolic syndrome and CIMT or arterial stiffness in men and women separately.*

Reference	n	n male/female	Age	Origin of the subjects	Definition of metabolic syndrome	Marker of atherosclerosis	Findings
Illgiseder et al. 2005	1588	1001/587	40–65 years	Austrian	NCEP	CIMT	Stronger association between CIMT and MetS in women than in men
Kawamoto et al. 2007a	868	388/480	14–104 years	Japanese	Japanese criteria	CIMT	MetS independently associated with CIMT only in women
Chen et al. 2008	810	456/354	>38 years	Chinese	NCEP	CIMT	MetS independently associated with CIMT only in women
Lee et al. 2010	1730	634/1096	>50 years	Korean	Modified NCEP	CIMT	MetS independently associated with CIMT only in women
Lin et al. 2010	1245	566/679	15–87 years	Chinese	NCEP with Asian modification	CIMT, carotid artery stiffness indices and PWV	MetS independently associated with CIMT only in women. Stronger association between arterial stiffness and MetS in women than in men
Skilton et al. 2007	1782	1101/681	30–80 years	French	NCEP, IDF, AHA	CIMT	IDF definition was independently associated with CIMT in women but none of the definitions independently associated with CIMT in men
Choi et al. 2004	368	119/249	Middle aged	Korean	NCEP (with modified abdominal obesity criteria)	Brachial ankle PWV	PWV significantly associated with MetS in women but not in men
Ferreira et al. 2007	313	160/153	20–25 years	Subjects from Northern Ireland	Cut-off values for MetS components derived from the study population	PWV in three different vascular segments	PWV significantly associated with MetS only in women

Protogerou et al. 2007	613	364/249	Middle aged (mean 58.8 years)	French	NCEP	Carotid femoral PWV	MetS independently associated with PWW only in women
Empana et al. 2007	5585	2124/3461	65–85 years	French	NCEP	CIMT	MetS associated similarly with CIMT in both sexes
Scuteri et al. 2010	6148	About 40% men	14–102 years	Italian	NCEP	CIMT, aortic PWV and carotid artery stiffness index	MetS associated similarly with markers of atherosclerosis in both sexes across all age groups
Ishizaka et al. 2009	3904	Approximately 2/3 men	Middle aged	Japanese	NCEP (body mass index used instead of waist circumference)	CIMT	Significant associations between MetS and CIMT only in men

MetS= metabolic syndrome, PWV= pulse wave velocity, CIMT= carotid artery intima-media thickness, NCEP= National Cholesterol Education Program, IDF= International Diabetes Federation, AHA= American Heart Association.

In the present study, MetS was found to be an independent determinant of CIMT together with age in both sexes, when it was included in the regression model instead of its components. However, the explained variance of the model was smaller in men (22%) than in women (29%). Moreover, when MetS was added into the regression model together with its components, it remained an independent determinant for CIMT in women but not in men. It should be kept in mind that the change in the explained variance of this model was rather small (1%) also in women. MetS was also independently associated with PWV in both sexes, the regression coefficient (β) being larger for women (1,047) than for men (0,703). In other words, a similar sex-related pattern was observed as with CIMT. When MetS was added into the regression model together with its components, it did not remain a significant predictor for PWW for either sex. Some authors have reported earlier that MetS is associated with early atherosclerosis independently of its components (Scuteri et al. 2004, Kawamoto et al. 2005). However, the hypothesis that MetS would increase cardiovascular risk more than the sum of its parts has been critically questioned. There is no convincing evidence supporting the hypothesis (Kahn et al. 2005, Bayturan et al. 2010).

The clinical significance of the findings in epidemiological studies is not straightforward. The clinical importance of the observed differences in CIMT can be estimated by comparing them to previous data. Prospective studies have shown that a 0.1mm increase in CIMT is associated with a 20% to 30% higher risk of subsequent CHD (Bots et al. 1997, Hodis et al. 1998, O'Leary et al. 1999). In a study by Burke et al. subjects with a previous myocardial infarction had a 0.07 mm greater CIMT compared to healthy controls (Burke et al. 1995). In another study subjects with a previous stroke had a 0.1 and 0.07 mm higher CCA IMT compared to subjects without stroke among women and men, respectively (Chambless et al. 2000). In the present study, the adjusted means for CIMT (adjustments for age, LDL-C, hsCRP and smoking) were 0.051 mm (men) and 0.074 mm (women) higher in subjects with MetS than in those without it (Figure 5.1, unpublished data). In other words, the increase in CIMT as mediated by MetS was 0.023 mm higher in women than in men. This is an indirect estimate of the clinical significance, but it supports the conclusion that the observed sex-specific difference is meaningful.

6.4.2 Metabolic syndrome as a risk factor beyond the Framingham Risk Score

Few studies have elaborated the association of MetS and CIMT after taking the Framingham Risk Score into account. Hassinen et al. found in a prospective

study with elderly women that MetS is associated with accelerated progression in CIMT independently of this risk score (Hassinen et al. 2006). Teramura et al. also suggested that MetS, as defined by Japanese criteria, would have additive predictive value on CIMT beyond the Framingham score values (Teramura et al. 2007). On the other hand, Ahluwalia et al. suggested that MetS would not have such an additive effect (Ahluwalia et al. 2006). To the best of the author's knowledge, sex-related differences in the associations between MetS, the Framingham Risk Score and CIMT have not been studied previously. In the present study, these associations were modified by sex. The study population was divided into four risk profile categories according to the Framingham score value and MetS status. In men, CIMT was significantly higher if the score value was high, regardless of the MetS status. In women, however, CIMT was significantly higher if MetS was present, regardless of the Framinghan Risk Score category. These findings suggest that MetS has a different, and in some circumstances stronger, association with early atherosclerosis in women than in men.

In conclusion, the present results suggest that MetS is, in both sexes, associated with early atherosclerosis independently of the risk factors that are not included in its definition. These associations are, however, modified by sex, and MetS may increase the risk of early atherosclerotic changes more in women than in men. In men, traditional risk factors, as defined by the Framingham Risk Score, are strong determinants of CIMT, and MetS appears to offer no additional information. In women, however, MetS may add to the risk of subclinical atherosclerosis beyond the score values. This additive relationship was observed particularly in women with a low risk score, indicating that MetS is an important risk factor for women with a low cardiovascular risk according to the traditional risk factors. The NCEP and IDF definitions were both similarly associated with PWV, which suggests that both definitions are able to identify subjects with increased arterial stiffness.

6.5 Coronary heart disease-associated locus on chromosome 9p21.3 and early atherosclerosis

The chromosome 9p21.3 locus has a robust association with CHD, which has been demonstrated with a wide range of populations (Burton et al. 2007, Helgadottir et al. 2007, McPherson et al. 2007, Samani et al. 2007, Broadbent et al. 2008, Schunkert et al. 2008). The SNP (rs1333049) included in the present study has had the strongest association with CHD in these genome-wide association studies. The region associated with CHD is located adjacent to genes that play a central role in the regulation of the cell cycle and may be related to atherosclerosis through

their role in transforming growth-factor-β-induced growth inhibition (Hannon and Beach 1994, Kalinina et al. 2004). In this context, it is relevant to examine the association of this locus with different stages of atherosclerosis. Interestingly, one study has also shown an association of the locus with abdominal aortic aneurysms as well as with intracranial aneurysms, suggesting that the mechanism of its effect on the vascular wall might be more complex than simple promotion of the development of atherosclerosis (Helgadottir et al. 2007). Furthermore, the SNP of interest has not been identified in genome-wide association studies investigating stroke (Matarin et al. 2007, Ikram et al. 2009) or peripheral arterial disease (Koriyama et al. 2010). On the other hand, in some candidate gene studies this particular SNP has been significantly associated with stroke (Smith et al. 2009) and peripheral arterial disease (Cluett et al. 2009).

The association of the 9p21.3 locus with early atherosclerosis has not been examined extensively. Studying the relationships between the risk factors and arterial wall thickness in a young predisease cohort provides a useful means of investigating the early impact of such determinants on atherosclerosis. Because genetic determinants could be active from a young age, the association of this polymorphism with CIMT was studied in a cohort of young adult subjects. It was also studied in a second cohort with a similar age range as in those in which the association of the locus with CHD was originally demonstrated. Somewhat surprisingly, no evidence was observed in support of an association between this polymorphism and any of the CIMT parameters in either age group. Similarly, there was no significant association between the polymorphism and brachial FMD in the Young Finns Study. After the publication of this study, others have reported similar results (Cunnington et al. 2009).

Some possible explanations for the lack of association between the studied SNP and early atherosclerosis must be discussed: 1) Both cohorts were population-based, ethnically homogeneous, and of Caucasoid origin where the association of CHD with this polymorphism has been well demonstrated (Burton et al. 2007, Helgadottir et al. 2007, McPherson et al. 2007, Samani et al. 2007, Schunkert et al. 2008). Therefore, the population structure does not offer a plausible explanation for the findings. 2) The measurements of CIMT and FMD were performed using standardised protocols by trained personnel. Therefore, it is not likely that the observations would be explained by imprecise measurements. 3) The power calculations in the present data have shown that there was 80% power at an alpha of 0.05 to detect 0.02 mm and 0.06 mm differences in CIMT between CC and GG subjects in the Young Finns Study and the Health 2000 cohort, respectively. This means that there was 99% power at an alpha of 0.01 to detect a 0.2 mm difference in both cohorts. Previous literature has demonstrated that an increase in CIMT by 0.1 mm would elevate the risk for future CHD by 20% to 30% (Bots et al.

1997, Hodis et al. 1998, O'Leary et al. 1999). According to a recent meta-analysis each copy of the risk allele (allele C) of this polymorphism is associated with a 24% increase in the risk of CHD (95% CI: 20% to 29%) (Schunkert et al. 2008). Therefore, the expected effect of this SNP on CIMT would be approximately 0.1 mm per allele, assuming that the association with CHD was mediated through a similar mechanism. Moreover, several previously reported associations between other cardiovascular risk factors and CIMT were easily detected in both cohorts. Based on this knowledge, it does not seem likely that inadequate power to detect an effect would explain the lack of association between the 9p21.3 locus and CIMT.

One plausible explanation for the lack of association between this CHD-associated locus and CIMT would be that it is related to the risk of CHD through mechanisms that are not reflected by changes in arterial wall thickness. Similarly, the findings suggest that this polymorphism does not enhance the risk of CHD primarily causing endothelial dysfunction at a young age. If the mechanism of the effect of the 9p21.3 locus on CHD is related to cell growth and turnover, it is possible that it affects coronary plaque stability or vulnerability rather than its development per se.

6.6 Association of IL6-174 G>C genotype with the risk factors and markers of early atherosclerosis

The effect of the IL6–174 G>C genotype (rs1800795) on circulating IL-6 levels is complex. Although individual studies have associated either the C or the G allele with increased circulating IL-6 levels, a recent joint analysis of participants from 17 studies found no such association (Huth et al. 2009). The authors of this analysis concluded that the genotype might yield an effect on IL-6 levels only in certain populations, such as diabetics. Indeed, the CC genotype has been related to elevated IL-6 levels in a large study of elderly subjects with a high prevalence of diabetes (Walston et al. 2007).

Several previous studies have found associations between this polymorphism, CVD events and cardiovascular risk factors. Allele C has been related to increased risk for myocardial infarction (Georges et al. 2001, Humphries et al. 2001, Chiappelli et al. 2005) in men as well as obesity (Berthier et al. 2003, Klipstein-Grobusch et al. 2006), insulin resistance (Kubaszek et al. 2003), elevated CRP level (Humphries et al. 2001, Sie et al. 2006), and increased all-cause mortality among the elderly (Bruunsgaard et al. 2004, Hurme et al. 2005). In some populations, on the other hand, allele C has been related to a decreased risk (Basso et al. 2002) or severity (Mysliwska et al. 2006) of CHD, a lower risk for stroke (Balding et al.

2004) and also better insulin sensitivity (Fernandez-Real et al. 2000) in comparison to allele G. Moreover, allele G has been suggested to contribute to the development of peripheral arterial disease (Flex et al. 2002, Libra et al. 2006).

All in all, the previous results seem quite controversial. In a recent meta-analysis, no significant association between the investigated polymorphism and the risk for CHD was found (Sie et al. 2006). Furthermore, the authors of a systematic review regarding its association with stroke stated that the literature is conflicting, which might reflect the complexity of IL-6 physiology (Tso et al. 2007). They could not show either of the alleles to universally contribute to the stroke risk. In a joint analysis of 21 different studies, allele C had a protective effect against T2DM (Huth et al. 2006), and in a more recent joint analysis carriers of this allele had lower fasting glucose levels independently of BMI (Huth et al. 2009). No significant association between IL6–174 G>C polymorphism and BMI was observed in the joint analysis by Huth et al. or in a meta-analysis by Qi et al. (Qi et al. 2007, Huth et al. 2009).

6.6.1 IL6-174 G>C genotype and the risk factors for atherosclerosis

The results of the present study suggest that the IL6–174 G>C polymorphism associates independently with total cholesterol, LDL-C, fasting plasma glucose, SBP and BMI in men. In women, however, no significant associations were observed between this genotype and cardiovascular risk factors.

In men, allele C was associated with decreased total cholesterol and LDL-C concentrations. The previous results in regard to the association of this polymorphism and lipid metabolism are limited and inconsistent. Some similarities with the present findings were reported by Barbieri et al. (Barbieri et al. 2005). In their population, including men and women, allele C was associated with lower total cholesterol levels than allele G. On the other hand, Cardellini et al. reported quite the opposite results in a population sample of obese subjects (Cardellini et al. 2005). They did not observe this association in normal-weight subjects. Henningsson et al. found an association between the CC genotype and low total cholesterol and LDL-C levels in women (Henningsson et al. 2006). There was no association in men between this polymorphism and the lipid pattern, and the authors suggested that there are sex-specific differences in these associations. Some studies have not found associations between this genotype and the lipid pattern (Lieb et al. 2004, Wernstedt et al. 2004, Moleres et al. 2009). However, possible sex-related differences have not been investigated in these studies.

There were no significant associations between the IL6–174 G>C polymorphism and HDL-C or triglyceride concentrations in the present study, which is in line with

some previous reports (Moleres et al. 2009). In a very small population Fernandez-Real et al. found an association between allele G and high triglyceride as well as low HDL-C concentrations (Fernandez-Real et al. 2000b). On the other hand, allele C has been associated with a lower HDL-C concentration in men (Hulkkonen et al. 2009) and a higher triglyceride concentration in women (Henningsson et al. 2006).

In the present study, an adverse association of allele C was observed with SBP, BMI and fasting plasma glucose level. The observed trend for increasing blood pressure with the increasing number of allele C copies is in line with previous studies by Humphries et al. (Humphries et al. 2001) and Hulkkonen et al. (Hulkkonen et al. 2009) with middle-aged British men and young Finnish men, respectively. There are also reports showing no association between this polymorphism and blood pressure (Lieb et al. 2004), and it has not been identified in large genome-wide association studies on blood pressure (Levy et al. 2009). The current results regarding BMI are in concert with some of the studies relating allele C with obesity (Berthier et al. 2003, Wernstedt et al. 2004, Klipstein-Grobusch et al. 2006). The observed associations between the polymorphism, fasting glucose and BMI differ, however, from the results of the recent joint analysis by Huth et al. (Huth et al. 2009). In fact, the present results are opposite to this analysis as regards fasting glucose levels. Possible differences in the populations might explain some of this discrepancy. The overall pattern in the present results is rather similar in comparison to the results reported by Hulkkonen et al. (Hulkkonen et al. 2009), who also performed their study on the Finnish population. The level of the effect size was also similar in these studies. In both studies allele C was associated with deterioration in multiple metabolic risk factors in men. If there truly are sex-specific differences in these associations, this might also explain some of the variation in the previous literature.

6.6.2 IL6-174 G>C genotype and the markers of early atherosclerosis

The IL6–174 G>C polymorphism was not associated with the markers of early atherosclerosis in the present cohort in either sex, or when men and women were analyzed together. This polymorphism has been previously associated with CIMT in British adults with a wide age range (Mayosi et al. 2005) and with arterial stiffness among young men in the Cardiovascular Risk in Young Finns study cohort (Hulkkonen et al. 2009). The article by Mayosi et al. also included a meta-analysis of the previous studies and the results were similar. There are several possible reasons for the lack of association between the mentioned polymorphism and early atherosclerosis among the middle-aged and elderly subjects included

in the present study. Among the most evident reasons are differences in age, baseline blood pressure, body mass or composition, and metabolic as well as pharmacological factors between the study cohorts. Furthermore, it is possible that the subtle allelic effects, which can be observed in young populations, are overrun by stronger lifestyle-related covariates in older cohorts. Post-hoc power calculations in the present data have shown that there was 80% power at an alpha of 0.05 to detect 0.08 mm and 0.07 mm differences in CIMT between CC and GG subjects in men and women, respectively. Therefore, smaller differences may not have been detected. On the other hand, the respective detectable difference in CIMT was 0.05 mm when the sexes were pooled together.

6.6.3 Clinical characteristics and the effects of the IL6-174 G>C genotype

The IL-6 gene is under complex regulation, and it is known that many factors, such as glucocorticoids, IL-1 and oestrogen (17-beta estradiol), affect its transcription activity (Pottratz et al. 1994, Fried et al. 1998). It has been reported that adipose tissue accounts for up to 30% of the total circulating concentrations of IL-6 in healthy subjects (Mohamed-Ali et al. 1997, Yudkin et al. 1999). One of the reasons that no associations between the IL6–174 G>C genotype and cardiovascular risk factors were observed in women in the current study may be related to the inhibitory effect of oestrogens on IL-6 transcription activity.

Allele C may be associated with a lower transcription peak after stimulation but a slower decline to baseline in comparison to allele G. As a result, there would be higher chronic levels of IL-6 in allele C carriers than in GG carriers and, on the contrary, higher levels in GG subjects in response to acute inflammation (Terry et al. 2000). It has been recently shown that this kind of genetic effect of this polymorphism on circulating IL-6 levels is clearly seen in normal-weight (BMI<25 kg/m^2) but not in overweight (BMI>25) subjects (Sanderson et al. 2009). Authors of this particular study also noted that the baseline IL-6 level was higher in overweight subjects than in those with a normal (body) weight, suggesting that there are mutual interactions between IL-6, body composition and inflammation (Sanderson et al. 2009). Moreover, these interactions were emphasized in another study with adolescent subjects, where allele C carriers were shown to have higher values of lipoprotein (a) and CRP concentrations as their percentage of body fat mass increased (Moleres et al. 2009). The authors concluded that subjects carrying the C allele might be at a greater risk of developing obesity-related disorders. IL-6 is a key inflammatory factor stimulating the expression of acute-phase proteins such as CRP in the liver (Heinrich et al. 1990, Keller et al. 1996). In a recent

study consisting of 5,924 participants, Sie et al. found significantly higher CRP levels in allele C carriers when compared to the non-carriers of this allele (Sie et al. 2006). The difference in the mean CRP levels between the CC and GG genotypes was approximately 0.25 mg/l. In the present study, however, no such association was observed. On the basis of the current and previous results, it is tempting to speculate whether the associations of the IL6-174 G>C genotype with plasma lipids, fasting glucose and BMI reflect direct effects of IL-6 in the liver intermediate metabolism (gluconeogenesis), cholesterol synthesis, and acute phase protein induction. These possible metabolic effects of IL-6 should be studied further.

In conclusion, there are significant associations between the IL6–174 G>C genotype and the levels of total cholesterol, LDL-C, fasting plasma glucose and BMI in middle-aged and elderly men. There also appears to be an increasing trend in blood pressure with the increasing number of allele C copies of this polymorphism in men. It must be stated that although the absolute effect size of this genotype for risk factor levels is relatively low, the genetic determinants presently studied are very common. Therefore, at the population level, this polymorphism might be an important genetic risk factor in men. However, the effect of the genotype on different risk factors seems to vary between positive and negative depending on the risk factor in question. Hence, the significance of the investigated polymorphism remains uncertain. There are possible sex-specific differences in the associations of the IL6–174 G>C genotype and cardiovascular risk factors. The effect of this genotype may also depend on such factors as the subject's body fat mass and metabolic as well as inflammatory state. The vast heterogeneity in the literature regarding the effect of this polymorphism on atherosclerosis could be explained by these factors.

6.7 Clinical implications and future research needs

The growing epidemic of a sedentary lifestyle and obesity has introduced new challenges in the work for the prevention of cardiovascular disease. Metabolic risk factors such as T2DM and MetS have gained vast amounts of attention in the field of cardiovascular research recently. Their role in the development of atherosclerosis has not, however, been fully clarified, and the whole concept of MetS has received much criticism (Kahn et al. 2005). There is little doubt that overweight and obesity-related disorders are risk factors for atherosclerosis. Their role independent of traditional risk factors has, however, been subject to much debate. The evidence is not convincing that MetS would be a greater cardiovascular risk factor than the sum of its parts (Bayturan et al. 2010). It has,

nevertheless, been suggested that it acts as an early warning sign especially in younger subjects with a relatively low risk according to the individual risk factors (Cameron et al. 2009).

The present results show that MetS and impaired glucose metabolism are risk factors for early atherosclerosis in both sexes. These findings are in line with earlier reports. However, the associations between metabolic risk factors and early atherosclerosis seem to be modified by sex. The present results suggest that these associations might be more pronounced in women. There have been some earlier indications of this (Salomaa et al. 1995, Iglseder et al. 2005), but the issue has not been substantially covered. According to the present results MetS is not an additional risk factor beyond its components in men, nor is the possible additional value very prominent in women either. The findings that the effects of risk factors differ between men and women are not surprising, given the fact that the sexes differ in genetic and hormonal background. One may speculate some possible explanations for these sex-related differences. Menopause and oestrogenic status are possible and commonly suggested mediators for sex-related differences in cardiovascular risk. Women in the present study were mostly postmenopausal, and it is reasonable to speculate that this might have increased their vulnerability to such risk factors as glucose intolerance and MetS. It has also been shown that the deteriorating tendency in other metabolic risk factors, such as central obesity, lipid profile and hypertension, is stronger in diabetic women than their male counterparts (Howard et al. 1998). Insulin resistance has been suggested to be the key element in this process of reversing the usually favourable female risk profile in diabetic women (Howard et al. 1998).

If women with metabolic risk factors truly are at greater risk of CVD, this warrants physicians and researchers to pay more and more attention to women's cardiovascular health as the prevalence of obesity increases. Further studies are needed to confirm the sex-specific differences and also to clarify the molecular mechanisms behind them.

Another rapidly growing field in cardiovascular research is genetics. Genetic risk factors for CVD have been studied vigorously in recent years. However, few clinical tools have been derived from this work, the main reason being the complexity of atherosclerosis as a disease. It is a multifactorial process whose molecular biology has not been fully discovered. Another important reason is that the effects of genetic risk factors may vary between populations. Other factors – such as obesity, smoking and hypertension – may also modify the effects. The interactions are not easy to control in scientific studies, because many of them are yet unidentified.

Previous literature regarding the effects of the IL6–174 G>C genotype on the risk factors and markers of early atherosclerosis emphasize the above-mentioned

problems. The results of the current study suggest that allele C of this genotype is associated with higher blood pressure, and with blood glucose level as well as obesity in men. Comparable results have been reported (Humphries et al. 2001, Berthier et al. 2003, Klipstein-Grobusch et al. 2006, Hulkkonen et al. 2009), but large joint and meta-analyses have yielded contradictory results (Qi et al. 2007, Huth et al. 2009). These confusing results may arise from population differences, as the effect of this polymorphism may also depend on the subject's metabolic and inflammatory state. The present results also suggest that there are sex-specific differences in the studied associations. The true role of the studied genotype in the pathogenesis of atherosclerosis needs to be elaborated further.

The chromosome 9p21.3 locus has been shown to have a robust association with CHD in a wide range of populations (Burton et al. 2007, Helgadottir et al. 2007, McPherson et al. 2007, Samani et al. 2007, Broadbent et al. 2008, Schunkert et al. 2008). Interestingly, no such association with early atherosclerosis was found in the present study. The reason for this might be that the effect of the locus may be related to advanced stages of the atherosclerosis process and not so much to its initiation. This finding emphasizes the fact that atherosclerosis and its complications are results of a long process. Risk factors may thus be associated with different parts of the process.

In the future, cardiovascular research needs to concentrate on more personalized approaches. Risk factors that need to be taken into account may depend considerably on sex, age, ethnicity and other factors. Even some of the traditional risk factors may have to be re-evaluated in specific populations.

7 SUMMARY AND CONCLUSIONS

Obesity is becoming a major cardiovascular risk factor. It is related to insulin resistance, metabolic syndrome and type 2 diabetes. The independent associations of metabolic syndrome and different stages of glucose intolerance with early atherosclerosis were studied in the population of the Health 2000 Survey. Possible sex-related differences in these relationships were one of the main areas of interest. Atherosclerosis also has a strong genetic basis that has not been thoroughly established. In the present thesis, two contemporary genetic variants were studied in detail. These particular genetic variants were identified utilizing different genetic approaches. The relationship between the coronary heart disease-associated locus on chromosome 9p21.3 (rs1333049) and early atherosclerosis was investigated among the populations of the Health 2000 Survey and the Cardiovascular Risk in Young Finns Study, because this polymorphism has emerged as having the strongest association with coronary heart disease in genome-wide association studies. The associations between the IL6–174 G>C genotype, early atherosclerosis and cardiovascular risk factors were evaluated in the Health 2000 Survey population, because this polymorphism has been strongly associated with cardiovascular risk factors and coronary heart disease in candidate gene studies. Moreover, IL-6 is closely connected to the inflammatory cascade, as are MetS and glucose intolerance, which were also included in the main focus of this thesis. The principal conclusions of the present study are as follows:

I There is a trend of increasing carotid atherosclerosis according to the worsening of glucose tolerance in both sexes. This trend is already seen in the non-diabetic stages of glucose intolerance. Impaired glucose tolerance generally associates with a worse profile in early carotid atherosclerosis when compared to impaired fasting glucose. A deterioration in the overall CVD risk factor profile in subjects with glucose intolerance plays an important part in mediating the observed associations. These results also suggest that women with glucose intolerance may have a greater risk for early atherosclerotic changes than men.

II Metabolic syndrome is, in both sexes, associated with early atherosclerosis independently of the risk factors that are not included in its definition among

middle-aged and elderly Finns. This association is modified by sex and may be more pronounced in women than in men. Metabolic syndrome is associated with increased carotid artery intima-media thickness especially in women with low cardiovascular risk as defined by traditional risk factors.

III No association between the chromosome 9p21.3 locus (rs1333049) and early carotid atherosclerosis was found in either young Finnish adults or middle-aged and elderly Finns. These results suggest that this locus is related to the risk of coronary heart disease through mechanisms that are not reflected by changes in arterial intima-media thickness. Similarly, the findings propose that this genetic locus does not enhance the risk of coronary heart disease, primarily causing endothelial dysfunction at a young age.

IV There were significant associations between the IL6–174 G>C genotype and the levels of total cholesterol, LDL-C, fasting plasma glucose and BMI among middle-aged and elderly men. This polymorphism also appears to be associated with systolic blood pressure in men. The results were, however, contradictory for the different risk factors in regard to which allele had a favourable association. No such associations were observed in women. Possible sex-specific differences in the associations of the IL6–174 G>C genotype and cardiovascular risk factors may partly explain the heterogeneity in the previous results regarding the effect of this genotype on atherosclerosis. The impact may also depend on factors such as body fat mass, and the metabolic as well as inflammatory state.

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Metabolic syndrome and arterial stiffness: The Health 2000 Survey

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Abstract

Metabolic syndrome and its components have been associated with arterial stiffness and cardiovascular disease. The objective of this study was to examine the independent influences of metabolic syndrome, its components, and other cardiovascular risk factors on arterial stiffness as well as to compare 2 definitions for metabolic syndrome (National Cholesterol Education Program [NCEP] and International Diabetes Federation [IDF]) in their ability to identify subjects with arterial stiffness. The study population consisted of 401 Finnish men and women aged 45 years and older who participated in a substudy of the Finnish population–based Health 2000 Survey. Pulse wave velocity (PWV) measured by whole-body impedance cardiography was used as a marker of elevated arterial stiffness. In multivariate models, systolic blood pressure, age, waist circumference, and fasting blood glucose ($P \leq .001$ for all) were independent determinants for PWV. In the models including metabolic syndrome instead of its components, the NCEP and IDF definitions were similarly associated with PWV ($P \leq .01$ for both), the other independent determinants being age, sex ($P < .001$ for both) and plasma C-reactive protein concentration ($P = .016$ and $P = .005$ in models containing the NCEP and IDF definitions, respectively). Systolic blood pressure, age, waist circumference, and fasting blood glucose level were independently associated with increased arterial stiffness. Metabolic syndrome determined increased arterial stiffness independently of other known cardiovascular risk factors. The NCEP and IDF definitions did not differ in their ability to identify subjects with increased arterial stiffness.

1. Introduction

Metabolic syndrome is a cluster of cardiovascular risk factors such as central obesity, hypertension, dyslipidemias, and glucose intolerance. It has been shown to be a predictor of type 2 diabetes mellitus [1], coronary heart disease [2], and mortality [3-5]. Metabolic syndrome has various definitions. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III proposed their widely used clinical definition for metabolic syndrome in 2001 [6]. Recently, the International Diabetes Federation (IDF) also published a worldwide definition of metabolic syndrome [7]. These definitions have 2 basic differences. First, the IDF definition has a significantly lower cutoff point for waist circumference than the NCEP definition. Second, the IDF definition makes the presence of increased waist circumference mandatory for diagnosis, whereas the NCEP definition considers waist circumference as important as the other components.

Arterial stiffness has been a strong independent predictor of coronary events and cardiovascular mortality in several

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patient groups [8-11]. It can be evaluated by measuring pulse wave velocity (PWV) along the arterial tree. The individual components of metabolic syndrome have been previously associated with increased arterial stiffness and higher PWV values [12,13]. Hypertension in particular has been linked to increased arterial stiffness [14]. Metabolic syndrome as a whole, mostly according to the NCEP criteria, has also been associated with arterial stiffness [13,15-18]. The NCEP and the IDF criteria have been compared in few studies so far. Both definitions have been similarly associated with coronary heart disease [19] and mortality [20]. To our knowledge, these criteria have not been compared regarding their ability to identify subjects with increased arterial stiffness.

The aim of this study was to evaluate the relationships among metabolic syndrome, its individual components, and arterial stiffness. Furthermore, we set out to discover whether the NCEP and the IDF definition can identify subjects with increased arterial stiffness similarly. We also intended to test the applicability of the whole-body impedance cardiography (ICG_{WB}) method for measuring PWV in a large epidemiological study.

2. Methods

2.1. Study population

We studied a subpopulation of a large Finnish cross-sectional health examination survey (the Health 2000 Survey) carried out in 2000-2001 [21]. The overall study cohort was a two-stage stratified cluster sample (8028 persons) representing the entire Finnish population aged 30 years and older. To study cardiovascular disease (CVD) and diabetes more thoroughly, a supplemental study was carried out (sample size, 1867; participation rate, 82%). The subjects, a subpopulation of the Health 2000 Survey, in the supplemental study were 45 years and older, and the study was executed in the catchment areas of the 5 Finnish university hospitals because specialized equipment was required. In the catchment areas of Tampere and Turku university hospitals, 401 individuals (176 men and 225 women; mean age, 58 years; range, 46-76 years) participated in the supplemental study and underwent the ICG_{WB} measurements. These individuals were selected to be our study group.

Waist circumference was not measured in the supplemental study; therefore, the Health 2000 Survey parameters were used to define the presence of metabolic syndrome. All the other measurements and laboratory tests used in the present study were collected from the data of the supplemental study. The mean time interval between the Health 2000 Survey and the supplemental study was 1 year and 4 months (range, 10-23 months). The mean ($\pm$SD) change in body weight during this time was 0.52 ($\pm$3.43) kg. Because this change was not clinically significant, it is likely that change in waist circumference was not clinically significant either. Because most of the women in our study population had reached menopause, we did not analyze premenopausal and postmenopausal women separately. To evaluate a possible conflicting influence of concomitant diseases on the associations between the risk factors and PWV, we also created a smaller sample excluding subjects with CVD and diabetes. Subjects with previous myocardial infarction or stroke, or with diagnosed diabetes, coronary heart disease, cardiac insufficiency, cardiac arrhythmia, hypertension, arterial stenosis, or thrombosis in a lower limb or other CVD were excluded. The subjects who had fasting plasma glucose concentration of 7 mmol/L or higher or who had 2-hour glucose value of 11.1 mmol/L or higher in the oral glucose tolerance test were excluded. From this smaller sample, we also excluded subjects who were on antihypertensive medication or statins. After these additional exclusions, 200 individuals free of CVD and diabetes remained with available PWV data.

2.2. Metabolic syndrome

We used 2 different criteria to define metabolic syndrome. According to the NCEP definition [6], a person has metabolic syndrome if at least 3 of the following criteria are met: waist circumference greater than 102 cm for men and greater than 88 cm for women; triglycerides, 1.7 mmol/L or greater; high-density lipoprotein (HDL) cholesterol, less than 1.03 mmol/L for men and less than 1.29 mmol/L for women; systolic blood pressure, 130 mm Hg or higher, and diastolic blood pressure, 85 mm Hg or higher; and fasting glucose, 5.6 mmol/L or higher. The fasting glucose threshold of the NCEP criterion was modified in 2004 [22].

According to the IDF definition [7], a person has metabolic syndrome if waist circumference is increased ($\geq$94 cm for men and $\geq$80 cm for women) and at least 2 of the following factors are present: triglycerides, 1.7 mmol/L or greater, or specific treatment of this lipid abnormality; HDL cholesterol, less than 1.03 mmol/L in men and less than 1.29 mmol/L in women, or specific treatment; systolic blood pressure 130 mm Hg or higher or diastolic blood pressure 85 mm Hg or higher, or treatment of previously diagnosed hypertension; fasting plasma glucose, 5.6 mmol/L or higher, or previously diagnosed type 2 diabetes mellitus. The IDF definition has ethnicity-specific cutoff points for waist circumference.

2.3. Pulse wave velocity

Pulse wave velocity was measured by ICG_{WB} using a commercially available circulation monitor device (CircMon B202, JR Medical, Tallinn, Estonia). Subjects were first interviewed and then electrodes (Blue Sensor type R-00-S; Medicotest, Ölstykke, Denmark) were applied while subjects were in the supine position for at least 15 minutes before the 10-minute PWV measurements. A pair of electrically connected current electrodes was placed on the distal part of the extremities just proximal to the wrists and ankles. Voltage-sensing electrodes were placed proximally to the current electrodes, with a distance of 5 cm

Table 1
Clinical and laboratory parameters of the study cohort (n = 400) with and without metabolic syndrome (MetS) according to 2 definitions (NCEP, IDF)

	MetS by NCEP definition			MetS by IDF definition		
	Yes (n = 156-162)[a]	No (n = 227-238)[a]	*P*	Yes (n = 175-182)[a]	No (n = 208-218)[a]	*P*
Age (y)	59.5 ± 7.7	57.6 ± 7.9	.017	59.5 ± 7.6	57.4 ± 8.0	.009
Sex (men, %)	44	44	.883	46	43	.555
Current smoking (%)	24	23	.748	24	23	.689
BMI (kg/m^2)	29.3 ± 3.9	25.6 ± 3.7	<.001	29.0 ± 3.8	25.5 ± 3.9	<.001
Waist circumference (cm)	101.5 ± 11.2	89.2 ± 10.8	<.001	100.9 ± 10.8	88.5 ± 11.0	<.001
Heart rate (beats/min)	64.9 ± 10.1	63.3 ± 10.7	.133	64.6 ± 9.7	63.4 ± 11.1	.260
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.7 ± 0.5	<.001	1.4 ± 0.4	1.7 ± 0.5	<.001
LDL cholesterol (mmol/L)	3.6 ± 0.9	3.3 ± 0.9	.008	3.5 ± 0.9	3.3 ± 0.9	.028
Total cholesterol (mmol/L)	5.7 ± 1.0	5.5 ± 0.9	.213	5.6 ± 1.0	5.6 ± 0.9	.429
Triglycerides (mmol/L)	1.7 ± 0.8	1.2 ± 0.5	<.001	1.7 ± 0.8	1.1 ± 0.5	<.001
Fasting glucose (mmol/L)	6.3 ± 1.7	5.5 ± 0.7	<.001	6.2 ± 1.6	5.5 ± 0.7	<.001
CRP (mg/L)	4.1 ± 5.6	2.2 ± 3.0	<.001	4.0 ± 5.4	2.1 ± 2.8	<.001
SBP (mm Hg)	142.3 ± 19.5	129.1 ± 18.4	<.001	140.2 ± 19.3	129.6 ± 19.1	<.001
DBP (mm Hg)	85.1 ± 9.2	81.0 ± 9.7	<.001	85.0 ± 8.7	80.1 ± 10.0	<.001
PP (mm Hg)	57.2 ± 15.4	48.2 ± 11.8	<.001	55.3 ± 15.4	48.9 ± 12.1	<.001

Values are means ± SD except values for sex and smoking, which are percentages. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.
[a] Variation of n is caused by the fact that some values are missing for some subjects.

between the centers of the electrodes. With this electrode configuration, the recorded heart-synchronous changes in impedance reflect the weighted sum of the pulsatile plethysmograms of the vessels between the electrodes, ie, almost the whole vascular system. The foot of the whole-body impedance cardiogram coincides with pulse transmission in the aortic arch, making it possible to estimate the beginning of pulse wave transmission in the arterial system. Similarly, with voltage sensing electrodes applied to any distal region between the current electrodes, pulse-related impedance changes can be recorded. In this study, the distal impedance plethysmogram was recorded from a popliteal artery at knee joint level. The active electrode was placed on the lateral side of the knee joint and the reference electrode on the calf, the distance between the electrodes being about 20 cm. The time difference between the feet of these impedance plethysmograms, recorded from the aortic arch and popliteal artery, was measured. The time resolution of the CircMon recordings was 5 milliseconds. The evaluation of the ICG_{WB} method and PWV measurement using ICG_{WB} has been described in detail previously [23,24]. Reproducibility values of the PWV measurements by ICG_{WB} (2.42 m/s) and Doppler ultrasound (2.17 m/s) are similar [24].

2.4. Waist circumference, body mass index, blood pressure, and smoking

Waist circumference was measured with subjects in the standing position by using the standards created for population health studies [25]. Height and weight were measured and body mass index (BMI) was calculated. In the Health 2000 Survey, blood pressure was measured from the right arm with a mercury sphygmomanometer (Mercuro 300, Speidel & Keller, Juningen, Germany). The first measurement was taken after subjects had rested at least 5 minutes in the sitting position. Korotkoff's first phase was used as the sign of systolic blood pressure and the fifth phase as the sign of diastolic pressure. The measurement was repeated 2 minutes after the first measurement. The average of the 2 measurements was used in the analysis. In the supplemental study, blood pressure was measured from the right arm after at least 10 minutes' rest. The measurement was taken 3 times with 1- to 2-minute intervals. The automatic Omron M4 manometer (Omron Matsusaka, Japan, and Omron Healthcare Europe, Hoofddorp, the Netherlands) was used in these measurements. The average of the 3 measurements was used in the analysis. Pulse pressure was calculated as the difference between the average systolic and the average diastolic blood pressure. Current smoking was evaluated with a questionnaire. Those who were currently smoking were defined as smokers and the rest of the subjects as nonsmokers. The smoking data used in the present study were collected from the Health 2000 Survey data.

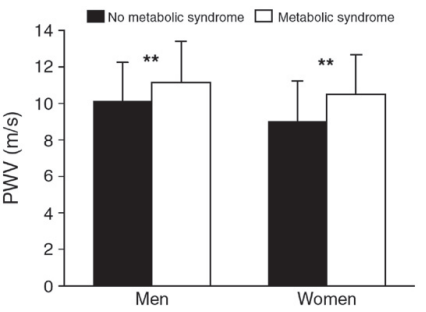

Fig. 1. PWV (mean and SD) in men and women with or without metabolic syndrome, according to the NCEP definition. $^{**}P < .01$. The pattern is essentially the same using the IDF definition.

Table 2
Univariate correlations between cardiovascular risk factors and PWV in the whole cohort (n = 393-401) and in the healthy subsample (n = 197-200)

	Whole cohort		Subsample	
	r	*P*	*r*	*P*
Age (y)	0.510	<.001	0.518	<.001
BMI (kg/m^2)	0.238	<.001	0.274	<.001
Waist circumference (cm)	0.343	<.001	0.353	<.001
HDL cholesterol (mmol/L)	−0.161	.001	−0.255	<.001
LDL cholesterol (mmol/L)	0.062	.217	0.208	.003
Total cholesterol (mmol/L)	0.028	.577	0.120	.090
Triglycerides (mmol/L)	0.199	<.001	0.174	.014
Fasting glucose (mmol/L)	0.341	<.001	0.252	<.001
CRP (mg/L)	0.226	<.001	0.214	.003
SBP (mm Hg)	0.627	<.001	0.694	<.001
DBP (mm Hg)	0.392	<.001	0.496	<.001
PP (mm Hg)	0.619	<.001	0.642	<.001
HR × PP	0.627	<.001	0.664	<.001

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; PP, pulse pressure.

2.5. Laboratory tests

Venous blood samples were drawn from the antecubital vein after an overnight fast. HDL cholesterol, total cholesterol, triglyceride, and glucose concentrations were determined enzymatically (Roche Diagnostics, Mannheim, Germany, for HDL; Olympus System Reagent, Hamburg, Germany, for total cholesterol, triglyceride, and glucose) with a clinical chemistry analyzer (Olympus, AU400, Hamburg, Germany). C-reactive protein (CRP) concentrations were determined by a chemiluminescent immunometric assay (Immulite, Diagnostic Products, Los Angeles, CA). Low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald formula.

2.6. Statistical analyses

Statistical analyses were performed using SPSS for Windows (version 13.0; SPSS, Chicago, IL). The skewed distributions of triglycerides and CRP were corrected logarithmically before statistical analyses. Chi-square and *t*-test analyses were calculated to compare categorical and continuous variables between the metabolic syndrome groups, respectively. Pearson correlation coefficients were used to examine the association between cardiovascular risk factors and PWV. Stepwise linear regression analysis was performed for continuous and dichotomous variables to examine independent relationships among metabolic syndrome, its components, other cardiovascular risk factors, and PWV.

3. Results

The prevalence of metabolic syndrome obtained by using the NCEP and IDF definitions was, respectively, 41% and 47% in men and 40% and 44% in women. For one participant, the waist circumference measurement was missing and the presence of metabolic syndrome could not be assessed. By both definitions, the subjects with metabolic syndrome were older, had higher BMI, waist circumference, LDL cholesterol, triglycerides, fasting plasma glucose, CRP and blood pressure and lower HDL cholesterol ($P < .05$ for all) than the subjects who did not have the syndrome. Subjects with and without metabolic syndrome did not differ in sex, resting heart rate, total cholesterol levels, or smoking habits ($P > .1$ for all). Twenty-eight percent of the study population was on antihypertensive medication, and 11% used statins. Selected clinical and demographic values of the study population are given in Table 1.

Men had significantly higher mean PWV than women ($P < .001$). For both sexes, the mean PWV was significantly higher in subjects with metabolic syndrome (using both definitions; $P < .01$) than those without the syndrome (Fig. 1 illustrates the data obtained with the NCEP definition). There was no statistically significant difference in the mean PWV ($P > .1$) of current smokers and nonsmokers. Age, systolic blood pressure, diastolic blood pressure, pulse pressure, product of heart rate and pulse pressure, waist circumference, BMI, and levels of HDL cholesterol, triglycerides, CRP, and fasting plasma glucose correlated statistically significantly with PWV (Table 2). Correlations between risk factors and PWV remained essentially similar in the smaller group excluding subjects with CVD and diabetes with the exception that the plasma LDL cholesterol level correlated statistically significantly with PWV (Table 2).

A stepwise linear regression model was performed to examine the relationships between cardiovascular risk

Table 3
A linear regression model for the relationships between cardiovascular risk factors and PWV in the whole cohort (n = 390) and in the healthy subsample (n = 196)

Risk variable	Whole cohort			Risk variable	Subsample		
	$\beta \pm$ SE	*P*	R^2 change (%)		$\beta \pm$ SE	*P*	R^2 change (%)
SBP (mm Hg)	.05 ± .00	<.001	40	SBP (mm Hg)	.06 ± .01	<.001	48
Age (y)	.10 ± .01	<.001	11	Age (y)	.10 ± .01	<.001	12
Fasting glucose (mmol/L)	.27 ± .07	<.001	3	Waist circumference (cm)	.02 ± .01	.042	1
Waist circumference (cm)	.03 ± .01	<.001	1				
R^2 (%)	55%				61		

Initial stepwise regression models included age, sex, waist circumference, fasting plasma glucose, HDL cholesterol, systolic blood pressure, CRP, triglycerides, smoking and LDL cholesterol (only in the sub sample) as independent variables. β indicates regression coefficient; R^2 change, change in adjusted R^2 value after addition of the respective variable in to the model; R^2, adjusted R^2 value of the whole model.

Table 4
Linear regression model for the relationships between metabolic syndrome (MetS), using 2 different definitions, other cardiovascular risk factors and PWV in the whole cohort (n = 392) and in the healthy sub sample (n = 197)

Risk variable	Whole cohort			Risk variable	Subsample		
	$\beta \pm$ SE	P	R^2 change (%)		$\beta \pm$ SE	P	R^2 change (%)
Age (y)	.14 ± .01	<.001	26	Age (y)	.13 ± .02	<.001	26
MetS using the NCEP definition	.92 ± .20	<.001	4	MetS using the NCEP definition	.84 ± .25	<.001	5
Sex	−.82 ± .19	<.001	3	Sex	−.72 ± .23	.002	2
CRP (mg/L)	.52 ± .21	.016	0.8				
R^2 (%)	34				33		
Age (y)	.14 ± .01	<.001	26	Age (y)	.13 ± .02	<.001	26
Sex	−.80 ± .19	<.001	3	MetS using the IDF definition	.78 ± .24	.001	4
MetS using the IDF definition	.67 ± .20	.001	3	Sex	−.69 ± .24	.004	3
CRP (mg/L)	.60 ± .21	.005	1				
R^2 (%)	33				33		

Initial stepwise regression models included age, sex, CRP, smoking, metabolic syndrome, according to the NCEP or the IDF definition and LDL cholesterol (only in the subsample) as independent variables. β indicates regression coefficient; R^2 change, change in adjusted R^2 value after addition of the respective variable in to the model; R^2, adjusted R^2 value of the whole model.

factors as independent variables and PWV (Table 3). The initial stepwise regression model included age, sex, waist circumference, fasting plasma glucose, HDL cholesterol, triglycerides, systolic blood pressure, CRP, and smoking as independent variables. In that model, systolic blood pressure, age, fasting blood glucose, and waist circumference explained 55% (adjusted R^2, 55%) of the variation in PWV. When the same model was adjusted by replacing systolic blood pressure with pulse pressure or with the product of heart rate and pulse pressure (data not shown), the results remained essentially the same (adjusted R^2, 53% and 55%, respectively) with the exception that sex was also an independent determinant of PWV. We used the same linear regression model (LDL cholesterol included) for the smaller sample excluding subjects with CVD and diabetes (Table 3). Systolic blood pressure, age, and waist circumference explained 61% of the variation in PWV. Therefore, in this smaller healthier population, fasting plasma glucose was not an independent factor determining PWV.

Another stepwise linear regression model was performed by using metabolic syndrome (both definitions separately) and other known cardiovascular risk factors (age, sex, CRP, and smoking) as independent variables (Table 4). Age, metabolic syndrome (using the NCEP or the IDF definition), sex, and CRP were independent determinants for PWV (adjusted R^2, 34% and 33% in the models containing the NCEP and the IDF definition, respectively). When CRP was excluded from the models, the results remained essentially the same. The same linear regression model (including LDL as independent variable) was used for the smaller sample that excluded subjects with CVD and diabetes (Table 4). Age, metabolic syndrome, and sex explained 33% (using the NCEP or the IDF definition) of the variation in PWV.

4. Discussion

Metabolic syndrome and its individual components are risk factors for atherosclerosis and CVD [1-5]. Arterial stiffness is also related to CVD and atherosclerosis [26] and has been a strong independent predictor of coronary events and cardiovascular mortality in several patient groups [8-11]. In this study, we examined the relationships between arterial stiffness measured by PWV and single cardiovascular risk factors as well as metabolic syndrome as a whole. Our aim was also to compare 2 different definitions for metabolic syndrome (NCEP and IDF) in their relations with arterial stiffness.

Age, systolic blood pressure, diastolic blood pressure, pulse pressure, product of heart rate and pulse pressure, BMI, waist circumference, and triglyceride, HDL cholesterol, fasting plasma glucose, and CRP levels correlated statistically significantly with PWV. The univariate associations were thus significant between PWV and all the components of metabolic syndrome. The strongest correlation was observed between systolic blood pressure and PWV, which is not surprising considering that both are connected to arterial stiffness. As expected, age was strongly correlated with PWV. In all, these findings are well in line with previously published data [14,27]. LDL cholesterol has also been linked with arterial stiffness [28,29]. In a smaller sample excluding subjects with CVD and diabetes, we also found a significant correlation between LDL cholesterol and PWV.

The mean PWV was significantly higher in the subjects with metabolic syndrome than in those without the syndrome, regardless of the definition used. This is in line with previous studies using the NCEP definition [13,14,16-18]. To our knowledge, this is the first study to examine the relationships between metabolic syndrome and arterial stiffness by using the IDF definition. In our study, men had significantly higher mean PWV than women. The results of the Framingham heart study were in agreement with the present study, the difference in PWV being small but statistically significant [30]. On the other hand, several other studies have not reported significant differences between the sexes [31,32].

Although we did find that many individual risk factors were associated with arterial stiffness, only some of them

appeared in the final regression models as independent determinants of PWV. As expected, systolic blood pressure and age were the strongest factors determining arterial stiffness—a finding that is in agreement with previous data [33]. In our study population, waist circumference and fasting plasma glucose were also independent determinants of arterial stiffness, results consistent with those of previous studies [13-15,34]. When metabolic syndrome was included in the regression model instead of its components, it was found to be an independent determinant of arterial stiffness (using both definitions) together with age, sex, and CRP concentration. In the smaller sample that excluded CVD and diabetes, fasting plasma glucose was not an independent factor determining PWV. This can be partly explained by the smaller sample size. On the other hand, metabolic syndrome remained as an independent factor even in this population free of CVD and diabetes. Similar results have been reported previously [18].

We did not find a significant difference between the NCEP and the IDF definitions in their ability to determine arterial stiffness. To our knowledge, this has not been studied previously. The main difference between these 2 definitions is that the latter makes central obesity mandatory for diagnosis. The IDF definition also has a lower waist circumference threshold. Because of this, the IDF definition produces a higher prevalence for metabolic syndrome than the NCEP definition. In our study population, the prevalence figures were 45.4% and 40.4%, respectively, which are relatively high when compared with most of the earlier studies. This is probably related to the age structure of our study population (older than 45 years). Nevertheless, this increases our understanding of the magnitude of metabolic syndrome as an important CVD risk factor.

C-reactive protein correlated significantly with PWV in the present study. The association of CRP and arterial stiffness has been reported previously [35-37]. Some [36,37] of the previous studies have suggested that CRP is associated with arterial stiffness independently of other CVD risk factors. In our population, however, CRP was not independently associated with arterial stiffness when all the other risk factors were taken into account. In the regression models with metabolic syndrome included instead of its components, CRP was an independent determinant. This is probably due to significant associations between CRP and all the components of metabolic syndrome.

Previously, PWV measurements have been done mostly by methods using Doppler ultrasound or mechanoelectrical pulse transducers [38,39]. PWV can also be measured by ICG_{WB}, which provides a handy and reliable tool for evaluating arterial stiffness on the basis of PWV simultaneously with cardiac output and related hemodynamic parameters. The ICG_{WB} method turned out to be applicable especially for large epidemiologic studies because it is not user dependent and does not require large personnel resources. The method is highly repeatable and reproducible [24].

In conclusion, our findings indicate that blood pressure, age, waist circumference, and fasting plasma glucose concentration are important independent factors for determining arterial stiffness in a middle-aged and elderly population. The NCEP and IDF definitions were both similarly associated with PWV, independently of other known cardiovascular risk factors. This suggests that both the NCEP and IDF definitions are able to identify subjects with increased arterial stiffness in a Finnish population. However, although metabolic syndrome is an important factor affecting cardiovascular health and its prevalence remarkably high in developed countries, its components have to be carefully evaluated as independent risk factors.

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II

Coronary Artery Disease Associated Locus on Chromosome 9p21 and Early Markers of Atherosclerosis

Nilesh J. Samani, Olli T. Raitakari, Kalle Sipilä, Martin D. Tobin, Heribert Schunkert, Markus Juonala, Peter S. Braund, Jeanette Erdmann, Jorma Viikari, Leena Moilanen, Leena Taittonen, Antti Jula, Eero Jokinen, Tomi Laitinen, Nina Hutri-Kähönen, Markku S. Nieminen, Y. Antero Kesäniemi, Alistair S. Hall, Janne Hulkkonen, Mika Kähönen and Terho Lehtimäki

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Coronary Artery Disease–Associated Locus on Chromosome 9p21 and Early Markers of Atherosclerosis

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Background—Genome-wide association studies have recently identified a locus on chromosome 9p21 that influences risk of coronary artery disease (CAD). The effect of the locus on early markers of atherosclerosis is unknown. We examined its association with carotid intima-media thickness (CIMT) and brachial flow-mediated dilatation (FMD).

Methods and Results—We genotyped 2277 individuals, age 24 to 39 years, from the Cardiovascular Risk in Young Finns Study with CIMT and FMD measurements and 1295 individuals, age 46 to 76 years, from the Health 2000 Survey with CIMT for rs1333049, the chromosome 9p21 variant showing the strongest association with CAD. Both mean and maximum CIMT were significantly higher ($P<0.001$) in the older subjects of the Health 2000 Survey compared with the Young Finns Study. However, there was no association of the rs1333049 genotype with either mean or maximum CIMT at either age ($P=0.959$ and 0.977 for the 2 phenotypes in the Young Finns Study and $P=0.714$ and 0.725 in the Health 2000 Survey). Similarly, there was no association of the locus with variation in FMD in the Young Finns cohort ($P=0.521$).

Conclusions—The chromosome 9p21 locus does not influence CAD risk through a mechanism that also affects CIMT or induces early changes in FMD. (***Arterioscler Thromb Vasc Biol*. 2008;28:1679-1683**)

Key Words: genetics ■ coronary artery diseases ■ atherosclerosis ■ carotid-intima media thickness ■ endothelial dysfunction

Coronary artery disease (CAD) has a significant genetic determination that has hitherto been poorly characterized. However, recent genome-wide association studies have identified several novel loci that are strongly associated with CAD. Specifically, a common variant located in a region adjacent to the cyclin dependent kinase inhibitors, *CDKN2A* (encoding p16INK4a) and *CDKN2B* (p15INK4b) on chromosome 9p21.3 has been associated with increased risk in 4 separate genome-wide association and follow-up studies.[1–5]

Clinical manifestations of CAD represent the end stage of a chronic process. As genetic variants are present from birth, their effects on markers of atherosclerosis may be discernible at an earlier stage. Carotid intima-media thickness (CIMT) is an accurately quantifiable and reproducible marker of atherosclerotic risk and predicts future cardiovascular events.[6,7] Similarly, impaired brachial artery flow-mediated dilatation (FMD) is another marker of atherosclerotic risk and predicts cardiovascular events.[8] In this study, we investigated the association of the CAD associated variant on chromosome 9p21.3 on CIMT in 2 population based cohorts of different ages, in combination spanning the age range from 24 to 76 years. We also examined its association with variation in FMD in young healthy subjects.

Materials and Methods

Subjects

We studied subjects from 2 population based cohorts—the Cardiovascular Risk in Young Finns Study and the Health 2000 Survey.[9,10]

The Cardiovascular Risk in Young Finns Study is a multi-center study of atherosclerotic risk factors of children and young adults (http://vanha.med.utu.fi/cardio/youngfinnsstudy/). The first cross-

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sectional study was conducted in 1980 and included 3596 healthy children and adolescents, age 3, 6, 9, 12, 15, and 18 years. Details of the study design have been presented elsewhere.[11] Thereafter, these subjects have been followed with periodic examinations. In 2001, 2620 individuals, who had then reached the age of between 24 to 39 years, were studied.[9] In addition to detailed risk factor assessments, ultrasound examination of CIMT and brachial endothelial function were carried out.[12,13]

The Health 2000 Survey was a large Finnish cross-sectional health examination survey carried out in 2000 to 2001. The overall study cohort was a 2-stage stratified cluster sample (8028 persons) representing the entire Finnish population age 30 years and above.[10] To study cardiovascular disease risk factors and diabetes more thoroughly, a supplemental study was carried out (sample size 1867 and participation rate 82%). The subjects in the supplemental study were 45 years and older, and the study was executed in the catchments areas of the 5 Finnish University Hospitals because specialized equipment was required. Carotid ultrasound examination was part of this supplemental study.[14] There were 1295 subjects (595 men and 700 women; mean age, 58 years; range, 46 to 76 years) with available carotid ultrasound data. These individuals were selected to be our study group for the present analysis.

Clinical Characteristics

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Blood pressure (BP) was measured using a random zero sphygmomanometer in the Young Finns Study and the automatic Omron M4 sphygmomanometer (Omron Healthcare Europe B.V.) in the supplemental Health 2000 Study. Values for systolic and diastolic blood pressure were defined by Korotkoff phases I and V, respectively. The averages of 3 measurements obtained after 5 minutes of sitting with 1 to 2 minutes between readings were used in analyses. Smoking habits were inquired with a questionnaire.

Laboratory Tests

In both studies, venous blood samples were taken after an overnight fast. Total cholesterol, HDL cholesterol, triglyceride, and glucose concentrations were determined enzymatically (Roche Diagnostics, GmbH for HDL; Olympus System Reagent for total cholesterol, triglyceride, and glucose) with a clinical chemistry analyser (Olympus, AU400). LDL cholesterol was calculated with the Friedewald formula.

Ultrasound Imaging

In the Young Finns Study, carotid ultrasound studies were performed using a high-resolution ultrasound system (Sequoia 512, Acuson) with 13.0 MHz linear array transducer. CIMT was measured about 10 mm from 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.[12] At least 4 measurements were taken at each scan of the common carotid artery incident with the R-wave of the continuously monitored ECG to derive mean and maximum CIMT. The scans were analyzed by 1 reader blinded to subjects' details. The between visit (2 visits 3 months apart) coefficient of variation of mean CIMT measurements for a subset of the subjects was 6.4%.[12]

To assess brachial artery FMD, the left brachial artery diameter was measured both at rest and during the reactive hyperemia.[13] Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 4.5 minutes, followed by release. Three measurements of arterial diameter were performed at end-diastole at a fixed distance from an anatomic marker at rest and at 40, 60, and 80 seconds after cuff release. The vessel diameter after reactive hyperemia was expressed as the percentage relative to the resting scan. The between-visit CV for brachial diameter was 3.2% and for FMD 26.0%.[13]

In the Health 2000 supplemental study carotid ultrasound examination of the right carotid artery was performed according to a standardized protocol using a 7.5 MHz linear array transducer. The examinations were performed by centrally trained and certified sonographers at 5 study locations around Finland.[14] CIMT measurements were performed off-line with the use of automated imaging processing software. One reader was responsible for reading all ultrasound images. Mean and maximum CIMT were again calculated. The intrareader reproducibility of the CIMT measurements was assessed by calculation of the CIMT twice from 571 randomly selected images of 108 study subjects several weeks apart. The mean difference of the 2 measurements was 0.001 mm (SD 0.123), and the intraclass correlation was 0.934 ($P<0.001$).[14]

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit (Qiagen Inc). rs1333049 was genotyped by allelic discrimination using a standard TaqMan assay (further details available on request). Fluorescence was detected post polymerase chain reaction (PCR) using the ABI Prism 7900HT Sequence Detector System and genotypes called using ABI Prism SDS software version 2.1 (ABI). For reference, in the genome-wide association studies,[1,2] the CAD associated (risk) allele for rs133309 was C.

Statistical Analysis

Univariate data comparisons between genotype groups (and between subjects in the 2 studies) were based on analysis of variance for continuous variables and Chi-square test for categorical variables. Because of skewed distributions, the values for triglycerides were log transformed. Multiple logistic regression analysis was used to identify clinical and laboratory variables that were independently associated with mean and maximum CIMT and to assess the effect of genotype on CIMT taking these variables into account. The statistical tests were performed with SPSS (version 14.00) for the Young Finns Study and SAS (version 8.1) for the Health 2000 cohort. 95% CI for allele frequencies were calculated using confidence interval calculation program, CIA (version 2.1.2). Power calculations were undertaken using the P/S (Power and Sample size calculator) program.

Results

The age range of the subjects was 24 to 39 years (55% female) in the Young Finns Study and 46 to 76 years (55% female) in the Health 2000 cohort. The frequency of the C allele for rs1333049 was 0.41 (95% CI: 0.40 to 0.43) in the Young Finns Study and 0.42 (95% CI: 0.40 to 0.44) in the Health 2000 subjects and the genotypes were in Hardy Weinberg equilibrium in both cohorts. The characteristics of the subjects in each study partitioned by genotype for rs1333049 are shown in Table 1. Overall, the older subjects from the Health 2000 survey had higher BMI, LDL, and HDL cholesterol levels and higher average systolic and diastolic blood pressures compared with the subjects in the Young Finns Study ($P<0.001$). However, there was no significant difference in any of these traits according to rs1333049 genotype in either age group (Table 1).

Both mean and maximum CIMT were higher in the Health 2000 cohort compared with the Young Finns subjects (Table 1). However, there was no effect of the rs1333049 genotype on either phenotype in either the Young Finns study ($P=0.959$ and $P=0.977$, respectively) or in the Health 2000 cohort ($P=0.714$ and $P=0.729$). Specifically we did not see a higher CIMT in subjects carrying the CAD-risk associated allele (C) in either cohort. Brachial FMD responses (available in Young Finns) were likewise similar across the genotypes (Table 1, $P=0.521$).

Table 1. Demographic and Phenotypic Characteristics of Subjects in the Cardiovascular Risk in Young Finns Study and in the Health 2000 Cohort Partitioned by Genotype for the Coronary Artery Disease Risk Variant on Chromosomes 9p21.3

	Cardiovascular Risk in Young Finns				Health 2000 Cohort			
Phenotype	GG	GC	CC	*P* Value	GG	GC	CC	*P* Value
Age, y	31.6 (5.0)	31.8 (5.0)	31.8 (5.0)	0.522	58.0 (8.1)	58.6 (8.2)	57.8 (7.5)	0.292
	790	1093	394		454	599	242	
Males, %	44	45	46	0.506	48	44	43	0.394
	790	1093	394		454	599	242	
BMI, kg/m^2	25.1 (4.3)	25.0 (4.4)	25.2 (4.5)	0.742	27.2 (4.6)	27.1 (4.3)	27.4 (4.5)	0.618
	784	1084	391		450	597	241	
LDL chol, mmol/l	3.27 (0.83)	3.30 (0.86)	3.24 (0.82)	0.470	3.39 (0.87)	3.40 (0.86)	3.43 (0.91)	0.839
	782	1074	389		450	586	237	
HDL chol, mmol/l	1.30 (0.32)	1.30 (0.32)	1.26 (0.30)	0.084	1.59 (0.43)	1.57 (0.42)	1.57 (0.45)	0.798
	790	1091	394		453	598	242	
Triglycerides, mmol/l	1.31 (0.73)	1.34 (0.92)	1.38 (0.89)	0.643	1.32 (0.66)	1.43 (1.14)	1.42 (0.82)	0.382
	790	1093	394		453	598	242	
Systolic BP, mm Hg	116.7 (12.8)	116.8 (13.4)	116.0 (12.7)	0.788	138.0 (22.2)	139.0 (21.3)	137.6 (21.3)	0.599
	785	1075	393		454	598	241	
Diastolic BP, mm Hg	70.7 (10.3)	70.8 (11.1)	70.9 (10.8)	0.980	84.5 (11.2)	84.3 (10.0)	84.3 (10.2)	0.932
	785	1075	393		454	598	241	
Smoking, %	25	23	22	0.506	24	22	22	0.485
	785	1093	394		454	599	242	
Mean CIMT, mm	0.58 (0.09)	0.58 (0.09)	0.58 (0.10)	0.959	0.93 (0.23)	0.93 (0.23)	0.94 (0.22)	0.714
	779	1082	390		454	599	242	
Max CIMT, mm	0.62 (0.10)	0.62 (0.10)	0.62 (0.10)	0.977	0.99 (0.21)	1.00 (0.22)	1.00 (0.21)	0.729
	779	1082	390		454	599	242	
FMD, %	7.84 (4.34)	8.04 (4.47)	8.12 (4.39)	0.521	NA	NA	NA	
	718	1015	362					

Values are mean (SD) or prevalence in %. The numbers below each value is the No. of subjects for which the data were available. CIMT indicates carotid intima media thickness; FMD, Brachial artery flow-mediated dilatation; NA, not available. *P* values are unadjusted values based on analysis of variance for continuous variables and Chi-square test for smoking and gender. The CAD-risk associated allele is the C allele.

The results of multivariate logistic regression analysis of mean CIMT in the 2 studies are shown in Table 2. In the Young Finns Study, there were highly significant independent effects of age, gender, BMI, and BP on CIMT and borderline significant effect of smoking. In the Health 2000 cohort, there were similarly significant independent effects of age, gender, and SBP on CIMT. HDL-cholesterol, and smoking but not BMI were also independently associated with CIMT in this cohort. Taking these factors into account there was no independent effect of the rs1333049 genotype on mean CIMT (Table 2). The results for maximum CIMT were similar (not shown).

Discussion

Within a short period of its identification, the chromosome 9p21 locus has been shown to have a robust association with CAD in a wide range of populations.[1–5,15] The risk-associated allele, defined by the C allele of rs1333049, or alleles of other SNPs in strong linkage disequilibrium with it examined in some studies, have consistently shown an increased risk of 25% to 40% per copy of allele. The region of association with CAD on chromosome 9p21 spans ≈50 to 60 kb[1–4] and is located adjacent to genes coding for the cyclin-dependent kinases p16/CDKN2A and p15/CDKN2B as well as p14/ARF. These genes play a central role in the regulation of the cell cycle and may be implicated in the pathogenesis of atherosclerosis through their role in transforming growth factor (TGF)-β–induced growth inhibition.[16,17] Interestingly, although the 9p21 locus itself does not contain a protein coding gene, recent studies have shown that it codes a large noncoding RNA, ANRIL, which is expressed in atherosclerotic tissue.[15,18] Furthermore, expression of ANRIL is coordinated with that of p14/ARF and possibly also p16/CDKN2A and p15/CDKN2B, in both physiological and pathological conditions,[18] suggesting that it may regulate the expression of these genes. Further studies are required, but this could provide a potential mechanism by which the locus affects CAD risk.

In this context, it is relevant to examine the association of the 9p21 locus with other forms of atherosclerotic and vascular disease as well as markers of atherosclerosis. Indeed, a recent study has also shown an association of the locus with abdominal aortic aneurysms as well as with intracranial aneurysms.[19] Among atherosclerosis-related phenotypes, CIMT has gained particular prominence, both because of the ease, accuracy, and reproducibility of its measurement

Table 2. Determinants of Mean CIMT in the Young Finns Study and the Health 2000 Cohort: Results From Multivariable Regression Analysis

	Young Finns Study		Health 2000 Cohort	
	Beta (error)	*P*	Beta (error)	*P*
Gender	0.0101 (0.0043)	0.017	0.0247 (0.0094)	0.009
Age	0.0050 (0.0004)	<0.001	0.0097 (0.0006)	<0.001
BMI	0.0024 (0.0005)	<0.001	0.0015 (0.0011)	0.190
SBP	0.0054 (0.0020)	0.010	0.0096 (0.0024)	<0.001
DBP	0.0060 (0.0025)	0.015	0.0036 (0.0050)	0.472
LDL cholesterol	0.0036 (0.0023)	0.121	0.0009 (0.0051)	0.863
HDL cholesterol	0.0004 (0.0066)	0.947	−0.0264 (0.0131)	0.045
Triglycerides	−0.0062 (0.0045)	0.161	0.0337 (0.0296)	0.255
Smoking	0.0086 (0.0048)	0.050	0.0242 (0.0107)	0.024
rs1333049 GG/GC vs CC	−0.0009 (0.0048)	0.845	0.0107 (0.0112)	0.337

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. The beta values are based on the following: age, per year increase; BMI, per 1 kg/m^2 increase; for SBP and DBP, per 10 mm Hg increase; for LDL, HDL, and triglycerides, per 1 mmol/l increase. The beta for triglycerides is for log-transformed values. The beta coefficients for the comparison of GG/GC vs CC genotypes is shown. The results were similar when modelling GG vs GC vs CC or GG vs CC. The CAD risk associated allele is the C allele.

as well as the evidence for its correlation with atherosclerotic burden and future cardiovascular events.[6,7,20,21] Furthermore, because genetic determinants could presumably be active from a young age, assessment of any association with CIMT in a young predisease cohort provides a useful means of investigating the early impact of such determinants on atherosclerosis. Therefore, we examined the association of the chromosome 9p21 locus in CIMT in 2 cohorts, including a cohort of young adult subjects as well as a cohort with an age-range similar to cohorts which demonstrated the association of the locus with CAD. Somewhat surprisingly, we found no evidence of an association of the locus with either mean or maximum CIMT in either age group.

A number of possible explanations for the lack of association of the 9p21 locus with CIMT need to be considered. It is unlikely that selection bias is a factor. Both cohorts were population based, ethnically homogeneous, and of Caucasoid origin where the association with 9p21 has been robustly demonstrated.[1–5] Similarly, it is unlikely that the lack of association reflects either imprecision of measurement of CIMT or adequate power to detect an effect. Prospective studies have shown that every 0.1-mm increase in CIMT is associated with a 20% to 30% higher risk of subsequent CAD.[7,22,23] In a recent meta-analysis of the association between rs1333049 and coronary artery disease, each copy of the risk allele (C) was associated with a 24% (95% CI: 20% to 29%) increased risk of coronary artery disease.[5] These estimates suggest that the expected effect of the rs1333049 on CIMT would be approximately 0.1 mm per allele if the association with CAD was mediated through a similar mechanism. Posthoc power calculations in our data showed that we had 80% power at an alpha of 0.05 to detect a 0.02-mm difference in mean CIMT between CC and GG subjects in the Young Finns Study and 0.06 mm in the Health 2000 cohort. Hence we had >99% power at an alpha of 0.01 to detect a 0.2 mm difference in CIMT between CC and GG subjects in both cohorts. Furthermore, we easily detected several previously reported effects of other cardiovascular risk factors on CIMT in both cohorts. Therefore, a plausible, and perhaps mechanistically more interesting, explanation is that the chromosome 9p21 locus affects risk of CAD through mechanisms that are not manifested in the carotid wall and reflected by changes in CIMT.

Endothelial dysfunction is believed to be an early event in atherosclerosis and may predate the development of clinical disease by several decades.[8,24,25] Reduction in FMD is a validated marker of endothelial dysfunction and predicts future cardiovascular events, at least in older adults.[8] Several traditional risk factors for atherosclerosis such as hypercholesterolaemia, diabetes, and hypertension correlate with reductions in FMD.[25] Although the lack of a significant association between the 9p21 locus and FMD in the Young Finns study does not exclude the possibility that such an effect will be observed in older subjects, our finding again suggests that this genetic locus does not enhance risk of CAD by itself primarily causing endothelial dysfunction at a young age.

The recent finding that the 9p21 locus is also associated with the development of intracranial aneurysms[19] suggests that the mechanism of its effect on the vascular wall is perhaps more complex than simply promoting the development of atherosclerosis. If the mechanism relates to cell growth and turnover as discussed earlier, it is possible that this affects coronary plaque stability or vulnerability rather than its development per se. Further studies are necessary to understand the mechanism(s) by which the chromosome 9p21 locus affects risk of CAD. In this regard, our finding of a lack of association of the locus with CIMT and with FMD at a young age provides valuable information in directing this search.

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Disclosures

None.

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III

Metabolic syndrome and carotid intima media thickness in the Health 2000 Survey

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ABSTRACT

Background and purpose: Metabolic syndrome has been associated with increased carotid intima-media thickness (CIMT) and cardiovascular disease (CVD). The objective of this study was to examine metabolic syndrome as a determinant of CIMT in men and women and to compare the Framingham risk score (FRS) and metabolic syndrome as risk factors for increased carotid atherosclerosis.
Methods: The study population consisted of 1353 Finnish men and women aged 45 years and above who participated in Finnish population-based Health 2000 Survey. CIMT was used as a marker of subclinical atherosclerosis. The National Cholesterol Education Program Adult Treatment Panel III criterion was used to define the presence of metabolic syndrome.
Results: In multivariable models, metabolic syndrome was an independent determinant of CIMT in both sexes ($p \leq 0.001$ for both). When metabolic syndrome was included in the regression models along with its components, it was an independent determinant of CIMT in women but not in men. After dividing the population into risk categories according to FRS and the presence of metabolic syndrome, FRS predominantly determined CIMT regardless of the presence of metabolic syndrome in men. In women, however, CIMT was significantly higher in subjects with metabolic syndrome than in those without it, independently of the FRS.
Conclusions: Metabolic syndrome is an independent determinant of CIMT in both sexes. In women but not in men, metabolic syndrome is associated with CIMT independently of its components. Metabolic syndrome provides additional information on a person's risk for early atherosclerosis beyond FRS in women but not in men.

1. Introduction

Metabolic syndrome is a cluster of cardiovascular risk factors such as central obesity, hypertension, dyslipidemias and glucose intolerance. It has been shown to be a predictor of type 2 diabetes [1], coronary heart disease (CHD) [2] and mortality [3–5]. Various definitions of metabolic syndrome have been proposed for clinical use. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III proposed their widely used clinical definition for metabolic syndrome in 2001 [6].

Carotid artery intima-media thickness (CIMT) is a well-established marker of subclinical atherosclerosis that can be

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measured non-invasively. Increased CIMT has been shown to predict CHD, cerebrovascular disease [7–9] and cardiovascular mortality [10]. The presence of metabolic syndrome has been linked to increased CIMT in several studies [11–16]. It has been suggested that metabolic syndrome is a stronger risk factor for subclinical atherosclerosis in women than in men [17]. The data regarding sex differences are, however, limited.

There is an ongoing debate whether metabolic syndrome provides useful information of the patient's cardiovascular risk in addition to the traditional risk factors. The Framingham risk score (FRS) is a clinical tool for quantifying an individual's CHD risk [18] based on traditional risk factors. There are few data comparing metabolic syndrome and FRS in their ability to identify subjects with increased CIMT [15,19,20]. To the best of our knowledge, possible sex differences regarding this issue have not been studied.

The objective of this study was to find out whether metabolic syndrome associates differently with CIMT in men and women. Furthermore, we set out to discover whether or not metabolic syndrome is a risk factor of CIMT beyond FRS.

2. Methods

2.1. Study population

We studied a subpopulation of a large Finnish cross-sectional health examination survey (the Health 2000 Survey) carried out in 2000–2001 [21]. The overall study cohort was a two-stage stratified cluster sample (8028 persons) representing the entire Finnish population aged 30 years and above. In order to study cardiovascular diseases (CVD) and diabetes more thoroughly, a supplemental study was carried out (sample size 1867 and participation rate 82%). The subjects in the supplemental study, a subpopulation of the Health 2000 Survey, were 45 years and older and the study was executed in the areas located within 150 km from the five Finnish University Hospitals, because specialized equipment was required. A carotid ultrasound examination was included in this supplemental study. There were 1353 subjects (607 men and 746 women; mean age, 58 years; range 46–76 years) with available carotid ultrasound data. These individuals formed our study group.

Waist circumference was not measured in the supplemental study; therefore, the Health 2000 Survey parameters were used to define the presence of metabolic syndrome. All the other measurements and laboratory tests used in the present study were collected from the (data of the) supplemental study. The mean time interval between the Health 2000 Survey and the supplemental study was 16 months (range 10–23 months). The mean (±S.D.) change in body weight during this time was 0.52 (±3.43) kg. Since this change was not clinically significant, it is likely that the change in waist circumference was not clinically significant either.

2.2. Metabolic syndrome and the Framingham risk scoring

We used the NCEP definition [6] to define the presence of metabolic syndrome. According to this definition, a person has metabolic syndrome if at least three of the following criteria are met: waist circumference at least 102 cm (men) or 88 cm (women), triglycerides ≥1.7 mmol/l, HDL cholesterol less than 1.03 mmol/l (men) or 1.29 mmol/l (women), blood pressure ≥130/≥85 mmHg and fasting plasma glucose ≥5.6 mmol/l. The fasting glucose threshold of the NCEP criterion was modified in 2004 [22]. To define FRS, we used CHD score sheets that have been previously reported [18]. FRS defined by these sheets gives an estimation of a subject's risk for CHD over a period of 10 years.

2.3. Carotid artery studies

High-resolution B-mode carotid ultrasound examination of the right carotid artery was performed according to a standardized protocol using a 7.5 MHz linear array transducer. The examinations were performed by centrally trained and certified sonographers at 6 study locations around Finland. CIMT measurements were performed off-line with the use of automated imaging processing software. One reader was responsible for reading all ultrasound images. Three summary measures of the CIMT were calculated: (1) the mean of the three average IMTs of the common carotid artery (mean CCA IMT), (2) the mean of the three average IMTs of the carotid bulb (mean bulb IMT), and (3) the mean of these two means (mean IMT). Mean IMT was used in the present study. This method has been described in detail previously [23]. The intra-reader reproducibility of the CIMT measurements was assessed. The reader measured the CIMT twice from 571 randomly selected images of 108 study subjects several weeks apart. The mean difference of the two measurements was 0.001 mm (S.D. 0.123), the intra-class correlation 0.934 ($p < 0.001$) and the coefficient of variance 9.2%.

2.4. Waist circumference, body mass index, blood pressure and smoking

Waist circumference was measured in the standing position using the standards created for population health studies [24]. Height and weight were measured and body mass index (BMI) calculated. In the Health 2000 Survey, blood pressure was measured with a mercury sphygmomanometer (Mercuro 300, Speidel & Keller, Juningen, Germany) from the right arm. The first measurement was carried out after at least five minutes of rest in the sitting position. Korotkoff's first phase was used as the sign of systolic blood pressure and the fifth phase as the sign of diastolic pressure. The measurement was repeated two minutes after the first measurement. The average of the two measurements was used in the analysis. In the supplemental study, blood pressure was measured after at least ten minutes rest from the right arm. The measurement was taken three times with 1–2-min intervals. The automatic Omron M4 manometer (Omron Matsusaka Co., Japan, Omron Healthcare Europe B.V., Hoofddorp, the Netherlands) was used. The average of the three measurements was used in the analysis. Pulse pressure was calculated as the difference between the average systolic and the average diastolic blood pressure. Current smoking was evaluated with a questionnaire. Those who were currently smoking were defined as smokers and the rest of the subjects as non-smokers. The smoking data used in the present study were collected from the Health 2000 Survey data.

2.5. Laboratory tests

Venous blood samples were drawn from the antecubital vein after an overnight fast. HDL cholesterol, total cholesterol, triglyceride and plasma glucose concentrations were determined enzymatically (Roche Diagnostics, GmbH, Mannheim, Germany for HDL; Olympus System Reagent, Hamburg, Germany for total cholesterol, triglycerides and glucose) with a clinical chemistry analyser (Olympus, AU400, Hamburg, Germany). High sensitivity C-reactive protein (hs-CRP) concentrations were determined using a chemiluminescent immunometric assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA). LDL cholesterol was calculated with the Friedewald formula.

2.6. Statistical analyses

The skewed distributions of triglycerides and hs-CRP were corrected logarithmically before statistical analyses. Chi-square and

Table 1
Clinical and laboratory parameters of the study cohort ($n = 1353$) with and without metabolic syndrome (MetS), according to NCEP definition

	MetS					
	Male			Female		
	Yes ($n = 225–241$)[a]	No ($n = 359–366$)[a]	p	Yes ($n = 257–267$)[a]	No ($n = 469–479$)[a]	p
Age (years)	58.7 ± 7.6	57.6 ± 8.0	0.075	61.3 ± 8.3	56.7 ± 7.7	<0.001
Smoking			0.242			0.775
Yes (%)	24.9	29.2		18.4	19.2	
No (%)	75.1	70.8		81.6	80.8	
Body mass index (kg/m^2)	29.7 ± 3.9	26.0 ± 3.2	<0.001	30.1 ± 4.9	25.3 ± 3.8	<0.001
Waist circumference (cm)	106.8 ± 10.1	94.2 ± 9.1	<0.001	97.5 ± 11.9	83.9 ± 10.4	<0.001
Heart rate (beats/min)	67.6 ± 12.9	65.3 ± 11.9	0.023	68.6 ± 11.6	67.9 ± 10.0	0.421
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.6 ± 0.4	<0.001	1.4 ± 0.3	1.9 ± 0.4	<0.001
LDL cholesterol (mmol/l)	3.4 ± 0.9	3.5 ± 0.9	0.208	3.6 ± 0.9	3.2 ± 0.8	<0.001
Total cholesterol (mmol/l)	5.4 ± 1.0	5.6 ± 1.0	0.147	5.8 ± 1.0	5.6 ± 0.8	0.002
Triglycerides (mmol/l)	2.0 ± 1.6	1.2 ± 0.6	<0.001	1.6 ± 0.8	1.0 ± 0.4	<0.001
Fasting glucose (mmol/l)	6.6 ± 1.6	5.8 ± 1.0	<0.001	6.1 ± 1.4	5.3 ± 0.5	<0.001
Hs-CRP (mg/l)	3.6 ± 5.2	2.3 ± 2.9	<0.001	4.2 ± 4.7	2.3 ± 4.3	<0.001
SBP (mmHg)	148.9 ± 20.7	136.4 ± 18.6	<0.001	147.3 ± 21.7	130.3 ± 20.7	<0.001
DBP (mmHg)	90.1 ± 11.0	85.4 ± 10.2	<0.001	85.3 ± 9.0	80.2 ± 9.6	<0.001
PP (mmHg)	58.7 ± 15.0	51.1 ± 12.1	<0.001	62.0 ± 17.2	50.1 ± 13.9	<0.001
Medication for hypertension	41.5	19.7	<0.001	46.1	20.5	<0.001
Statin medication	15.4	11.5	0.165	14.6	8.6	0.010

Values are means ± S.D. except smoking and medication which are expressed as %. NCEP: National Cholesterol Education Program, Hs-CRP: high sensitivity C-reactive protein, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure.
[a] Variation of n is due to missing data for some subjects.

t-test analyses were performed to compare categorical and continuous variables between the subjects with and without metabolic syndrome, respectively. Pearson's correlation coefficients were used to examine the association between cardiovascular risk factors and CIMT. Interaction between sex and the prevalence of metabolic syndrome on CIMT was analyzed using analysis of covariance (ANCOVA). Stepwise linear regression analysis was performed for continuous and dichotomous variables to examine independent relationships between metabolic syndrome, its components, other cardiovascular risk factors and CIMT. Analysis of variance (ANOVA) was used to examine CIMT in subjects in different risk profile categories. We used Tukey's correction as a post-hoc test in ANOVA to examine the differences between the risk profile categories. Statistical analyses were performed using SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL, USA).

3. Results

The prevalence of metabolic syndrome using the NCEP definition was 40% in men and 36% in women. The subjects with metabolic syndrome were more frequently on antihypertensive medication, in addition to having higher BMI, waist circumference, triglycerides, fasting plasma glucose, hs-CRP and blood pressure as well as lower HDL cholesterol ($p < 0.05$ for all) than the subjects without the syndrome in both sexes (Table 1). Women with metabolic syndrome were older, used statins more often and had higher LDL and total cholesterol than women not suffering from the syndrome. In men these variables did not differ significantly according to the presence of metabolic syndrome. Subjects with and without metabolic syndrome did not differ in smoking habits ($p > 0.1$).

A borderline significant interaction was found between sex and the prevalence of metabolic syndrome in determining CIMT ($p = 0.058$). Because of this and our original hypothesis that the effect of metabolic syndrome on CIMT might be different between the sexes, we analyzed men and women separately. For both sexes, the mean CIMT was significantly ($p < 0.001$ for both) higher in subjects with metabolic syndrome (1.02 and 0.98 mm in men and women, respectively) than in those without the syndrome (0.94 and 0.85 mm in men and women, respectively). There was no statistically significant difference in the mean CIMT ($p > 0.1$ for both sexes) of current smokers and non-smokers. The correlation of age, systolic blood pressure, pulse pressure, HDL cholesterol, triglycerides, fasting plasma glucose, hs-CRP and FRS with CIMT was statistically significant in both sexes (Table 2). The correlation of BMI, waist circumference, LDL cholesterol, total cholesterol and diastolic blood pressure with CIMT was statistically significant in women but not in men.

Stepwise linear regression models were constructed for men and women separately to examine the influence of known cardiovascular risk factors on CIMT as independent variables (Table 3, model A). The initial stepwise regression models included variables that had significant univariate correlation with CIMT. In men, age and triglycerides were independent predictors explaining 24% of the variation in CIMT. In women, age, pulse pressure and triglycerides were independent predictors explaining 29% of the variation in CIMT. We performed the same regression analyses

Table 2
Correlations between cardiovascular risk factors and CIMT in males ($n = 496–607$[a]) and females ($n = 628–746$[a])

	Men		Women	
	r	p	r	p
Age (years)	0.464	<0.001	0.515	<0.001
Body mass index (kg/m^2)	0.045	0.267	0.171	<0.001
Waist circumference (cm)	0.077	0.057	0.189	<0.001
HDL cholesterol (mmol/l)	−0.131	0.001	−0.157	<0.001
LDL cholesterol (mmol/l)	−0.069	0.094	0.131	<0.001
Total cholesterol (mmol/l)	−0.055	0.174	0.113	0.002
Triglycerides (mmol/l)	0.161	<0.001	0.231	<0.001
Fasting glucose (mmol/l)	0.116	0.004	0.134	<0.001
Hs-CRP (mg/l)	0.122	0.003	0.111	0.003
SBP (mmHg)	0.137	0.001	0.352	<0.001
DBP (mmHg)	−0.008	0.849	0.148	<0.001
PP (mmHg)	0.208	<0.001	0.402	<0.001
FRS	0.370	<0.001	0.428	<0.001

CIMT: carotid intima-media thickness, r: Pearson's correlation coefficient, Hs-CRP: high sensitivity C-reactive protein, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, FRS: Framingham risk score value.
[a] Variation of n is due to missing data for some subjects.

Table 3
Linear regression models for the relationships between cardiovascular risk factors and CIMT in males and females

Risk variable	CIMT		
	$\beta \pm$ S.E.	p	R^2 change (%)
Model A			
Male (n = 594)			
Age (years)	0.014 ± 0.001	<0.001	21
Triglycerides (mmol/l)	0.205 ± 0.041	<0.001	3
R^2 (%)	24		
Female (n = 720)			
Age (years)	0.010 ± 0.001	<0.001	26
PP (mmHg)	0.002 ± <0.001	<0.001	3
Triglycerides (mmol/l)	0.107 ± 0.037	0.004	1
R^2 (%)	29		
Model B			
Male (n = 594)			
Age (years)	0.014 ± 0.001	<0.001	21
Triglycerides (mmol/l)	0.205 ± 0.041	<0.001	3
R^2 (%)	24		
Female (n = 720)			
Age (years)	0.010 ± 0.001	<0.001	26
PP (mmHg)	0.002 ± <0.001	<0.001	3
Metabolic syndrome	0.059 ± 0.015	<0.001	2
R^2 (%)	30		

CIMT: carotid intima-media thickness, β: regression coefficient, S.E.: standard error, PP: pulse pressure, R^2: adjusted R square value of the whole model, R^2 change: change in adjusted R square value after addition of the respective variable in to the model. Initial stepwise regression model for men included age, fasting plasma glucose, HDL cholesterol, pulse pressure, high sensitivity-CRP, triglycerides (models A and B) and metabolic syndrome (model B only) as independent variables. Initial stepwise regression model for women included age, waist circumference, fasting plasma glucose, HDL cholesterol, pulse pressure, high sensitivity-CRP, triglycerides, LDL cholesterol (models A and B) and metabolic syndrome (model B only) as independent variables.

with metabolic syndrome included in the models as an independent variable (Table 3, model B). In men this did not change the results, and metabolic syndrome was not an independent predictor of CIMT in this model. In women, however, metabolic syndrome was an independent predictor ($p < 0.001$) of CIMT together with age and pulse pressure ($R^2 = 0.30$). Another stepwise linear regression model was performed for both sexes using metabolic syndrome (instead of its components) and other known cardiovascular risk factors (that significantly correlated with CIMT in univariate models) as independent variables (Table 4). Age and metabolic syndrome were independent determinants for CIMT in both sexes (adjusted R^2 0.22 and 0.29 in men and women, respectively). We also performed all the above mentioned regression models using the presence of antihypertensive medication and statin medication as independent variables. The main findings remained similar (data not shown).

In order to examine the associations of metabolic syndrome and FRS with CIMT, we divided the population into four risk profile groups. In the first group, subjects had low FRS (indicating a 10-year CHD risk less than 10%) and no metabolic syndrome. The second group consisted of subjects with low FRS and metabolic syndrome. In the third group, subjects had high FRS (indicating a 10-year CHD risk greater than 10%) and no metabolic syndrome. The fourth group consisted of subjects with metabolic syndrome and high FRS. In both sexes, there were significant differences in CIMT between the risk groups ($p < 0.001$). In men CIMT was significantly higher in both of the high-FRS groups than in any of the low-FRS groups, regardless of metabolic syndrome ($p < 0.01$) (Fig. 1). In women who had low FRS and metabolic syndrome, CIMT was significantly higher than in women with low FRS without the syndrome ($p = 0.012$). A similar pattern was observed in women with high FRS ($p = 0.007$).

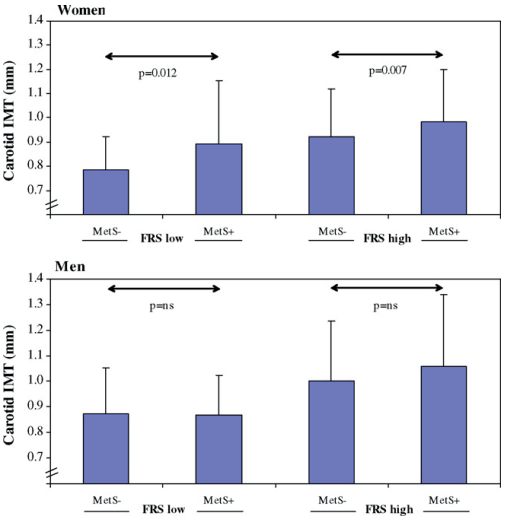

Fig. 1. Carotid IMT (mean and S.D.) according to the risk profile group in both sexes. FRS = Framingham risk score and MetS = metabolic syndrome.

4. Discussion

Metabolic syndrome and its individual components are risk factors for atherosclerosis and CVD [1–5]. CIMT is a well-known marker of subclinical atherosclerosis and predicts future CHD and myocardial infarction [7–9]. The association between metabolic syndrome and CIMT has been reported in several populations [11–16]. It has been suggested that this association is stronger in women than in men [17]. In the present study, our primary target was to investigate these possible sex differences in the associations of metabolic syndrome and CIMT. We were especially interested in the influence of metabolic syndrome on CIMT beyond traditional

Table 4
Linear regression model for the relationships between metabolic syndrome, cardiovascular risk factors and CIMT

Risk variable	CIMT					
	Male (n = 597)			Female (n = 722)		
	$\beta \pm$ S.E.	p	R^2 change (%)	$\beta \pm$ S.E.	p	R^2 change (%)
Age (years)	0.014 ± 0.001	<0.001	21	0.012 ± 0.001	<0.001	26
Metabolic syndrome	0.064 ± 0.018	<0.001	2	0.074 ± 0.014	<0.001	3
R^2	22%			29%		

CIMT: carotid intima-media thickness, β: regression coefficient, S.E.: standard error, R^2: adjusted R square value of the whole model, R^2 change: change in adjusted R square value after addition of the respective variable in to the model. Initial stepwise regression model for men included age, high sensitivity-CRP and metabolic syndrome as independent variables. Initial stepwise regression model for women included age, high sensitivity-CRP, LDL cholesterol and metabolic syndrome as independent variables.

risk factors defined by such risk stratification tools as FRS. Indeed, our data suggest that the associations between metabolic syndrome, FRS and early atherosclerosis are modified by sex.

Age, HDL cholesterol, triglycerides, fasting plasma glucose, hs-CRP, systolic blood pressure, pulse pressure and FRS had statistically significant correlations with CIMT in univariable models for both sexes. BMI, waist circumference, LDL cholesterol, total cholesterol and diastolic blood pressure had statistically significant univariate correlations with CIMT in women but not in men. Comparable associations between CIMT and cardiovascular risk factors have been reported previously [25–29]. The mean CIMT was significantly higher in subjects with metabolic syndrome than those without the syndrome in both sexes. This is in line with previously reported data [16,30,31]. In women the univariate correlations between cardiovascular risk factors and CIMT were stronger than in men. Nishida et al. have reported comparable results [28].

Although we did find that many individual risk factors were associated with CIMT, only some of them appeared in the final regression models as independent determinants of CIMT. As expected, age was clearly the strongest factor determining CIMT in both sexes—a finding which is in agreement with previous data [13,15]. In our study population, triglyceride concentration was also independently associated with CIMT in men. In women, pulse pressure and triglycerides appeared in the final model as independent predictors of CIMT. The final model for women was somewhat stronger, explaining 29% of the variance in CIMT, whereas the adjusted R^2 in men was 24%.

It has been reported earlier that metabolic syndrome is independently associated with CIMT [13]. In the present study, when metabolic syndrome was included in the regression models instead of its components, it was found to be an independent determinant of CIMT together with age in both sexes. However, the adjusted R^2 dropped to 0.22 in men while remaining at 0.29 in women. When metabolic syndrome was included in the regression models together with its components, it was found to be an independent determinant for CIMT in women but not in men. Similar findings have been reported previously [13,27], but in these studies sexes have not been analyzed separately. Previous data regarding sex differences in the effect of metabolic syndrome on CIMT are limited. Iglseder et al. reported that the effect of metabolic syndrome on CIMT was stronger in women than in men [17]. Nishida et al. showed that the increase in the number of metabolic syndrome components was associated more strongly with CIMT in women than in men [28], the findings being in concert with the present results. Skilton et al. did not find sex differences in the associations between metabolic syndrome and CIMT when the NCEP definition was used, although there were sex differences when other definitions for metabolic syndrome were used [16]. Empana et al. did not find sex differences in the associations between CIMT and metabolic syndrome [30]. To sum up the above, some evidence suggests that metabolic syndrome has a more pronounced influence on CIMT in women than in men. The matter has, however, been controversial.

We divided our study population into four risk profile groups according to FRS and metabolic syndrome. We defined CHD risk to be low if the risk was less than 10% according to FRS. In men CIMT was significantly higher if FRS was high, regardless of the presence of metabolic syndrome. In women, however, CIMT was significantly higher if metabolic syndrome was present, regardless of the FRS category. There are few studies that have elaborated the effects of metabolic syndrome on CIMT after taking FRS into account. Hassinen et al. found in a prospective study of elderly women that metabolic syndrome was associated with accelerated progression of CIMT independently of FRS [19]. Teramura et al. recently suggested that metabolic syndrome, as defined by Japanese criteria, has some additive predictive value on CIMT beyond FRS [20]. However, Ahluwalia et al. reported that metabolic syndrome was not associated with CIMT independently of FRS [15]. To the best of our knowledge, this is the first study to report sex differences in the effects of metabolic syndrome and FRS on CIMT.

There were also certain study limitations. Corresponding to the majority of Finnish population, our study group consisted of white Caucasians being racially homogenous. Thus, the present results may not be applicable to other ethnic groups than white Caucasians. Only Hs-CRP was used as a marker of low-grade systemic inflammation in the present study. Including larger set of pro- and anti-inflammatory markers may provide deeper insight into the role of inflammation in the pathophysiology of metabolic syndrome. Also measurement of other relevant biochemical indices such as adhesion molecules and adipokines may provide new information about the mechanisms behind the association between metabolic syndrome and early atherosclerosis.

In conclusion, our findings suggest that metabolic syndrome is associated with CIMT independently of other known cardiovascular risk factors in both sexes in a middle-aged and elderly population. This association is, however, modified by sex. Metabolic syndrome is associated with CIMT independently of its components in women but not in men. In men, traditional risk factors as defined by FRS are strong determinants of CIMT, with metabolic syndrome offering little additional information. In women, however, metabolic syndrome is an additional risk factor for subclinical atherosclerosis beyond FRS. This is the case especially in women with low FRS. These findings indicate that metabolic syndrome should be clinically evaluated especially in women with low CVD risk according to traditional risk factors.

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IV

Interleukin-6 promoter polymorphism and cardiovascular risk factors: The Health 2000 Survey

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ABSTRACT

Objective: Inflammatory factors modify the risk of cardiovascular diseases and atherosclerosis. The single base genetic polymorphism in the promoter region of inflammatory cytokine interleukin-6 (*IL6* −174 G>C, rs1800795) is associated with the variation of IL-6 production. The aim of this study was to investigate whether *IL6* −174 G>C is associated with the risk factors and early markers of atherosclerosis.
Methods: As part of Finnish Health 2000 Study, we performed carotid artery ultrasound examinations, *IL6* −174 G>C genotyping and cardiovascular risk factor determination for 1334 subjects aged 46–76 years.
Results: In men, serum total cholesterol was higher in *IL6* −174 GG (5.70 ± 0.88 mmol/L) than in the GC (5.51 ± 0.98 mmol/L) or CC (5.38 ± 0.97 mmol/L, mean ± SD, $p = 0.0059$) groups. The same order was seen in LDL-C (GG 3.64 ± 0.83 mmol/L, GC 3.41 ± 0.88 mmol/L, CC 3.30 ± 0.91 mmol/L, $p = 0.0017$). The opposite association was observed with plasma fasting glucose levels (GG 5.93 ± 0.97, GC 6.11 ± 1.34, CC 6.34 ± 1.59 mmol/L, $p = 0.043$) and BMI (GG 26.8 ± 3.42, GC 27.5 ± 4.32, CC 28.0 ± 3.81 kg/m^2, $p = 0.027$). *IL6* −174 allele C homozygous men indicated a trend towards higher systolic blood pressure. *IL6* −174 G>C was not associated with carotid artery compliance, intima media thickness or CRP. The effect size of *IL6* −174 G>C on cardiovascular risk factors was not significant in women. These results suggest that *IL6* −174 G>C modifies the levels of several metabolic risk factors of atherosclerosis in men.

1. Introduction

Inflammation plays a key role in pathogenesis of atherosclerosis [1]. Interleukin-6 (IL-6) is a pleiotropic inflammatory cytokine which has been associated with atherosclerosis and cardiovascular disease [2,3]. A single G>C base exchange polymorphism in the promoter region of the IL-6 gene (*IL6* −174 G>C) is associated with IL-6 production. In the initial studies *IL6* −174 allele G homozygous and G/C heterozygous subjects have shown a higher expression of IL-6 protein, higher transcriptional activity and higher inducible IL-6 responses than subjects homozygous for *IL6* allele C [4–6]. The effect of *IL6* −174 G>C on circulating IL-6 is more complex and may be dependent on the presence of immune challenge, age and BMI of subjects as well as physiological and psychosocial stress and various metabolic factors [7,8]. *IL6* −174 G>C has been associated with the risk of ischemic cerebrovascular events [9] and coronary heart disease in men [10,11]. The frequency of *IL6* allele C seems to decrease among old people, suggesting an association with mortality [12,13]. Allele C has also been associated with high blood pressure [11,14] and high levels of C-reactive protein [11,15]. A recent joint analysis conducted on the basis of data from 17 studies suggested association of IL-6 G>C with fasting glucose levels independently of BMI [7].

Established cardiovascular risk factors such as serum cholesterol, blood pressure, obesity and smoking are associated with increased arterial stiffness [16]. Ultrasonographically measured carotid artery compliance and common carotid intima media thickness (IMT) are markers of early atherosclerosis [17–19]. *IL6* −174 G>C genotypes have been associated with carotid artery compliance and carotid IMT [14,20].

In spite of intensive research, the role of *IL6* −174 G>C as a risk factor for cardiovascular diseases has remained inconsistent [15,21], and several authors have expressed a need for further studies [11,14,21,22]. We have previously found that IL-6 G>C

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is associated with carotid artery compliance, systolic (SBP) and diastolic (DBP) blood pressure, serum high-density lipoprotein cholesterol (HDL-C) in Finnish young male subjects aged 24–39 years ($n = 996$) [14]. In order to test whether *IL6* −174 G>C genotypes exert influence on the risk factors and early markers of atherosclerosis in the middle-aged to elderly population, we have now analysed promoter region polymorphism of *IL6* −174 G>C in 1334 Finnish participants of the Health 2000 Study. The age range of subjects in our study cohort was 46–76 years.

2. Materials and methods

2.1. Subjects

The study sample was drawn from participants of a large Finnish multidisciplinary epidemiological cross-sectional health examination survey (the Health 2000 Survey) carried out in Finland from autumn 2000 to spring 2001 [23]. The overall study cohort was a two-stage stratified cluster sample (8028 persons) representing the entire Finnish population aged 30 years and above. In order to study cardiovascular diseases and diabetes more thoroughly, a supplemental study was carried out (sample size 1867 and participation rate 82%). The subjects in the supplemental study, a subpopulation of the Health 2000 Survey, were 46 years and older and the study was executed in the areas located within 150 km from the five Finnish University Hospitals, because specialized equipment was required. A carotid ultrasound examination was included in this supplemental study. Moreover, current medication data was collected by questionnaire. There were 1353 subjects (607 men and 746 women; mean age, 58 years; range, 46–76 years) with available carotid ultrasound data. Genotyping was successful on 1334 of these subjects (98.6%), which formed our study group. For 19 subjects genotyping could not be completed because of signs of DNA degradation, or protein interference in DNA sample resulting in poor signal to noise ratio in fluorescence based analysis (see methods below). The characteristics of the participants are presented in Table 1.

2.2. Carotid artery studies

High-resolution B-mode carotid ultrasound examination of the right carotid artery was performed according to a standardized protocol using a 7.5 MHz linear array transducer. The examinations were performed by centrally trained and certified sonographers at six study locations around Finland. Carotid IMT measurements were performed off-line with the use of automated imaging processing software. One reader was responsible for reading all ultrasound images. Three summary measures of the carotid IMT were calculated: (1) the mean of the three average IMTs of the common carotid artery, (2) the mean of the three average IMTs of the carotid bulb, and (3) the mean of these two means (mean IMT). Mean IMT was used in the present study. This method has been described in detail previously [24]. The intra-reader reproducibility of the carotid IMT measurements was assessed. The reader measured the carotid IMT twice from 571 randomly selected images of 108 study subjects several weeks apart. The mean difference of the two measurements was 0.001 mm (SD 0.123), the intra-class correlation 0.934 ($p < 0.001$) and coefficient of variation 9.2%.

Table 1
Clinical and laboratory characteristics for the cases for which interleukin-6 G>C −174 genotype was determined ($n = 727$ female, $n = 607$ male). Values were measured at the time of cross section (during the years 2000–2001).

Parameter	Female, mean ± SD	Male, mean ± SD
Age (years)	58.7 ± 8.29	58.0 ± 7.78
BMI (kg/m^2)	27.1 ± 4.83	27.5 ± 4.03
Carotid IMT (mm)	0.90 ± 0.21	0.97 ± 0.24
Carotid artery compliance (%/10 mmHg)	0.95 ± 0.53	0.88 ± 0.41
SBP (mmHg)	136 ± 22.5	141 ± 20.6
DBP (mmHg)	81.9 ± 9.65	87.3 ± 10.7
Total cholesterol (mmol/L)	5.66 ± 0.92	5.51 ± 0.97
HDL-C (mmol/L)	1.70 ± 0.43	1.43 ± 0.38
LDL-C (mmol/L)	3.38 ± 0.87	3.43 ± 0.88
Triglycerides (mmol/L)	1.29 ± 0.65	1.52 ± 1.16
CRP (mg/L)	3.01 ± 4.63	2.79 ± 4.01
Fasting insulin (mmol/L)	8.99 ± 6.01	10.7 ± 7.84
Fasting glucose (mmol/L)	5.63 ± 0.99	6.14 ± 1.36

Abbreviations: BMI, body mass index; IMT, intima media thickness; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein.

2.3. Laboratory tests

Venous blood samples were drawn from the antecubital vein after an overnight fast. HDL-C, total cholesterol, triglyceride and plasma glucose concentrations were determined enzymatically with a clinical chemistry analyser. Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula. More detailed description of methodology has been published recently in this journal [25].

2.4. IL6 genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit (Qiagen Inc., Hilden, Germany) in 2001. *IL6* −174 G>C genotyping was performed by employing the 5′-nuclease assay and fluorogenic allele-specific TaqMan probes and primers [26] as well as the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

2.5. Statistical analysis

Statistical analyses were initially carried out using Statistica for Windows 6.0 (StatSoft Inc., Tulsa, OK, USA), and SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA), with Kruskall–Wallis analysis of variance and Mann–Whitney U-test. Hardy–Weinberg equations were calculated by Arlequin [27]. As the *IL6* −174 G>C genetic effect on carotid artery compliance, blood pressure and HDL-C has previously been shown to be dependent on sex [14], analyses were carried out separately for males and females. After nonparametric analyses, we evaluated the extent of the genetic effect of *IL6* −174 G>C on total cholesterol, LDL-C, HDL-C, triglycerides, systolic and diastolic blood pressure as well as fasting insulin and glucose levels and BMI. For this purpose we used multivariate linear regression analysis. In addition to *IL6* genotype, known common independent risk factors and common confounding factors were included in the model. Lists of these covariates are presented in Table 3. Before the analysis the regression models were assessed for the multicollinearity. Variance inflation factors for covariates in the presented models were at acceptable level, ranging from 1.023 to 1.735 (tolerances ranging from 0.576 to 0.988). The skewed distribution of triglycerides was corrected logarithmically before regression analyses. As the tests in the current study were done under a special hypotheses (post hoc) based on our previous study [14], p values less than 0.05 were considered significant. However, the data in Table 2 could be also referred by multiple alternative significance levels after adjusting with Bonferroni correction ($p < 0.0042$), Sidak's correction ($p < 0.0041$) or Scheffé's adjustment ($p < 0.0022$).

3. Results

The distribution (prevalence given in parenthesis) of *IL6* −174 G>C genotypes in our population was as follows: GG, $n = 258$

Table 2
The association of IL-6 −174 G>C with the risk factors and early markers of atherosclerosis (n = 727 female, n = 607 male).

Parameter	Men, mean ± SD				Women, mean ± SD			
	IL-6 genotype				IL-6 genotype			
	GG	GC	CC	p value*	GG	GC	CC	p value*
Total cholesterol (mmol/L)	5.70 ± 0.88	5.51 ± 0.98	5.38 ± 0.97	<0.01	5.75 ± 0.95	5.66 ± 0.93	5.60 ± 0.89	0.33
HDL-C (mmol/L)	1.47 ± 0.34	1.43 ± 0.40	1.38 ± 0.38	0.06	1.70 ± 0.40	1.70 ± 0.45	1.72 ± 0.43	0.76
LDL-C (mmol/L)	3.64 ± 0.83	3.41 ± 0.88	3.30 ± 0.91	<0.01	3.49 ± 0.91	3.38 ± 0.88	3.32 ± 0.83	0.28
Triglycerides (mmol/L)	1.34 ± 0.75	1.56 ± 1.41	1.58 ± 0.87	0.05	1.29 ± 0.70	1.31 ± 0.66	1.24 ± 0.59	0.45
SBP (mmHg)	137 ± 17.2	142 ± 20.2	144 ± 23.2	0.08	135 ± 21.3	136 ± 23.1	137 ± 22.3	0.65
DBP (mmHg)	85.4 ± 9.6	88.0 ± 10.6	87.7 ± 11.5	0.09	81.5 ± 9.6	81.3 ± 9.7	82.9 ± 9.5	0.13
IMT (mm)	0.96 ± 0.23	0.97 ± 0.24	0.97 ± 0.23	0.88	0.89 ± 0.21	0.91 ± 0.22	0.89 ± 0.19	0.51
CAC (%/10 mmHg)	0.95 ± 0.46	0.85 ± 0.38	0.91 ± 0.43	0.30	0.95 ± 0.57	0.98 ± 0.52	0.90 ± 0.51	0.15
CRP (mg/L)	3.03 ± 5.59	2.66 ± 2.93	2.84 ± 4.39	0.49	3.34 ± 5.78	3.01 ± 4.77	2.83 ± 3.54	0.90
Fasting insulin (mmol/L)	9.06 ± 5.24	10.8 ± 8.41	11.6 ± 8.25	0.02	8.63 ± 4.88	9.01 ± 5.71	9.17 ± 7.05	0.70
Fasting glucose (mmol/L)	5.93 ± 0.97	6.11 ± 1.34	6.34 ± 1.59	0.04	5.51 ± 0.73	5.64 ± 1.06	5.69 ± 1.00	0.39
BMI (kg/m^2)	26.8 ± 3.42	27.5 ± 4.32	28.0 ± 3.81	0.03	26.9 ± 4.27	27.2 ± 4.60	27.2 ± 5.48	0.82

Abbreviations: BMI, body mass index; IMT, intima media thickness; CAC, carotid artery compliance; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein.
* Kruskall–Wallis ANOVA test for a trend (2 degrees of freedom).

Table 3
Multivariate model of the relationships between total cholesterol, LDL-C, systolic blood pressure and fasting glucose levels adjusted with common cardiovascular risk factors in men (n = 727).

Parameter	Total cholesterol, coefficient ± SE	LDL-C, coefficient ± SE	SBP, coefficient ± SE	Fasting glucose level, coefficient ± SE
IL6 genotype	−0.123 ± 0.056*	−0.133 ± 0.051**	2.567 ± 1.167*	0.176 ± 0.077*
Age	−0.011 ± 0.005*	−0.007 ± 0.005	0.407 ± 0.110***	0.014 ± 0.007*
BMI	0.012 ± 0.011	0.017 ± 0.011	0.945 ± 0.238***	0.074 ± 0.014***
Smoking	0.021 ± 0.087	0.016 ± 0.080	−3.344 ± 1.814	0.069 ± 0.121
Fasting glucose level	−0.010 ± 0.034	−0.067 ± 0.033*	2.404 ± 0.699***	
Fasting insulin level	−0.014 ± 0.006*	−0.011 ± 0.006	0.054 ± 0.133	
Total cholesterol			3.529 ± 0.861***	
Statin treatment	−0.594 ± 0.116***	−0.643 ± 0.107***		
Antihypertensive treatment			−0.652 ± 1.986	
Whole model R^2	9.1%	11.8%	13.7%	6.7%

Abbreviations: For IL6 genotype coefficient is for a 1-unit change in allele G content (null or 1 or 2 copies of allele G). For smoking coefficient is for categorical change from nonsmoker to smoker and for statin and antihypertensive treatments coefficient is for categorical change of being with or without medication. SE, standard error; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; BMI, body mass index.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

(19.3%); GC, n = 685 (51.3%); CC, n = 391 (29.3%). Allele frequency for allele G was 0.450 and for allele C 0.550. These figures are similar with previously published figures on the Finnish population [6,14] and other European populations [7]. As expected, the distributions of individual *IL6* alleles followed the Hardy–Weinberg equation (p = 0.21).

In men we found several significant associations between *IL6* −174 G>C genotype and cardiovascular risk factors. Serum total cholesterol levels and LDL-C levels increased according to *IL6* −174 G>C genotype in the order GG > GC > CC (Table 2). Opposite associations (GG < GC < CC) were observed with serum triglyceride, fasting insulin and fasting glucose concentrations as well as BMI (Table 2). *IL6* −174 allele G homozygocity seemed to have beneficial effect on HDL-C, SBP and DBP, but these findings were borderline significant (Table 2). We found no significant association between *IL6* −174 G>C genotypes and carotid artery compliance, carotid IMT or CRP in this cohort. In order to evaluate the independent effect of *IL6* −174 G>C on these parameters, linear regression analyses were carried out. In the multivariate models *IL6* −174 G>C remained as an independent predictor of serum total cholesterol and LDL-C concentrations when adjusted by age, BMI, current smoking, fasting plasma glucose and insulin concentrations and statin treatment (Table 3). Moreover, the trend of effect of *IL6* −174 G>C on systolic blood pressure increased in significance (p = 0.028) in the multivariate model when adjusted by age, BMI, current smoking, fasting plasma glucose and insulin, total cholesterol and antihypertensive treatment (Table 3). IL-6 −174 G>C was also associated with fasting glucose concentrations when adjusted with age, BMI and current smoking (Table 3) and with BMI when adjusted with age, current smoking, fasting glucose and total cholesterol levels (p = 0.043, B 0.470, SE 0.232, whole model R^2 7%). The trends for *IL6* −174 G>C effect on HDL-C, plasma triglyceride levels and diastolic blood pressure could not be reassured (p = 0.139–0.652). When linear regression model R^2 values were calculated with and without the *IL6* genotype, the *IL6* genotype explained 0.7–1.3% of variation observed in total cholesterol, LDL-C, fasting glucose, BMI and SBP levels. The level of *IL6* −174 G>C effect size is thus similar to that which was observed in our previous study in young male subjects [14]. However, as the overall R^2 values in the current regression models were quite low the true effect size is expected to be below these rough estimates in the current cohort. Among women there were no significant association between *IL6* −174 G>C genotypes and cardiovascular risk factors or the above-mentioned early markers of atherosclerosis (Table 2).

4. Discussion

In this study, we have shown that the *IL6* −174 G>C genotype is an independent predictor of total cholesterol, LDL-C, fasting glucose levels and BMI in the middle-aged to elderly men.

Several previous studies have found association between *IL6* −174 G>C promoter polymorphism, atherosclerosis and cardiovascular risk factors [9–12,14,15,22,28–30]. The data concerning the

role of IL-6 in lipid metabolism is more limited. Fernandez-Real et al. reported association between *IL6* −174 allele G and high triglycerides, VLDL, FFA and low HDL-C, but the size of the study population was very small [31]. Henningsson et al. found association between the *IL6* CC genotype and low total cholesterol, LDL-C and triglycerides in women. There was no association in men between *IL6* −174 G>C genotype polymorphism and lipid pattern, but *IL6* −174 C carriers tended to display elevated triglycerides [32]. In the present study, we observed some similarity with the results of previous studies in which *IL6* −174 C allele carriers was shown to have higher triglycerides [32], lower HDL-C [14] and higher SBP [11,14,33], but after adjusting for other risk factors only a trend for SBP remained in the regression models. This trend for *IL6* −174 allele C and higher SBP is in line with observation by Humphries et al. in middle-aged men as well as our previous observation in young male subjects [11,14]. Moreover, our results of association between *IL6* −174 G>C and fasting glucose in our current middle-aged to elderly cohort are in line with the results of the recent joint analysis concluding that *IL6* −174 G>C is associated with fasting glucose levels independent of BMI [7].

IL6 gene is under complex regulation, with many factors known to exert impact in transcription activity such as glucocorticoids, IL-1 and estrogen (17-beta estradiol) [34,35]. Some studies have shown that adipose tissue accounts for up to 30% of the total circulating concentrations of IL-6 in healthy subjects [36,37]. The inhibitory effect of estrogen (estrogen released from fat tissue or hormone-producing organs) on *IL6* transcription activity may be one of the reasons why there was no association between *IL6* −174 G>C genotypes and the cardiovascular risk factors in women in this study. *IL6* −174 allele C may mediate a lower transcription peak after stimulation but a slower decline to baseline than the allele G. This would result in higher chronic levels of IL-6 in −174 C allele carriers compared to GG carriers, but higher levels in GG subjects in response to acute inflammation [38]. Moreover, it has been recently shown that this kind of genetic effect of IL-6 G>C for circulating IL-6 is clearly seen in subjects of normal weight ($BMI < 24.9\,kg/m^2$) but not in those who are overweight (BMI > 25) [8]. It is noticeable in this recent study that the baseline IL-6 were higher in overweight subjects than in normal subjects supporting the above-mentioned ideas about these mutual interactions between IL-6, body composition and inflammation [8]. It is also well known that IL-6 is a key inflammatory factor, which stimulates the expression of acute phase proteins such as CRP in liver [39,40]. In a recent Rotterdam study consisting 6434 participants, Sie et al. found significant association between *IL6* −174 allele C carriers and higher CRP levels compared to subjects without this allele [15]. On the basis of our current and previous results and observations of others concerning *IL6* and CRP, it is tempting to speculate whether the associations with *IL6* G>C and plasma lipids, fasting glucose and BMI are reflecting direct effects of *IL6* in liver intermediate metabolism (gluconeogenesis), cholesterol synthesis and acute phase protein induction. As it is, the metabolic effects of IL-6 should be studied further.

IL6 −174 G>C has been previously associated with IMT [20] and our previous results suggested an association of *IL6* −174 G>C with carotid artery compliance among young men in the Cardiovascular Study of Young Finns cohort [14]. However, among middle-aged to elderly subjects studied herein, no association with carotid IMT or carotid artery compliance could be observed. There are several potential reasons for this, most evident being differences in age, baseline blood pressure, body mass and body composition as well as metabolic and pharmacological interference in these study cohorts. It is also possible that the effect of *IL6* in atherosclerosis is different in the initiation and development of atherosclerosis and the subtle allelic effects which can be observed in young populations are masked by more stronger lifestyle-related covariates in older cohorts. Unfortunately this subject cannot be reliably analysed in our cross-sectional study, and intensive long span follow-up studies are needed to explore these issues further.

In conclusion, we have found association between *IL6* −174 G>C genotypes and total cholesterol levels, LDL-C, fasting glucose levels as well as BMI in middle-aged to elderly men, and there appears to be a trend between *IL6* −174 G>C genotypes and SBP. It is noteworthy that in spite of the relatively low absolute effect size of *IL6* −174 genotype for risk factor levels, the genetic determinants studied here are very common, the prevalence of the *IL6* −174 G>C allele C being 55%. Therefore, at population level, *IL6* polymorphism seems to be an important genetic risk factor for men. Further studies are needed to explain the metabolic effects of *IL6* in men and women.

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Carotid artery intima-media thickness and elasticity in relation to glucose tolerance

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Abstract

Aims: The association of diabetes and subclinical atherosclerosis is well established. The effect of non-diabetic glucose intolerance on early atherosclerosis is not as straightforward, and the data regarding sex-related differences in this matter is limited. Therefore, our aim was to investigate these associations in men and women separately.

Methods: We studied 1,304 Finnish men and women over 45 years of age who participated in the Finnish Health 2000 Survey. Ultrasonically determined carotid artery intima-media thickness and elasticity were used as markers of early atherosclerosis. Glucose tolerance was categorized according to the American Diabetes Association criteria for diabetes mellitus.

Results: Age-adjusted means for carotid artery intima-media thickness and elasticity indices were significantly ($p<0.05$) associated with glucose tolerance status in both sexes. There was a trend of increasing early atherosclerosis with the worsening of glucose tolerance in men and women. These associations were weakened in both sexes after further adjustments for other cardiovascular risk factors. In women, but not in men, significant ($p<0.05$) associations between glucose tolerance status and carotid artery elasticity were seen even after these further adjustments.

Conclusions: Diabetes and non-diabetic glucose intolerance are associated with increased early carotid atherosclerosis compared to normal glucose tolerance in both sexes. Our results suggest that women with glucose intolerance may be in greater risk than men.

Keywords: arteriosclerosis; carotid artery diseases; diabetes; glucose intolerance

Introduction

Diabetes (DM) is a known risk factor for cardiovascular disease (CVD) morbidity and mortality. The CVD risk is roughly 2- to 4-fold in diabetic subjects in relation to their healthy counterparts [1]. The American Diabetes Association (ADA) diagnostic criteria for DM recognize two intermediate metabolic states between normal glucose tolerance (NGT) and DM [2]. These pre-diabetic states known as impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) represent two distinct subgroups of abnormal glucose metabolism. It has been shown recently that impaired insulin release is a predominant feature in IFG, whereas peripheral insulin resistance is characteristic to IGT [3]. There is evidence that even these pre-diabetic abnormalities in glucose metabolism increase the risk for all-cause and CVD mortality [4].

Carotid artery intima-media thickness (CIMT) is a well-established marker of subclinical atherosclerosis that can be measured non-invasively. Increased CIMT has been shown to predict coronary heart disease (CHD), cerebrovascular disease [5,6] and cardiovascular mortality [7]. Carotid artery elasticity as a marker of arterial stiffness has also been used as a surrogate in determining early atherosclerosis, and it has been related to CVD mortality [8].

It is known that subjects with type 2 diabetes (T2DM) have increased CIMT [9,10] and carotid artery stiffness (CAS) [11,12] in comparison to subjects with NGT. Previous reports also suggest that CIMT would be increased in subjects with IGT as compared to those with NGT [13]. Non-diabetic glucose intolerance has been linked to increased CAS in some [11,14] but not all [12,15] of the studies. There is no convincing evidence that IFG alone would be a significant risk factor for early carotid atherosclerosis [15,16].

DM is a stronger risk factor for CVD events in women than in men [17]. Some reports have also suggested that the effect of glucose metabolism impairment on early carotid atherosclerosis would be more pronounced in women than in men [11,18]. Data regarding sex-related differences is, however, limited and other studies have not confirmed this hypothesis [10,19]. Therefore, the objective of the present study was to provide a comprehensive investigation of the associations between impaired glucose metabolism and early carotid atherosclerosis and to determine whether these associations are different in men and women.

Methods

Study population

Our study population was part of a large Finnish cross-sectional health examination survey (the Health 2000 Survey) carried out in 2000–2001. The overall study cohort was a two-stage stratified cluster sample (8,028 persons) representing the entire Finnish population aged 30 years and above. In order to study CVD and DM more thoroughly, a supplemental study was carried out (sample size 1,867 and participation rate 82%). Subjects in this subpopulation of the Health 2000 Survey were 45 years and older. A carotid ultrasound examination and an oral glucose tolerance test (OGTT) were performed in this population sample. Subjects with type 1 DM were excluded from our study. After this exclusion there were 1,304 subjects (579 men and 725 women; mean age, 58 years; range, 46–76 years) with available ultrasound data. These individuals formed the study group of this report. 1,173 of the subjects (523 men and 650 women) had available data for carotid artery elasticity determination.

The study protocol of the Health 2000 survey was approved by the Epidemiology Ethics Committee of the Helsinki and Uusimaa hospital region. The participants of the survey signed an informed consent to participate.

Smoking and blood pressure

Current smoking was evaluated with a questionnaire. Blood pressure was measured from the right arm. The measurement was taken three times with 1–2-minute intervals. The automatic digital Omron M4 oscillometric manometer (Omron Matsusaka Co, Japan, Omron Healthcare Europe B.V., Hoofddorp, the Netherlands) was used. The average of the three measurements was used in the analysis.

Glucose tolerance

Subjects using insulin were considered to have T2DM and they did not participate in OGTT. Subjects with oral hypoglycemic medication or a previous T2DM diagnosis were considered to have T2DM, regardless of their OGTT results. The ADA criteria for DM [2] were used in the classification of subjects with no previously diagnosed diabetes as follows: 1) subjects with fasting venous plasma glucose of ≥7.0 mmol/l or 2-h venous plasma glucose of ≥11.1 mmol/l in an OGTT were considered to have T2DM; 2) subjects with fasting venous plasma glucose of <7.0 mmol/l and 2-h venous plasma glucose of 7.8–11.0 mmol/l in an OGTT were considered to have IGT; 3) subjects with fasting venous plasma glucose of 5.6–6.9 mmol/l and 2-h venous plasma glucose of <7.8 mmol/l in an OGTT were considered to have IFG; and, finally, 4) subjects with fasting venous plasma glucose of <5.6 mmol/l and 2-h venous plasma glucose of <7.8 mmol/l in an OGTT were considered to have NGT.

In this classification, subjects with isolated IGT (normal fasting glucose) and those with both IFG and IGT are categorized into the same group (IGT group). IFG and IGT are considered as distinct entities and we would have preferred to report the results for isolated IFG and isolated IGT separately. However, the number of subjects with isolated IGT was very small. Therefore, we present the results with four glucose tolerance categories, as described above.

Laboratory tests

Venous blood samples were drawn from the antecubital vein after an overnight fast. HDL cholesterol, total cholesterol and triglyceride concentrations were determined enzymatically (Roche Diagnostics, GmbH, Mannheim, Germany for HDL; Olympus System Reagent, Hamburg, Germany for total cholesterol and triglycerides) with a clinical chemistry analyser (Olympus, AU400, Hamburg, Germany). High-sensitivity C-reactive protein (hs-CRP) concentrations were determined using a chemiluminescent immunometric assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA). LDL cholesterol was calculated with the Friedewald formula. In the OGTT, subjects were given 75 g of glucose in a 10% solution. Venous blood samples for glucose determination were taken before and 2 h after the glucose load. Plasma glucose was determined by the glucose dehydrokinase method (Diagnostica Merck, Germany) in a clinical chemistry analyzer (Konelab, Finland).

Carotid artery studies

High-resolution B-mode carotid ultrasound examination of the right carotid artery was performed according to a standardized protocol using 7.5 MHz or 10 MHz linear array transducer. Intima-media thickness was measured in the distal common carotid artery (CCA) and the carotid bulb with commercially available software (PROWIN 23.1). The mean of these two measurements was used as a measure of CIMT. This protocol has been described in detail earlier [20].

We calculated three carotid artery elasticity parameters to be used as markers for CAS. For the elasticity calculations, the computer software PROWIN 23.1 was used to determine the arterial

diameter over the distal 1 cm length of the CCA from peak systole and end-diastole images. The beginning of the carotid artery bulb (the site where the two parallel walls of the CCA diverge) was used as an anatomical landmark. Systolic and diastolic arterial diameters were calculated as the mean of three average systolic and diastolic arterial diameters, respectively. The following indices of arterial elasticity were calculated:

YEM (mmHg) = [Ep x DAD/(2 x IMT)]
SI = Ln (SBB/DBP)/(ADC/DAD)
CAC (%/10 mmHg) = 100 x 10 x [(ADC/DAD)/PP]

where YEM = Young's elastic modulus; Ep = Peterson's elastic modulus = (PP x DAD)/ADC; PP = pulse pressure = systolic blood pressure - diastolic blood pressure; ADC (arterial diameter change) = systolic arterial diameter (SAD) - diastolic arterial diameter (DAD); IMT = intima-media thickness of the far wall of the CCA at end-diastole; SI = beta stiffness index; SBP = systolic blood pressure; DBP = diastolic blood pressure; and CAC = carotid artery compliance.

We used several elasticity indices, because they measure different aspects of arterial elasticity. CAC reflects the ability of the carotid artery to expand as a response to PP, caused by cardiac contraction and relaxation [21]. SI is a marker of arterial elasticity that is considered to be relatively independent of blood pressure [22]. YEM, on the other hand, is a measure of arterial wall stiffness that is independent of IMT [11].

Statistical methods

Statistical analyses were performed using SPSS for Windows (version 16.0.1; SPSS Inc., Chicago, IL, USA). Interactions were tested using the SPSS general linear model. The skewed distributions of triglycerides and hs-CRP were corrected logarithmically before statistical analyses. Analysis of variance (ANOVA) was used in testing differences between unadjusted group means with Dunnett's T3- correction for multiple comparisons. Chi-square test (χ^2) was employed in the assessment of the differences in prevalence rates. Adjustment for age and other cardiovascular risk factors was performed using the SPSS general linear model (analysis of covariance = ANCOVA). In the case of a significant ANCOVA p value for glucose tolerance, Fisher's least significant difference test was used to evaluate differences between the NGT group and the other groups.

Results

The interaction between sex and glucose tolerance, in determining CIMT and carotid artery elasticity, was tested. A significant interaction ($p < 0.05$) was observed in relation to CIMT and CAC and the results are reported separately for men and women.

The clinical characteristics and the unadjusted means for the markers of carotid atherosclerosis according to glucose tolerance status are presented in Tables 1 and 2 for men and women, respectively. Of the 125 (66 men and 59 women) diabetic subjects, 51 (28 men and 23 women) had previously diagnosed T2DM. Of these 51, 11 (6 men and 5 women) subjects used insulin and 31 (20 men and 11 women) were on oral hypoglycaemic medication. There was an overall tendency for both sexes towards a deteriorating trend in various CVD risk factors and in the markers of carotid atherosclerosis with the worsening of glucose tolerance.

Age-adjusted means in the markers of early atherosclerosis according to glucose tolerance status are shown in Table 3. In 56 men and 75 women carotid artery elasticity indices could not be determined. Therefore, the amount of

subjects in the analysis was smaller in regard to these indices as compared to CIMT. Significant differences between the groups were seen in all variables and in both sexes (ANCOVA $p<0.05$ for all). In men, CIMT was significantly increased only in the T2DM group as compared to the NGT group. In women, however, mean CIMT increased with the worsening of glucose tolerance, and all the other groups had significantly higher CIMT than the NGT group. Mean CAC was significantly lower in all glucose intolerance groups and mean YEM significantly higher in the IGT and T2DM groups when compared to the NGT group in both sexes. In women, the T2DM and IGT groups had significantly higher mean SI than the NGT group, while in men only the T2DM group differed significantly from the NGT group in regard to SI.

The results of the second ANCOVA model with adjustments for age and other CVD risk factors are presented in Table 4. Some subjects had missing values for some of the adjusting factors. Therefore, slightly smaller number of cases was included in this model than in the

TABLE 1. *Clinical characteristics of the male study subjects*

	Glucose tolerance				
	NGT	IFG	IGT	T2DM	ANOVA
	n = 152-174[a]	n = 215-235[a]	n = 97-104[a]	n =58-66[a]	p-value
Age (years)	57.8±8.1	56.7±7.0	59.5±8.2	61.3±7.8*	<0.001
Smoking (%)	30.5	29.4	20.2	24.2	0.230
SBP (mmHg)	135.8±19.2	140.4±18.5	146.2±21.9§	150.8±23.2¶	<0.001
DBP (mmHg)	84.8±10.0	88.3±9.4§	88.1±12.0	88.6±13.5	0.004
BMI (kg/m^2)	26.0±3.0	27.5±3.9¶	28.4±3.9¶	30.1±4.5¶	<0.001
Total cholesterol (mmol/l)	5.5±0.9	5.6±0.9	5.7±1.0	5.3±1.1	0.075
LDL cholesterol (mmol/l)	3.5±0.8	3.5±0.9	3.5±0.9	3.0±1.0*	0.001
HDL cholesterol (mmol/l)	1.5±0.4	1.4±0.4	1.4±0.4	1.3±0.4§	0.001
Triglycerides (mmol/l)	1.2±0.6	1.4±0.6	1.6±0.9§	2.3±1.8¶	<0.001
Fasting glucose (mmol/l)	5.2±0.2	6.0±0.3¶	6.0±0.4¶	8.3±2.2¶	<0.001
2 h glucose (mmol/l)	5.3±1.2	5.7±1.1*	8.9±0.9¶	14.3±3.8¶	<0.001
Hs-CRP	2.5±2.9	2.4±2.9	3.4±5.9	4.2±5.9§	0.001
Antihypertensive med (%)	21.8	20.4	34.6	62.1	<0.001
Statins (%)	12.1	9.4	15.4	25.8	0.005
Previous CVD (%)	11.5	9.8	16.3	27.3	0.002
CIMT (mm)	0.950±0.197	0.934±0.209	0.989±0.261	1.127±0.351§	<0.001
YEM (mmHg)	6591±4146	7008±3951	8575±8681	9402±6207*	0.001
SI	3.59±0.49	3.63±0.43	3.69±0.51	3.84±0.52*	0.005
CAC (%/10 mmHg)	0.98±0.45	0.90±0.40	0.83±0.41	0.71±0.37¶	<0.001

Values are unadjusted means ± SD except the prevalence rates which are expressed as %.

[a] = Variation in n is caused by some missing data. NGT= normal glucose tolerance, IFG= impaired fasting glucose, IGT= impaired glucose tolerance, T2DM= type 2 diabetes, SBP= systolic blood pressure, DBP= diastolic blood pressure, BMI= body mass index, 2 h glucose= plasma glucose 2 h after glucose load in oral glucose tolerance test, hs-CRP= high sensitivity C-reactive protein, antihypertensive med= % of subjects using antihypertensive medication, statins= % of subjects using statins, previous CVD= % of subjects with previous knowledge of coronary heart disease, stroke, or arterial stenosis, or thrombosis in a lower limb, YEM = Young's elastic modulus, SI = beta stiffness index, CAC = carotid artery compliance.

*$p<0.05$, §$p<0.01$, ¶$p<0.001$, pairwise comparison with the NGT group; Dunnett's T3 correction for multiple comparisons in ANOVA.

TABLE 2. *Clinical characteristics of the female study subjects*

	Glucose tolerance				
	NGT	IFG	IGT	T2DM	ANOVA
	n = 336-371[a]	n =160-176[a]	n =105-119[a]	n =49-59[a]	p-value
Age (years)	56.5±7.6	58.7±8.3*	60.4±8.4¶	63.8±7.6¶	<0.001
Smoking (%)	20.8	19.9	15.1	13.6	0.373
SBP (mmHg)	129.0±20.8	138.1±19.7¶	146.6±21.5¶	155.6±22.2¶	<0.001
DBP (mmHg)	79.7±9.8	83.3±8.4¶	85.7±9.4¶	85.6±8.2¶	<0.001
BMI (kg/m^2)	25.6±4.3	27.4±4.7¶	28.9±4.9¶	30.6±4.5¶	<0.001
Total cholesterol (mmol/l)	5.6±0.9	5.8±0.9	5.8±0.9	5.5±1.0	0.053
LDL cholesterol (mmol/l)	3.3±0.8	3.5±0.9	3.5±0.8	3.3±0.9	0.078
HDL cholesterol (mmol/l)	1.8±0.4	1.7±0.4	1.6±0.4¶	1.4±0.3¶	<0.001
Triglycerides (mmol/l)	1.1±0.5	1.3±0.6¶	1.5±0.7¶	1.8±0.7¶	<0.001
Fasting glucose (mmol/l)	5.1±0.3	5.9±0.3¶	5.7±0.5¶	7.3±2.2¶	<0.001
2 h glucose (mmol/l)	5.6±1.1	6.1±1.0¶	9.0±0.8¶	14.1±4.0¶	<0.001
Hs-CRP	2.4±4.2	2.3±2.4	5.0±6.8¶	4.5±4.5¶	<0.001
Antihypertensive med (%)	17.5	29.5	40.3	69.5	<0.001
Statins (%)	6.7	9.7	13.4	23.7	<0.001
Previous CVD (%)	5.4	11.9	12.6	15.3	0.006
CIMT (mm)	0.852±0.175	0.923±0.226§	0.957±0.227¶	0.998±0.255¶	<0.001
YEM (mmHg)	5824±3757	6487±3772	7577±4144§	10424±7142¶	<0.001
SI	3.56±0.46	3.65±0.44	3.75±0.47§	3.92±0.47¶	<0.001
CAC (%/10 mmHg)	1.10±0.58	0.89±0.39¶	0.78±0.43¶	0.61±0.32¶	<0.001

Values are unadjusted means ± SD except the prevalence rates which are expressed as %.

[a] = Variation in n is caused by some missing data. NGT= normal glucose tolerance, IFG= impaired fasting glucose, IGT= impaired glucose tolerance, T2DM= type 2 diabetes, SBP= systolic blood pressure, DBP= diastolic blood pressure, BMI= body mass index, 2 h glucose= plasma glucose 2 h after glucose load in oral glucose tolerance test, hs-CRP= high sensitivity C-reactive protein, antihypertensive med= % of subjects using antihypertensive medication, statins= % of subjects using statins, previous CVD= % of subjects with previous knowledge of coronary heart disease, stroke, or arterial stenosis, or thrombosis in a lower limb, YEM = Young's elastic modulus, SI = beta stiffness index, CAC = carotid artery compliance.

*p <0.05, §p < 0.01, ¶p <0.001, pairwise comparison with the NGT group; Dunnett's T3 correction for multiple comparisons in ANOVA.

age-adjusted model. Among men, none of the parameters differed significantly between the groups (ANCOVA p>0.1 for all) after these further adjustments. In women, adjusted means in CIMT did not significantly differ between the groups in this model (p=0.341) but in the elasticity indices significant differences were observed (ANCOVA p<0.05 for all). In women, the T2DM group had significantly higher YEM and SI when compared to the NGT group (p<0.05 for all). The T2DM and IFG groups had significantly lower CAC than the NGT group. In both ANCOVA models the change across the groups in women appeared to be larger in the elasticity indices than in men.

When the second ANCOVA model was further adjusted with subjects' status in regard to antihypertensive and statin medications as well previous CVD (previous knowledge of CHD, stroke, or arterial stenosis or thrombosis in a lower limb) the main findings remained essentially similar (data not shown). The only

TABLE 3. *Carotid artery intima-media thickness (CIMT) and elasticity indices according to glucose tolerance status in men and women, adjusted for age.*

	Glucose tolerance				
	NGT	IFG	IGT	T2DM	ANCOVA
Men	n =152-174[a]	n =215-235[a]	n =97-104[a]	n =59-66[a]	p-value
CIMT (mm)	0.953±0.016	0.953±0.014	0.969±0.021	1.083±0.026¶	<0.001
YEM (mmHg)	6589±437	7191±371	8397±549*	9032±709§	0.007
SI	3.59±0.04	3.65±0.03	3.66±0.05	3.79±0.06§	0.045
CAC (%/10 mmHg)	0.98±0.03	0.88±0.03*	0.86±0.04*	0.76±0.05¶	0.002
Women	n=336-371[a]	n=160-176[a]	n=105-119[a]	n=49-59[a]	
CIMT (mm)	0.875±0.009	0.918±0.014§	0.930±0.017§	0.928±0.024*	0.005
YEM (mmHg)	6043±223	6433±320	7257±398§	9782±586¶	<0.001
SI	3.59±0.02	3.64±0.03	3.70±0.04*	3.83±0.06¶	0.001
CAC (%/10 mmHg)	1.06±0.03	0.90±0.04¶	0.84±0.05¶	0.73±0.07¶	<0.001

Values are age-adjusted means ± SE. [a] = variation in n is caused by missing elasticity data for some subjects. NGT= normal glucose tolerance, IFG= impaired fasting glucose, IGT= impaired glucose tolerance, T2DM= type 2 diabetes, YEM = Young's elastic modulus, SI = beta stiffness index, CAC = carotid artery compliance.

*p <0.05, §p < 0.01, ¶p <0.001, pair wise comparison with the NGT group; Fisher's least significant difference test was used if a significant ANCOVA p value for glucose tolerance was observed.

exception was that in regard to SI, the ANCOVA p value was borderline (p=0.067 vs. p=0.038 in the previous model) significant in women.

Discussion

In the present study, we have shown that the extent of early carotid atherosclerosis differs in men and women according to glucose tolerance status. Our findings suggest that impairment in glucose metabolism might have stronger association with early atherosclerosis in women than in men.

A deteriorating trend in CIMT [19,23,24] and CAS [12] according to the worsening of glucose tolerance has been reported previously. We observed this kind of trend in both sexes after the adjustment for age. It has been shown that subjects with T2DM [9,10,25] and, to a lesser extent, those with IGT [13] have significantly increased early carotid atherosclerosis in comparison to those with normal glucose metabolism. In regard to T2DM our results were comparable in both sexes after the adjustment for age. With reference to IGT, however, some sex-related differences were seen. In women IGT was associated with significant deterioration in all the parameters in comparison to NGT, while in men this was seen only in YEM and CAC. In the present study even IFG was related to decreased CAC in both sexes and to increased CIMT in women after age had been taken into account. In a study by van Popele et al., increased CAS was reported in subjects with IFG in comparison to those with NGT [14]. However, most of the previous studies have not confirmed this finding regarding IFG and early carotid atherosclerosis [15,16].

After further adjustment for other CVD risk factors, the associations of impaired glucose metabolism and early carotid atherosclerosis weakened substantially in both sexes. Statistically

TABLE 4. *Carotid artery intima-media thickness (CIMT) and elasticity indices according to glucose tolerance status in men and women, adjusted for age and other cardiovascular risk factors*

	Glucose tolerance				
	NGT	IFG	IGT	T2DM	ANCOVA
Men	n =149-171[a]	n =213-233[a]	n =94-100[a]	n =51-57[a]	p-value
CIMT (mm)	0.972±0.017	0.965±0.014	0.978±0.022	1.046±0.031	0.101
YEM (mmHg)	6969±457	7062±381	7969±567	8222±783	0.317
SI	3.59±0.04	3.64±0.03	3.64±0.05	3.72±0.07	0.461
CAC (%/10 mmHg)	0.95±0.03	0.89±0.03	0.90±0.04	0.88±0.06	0.587
Women	n=335-369[a]	n=157-173[a]	n=103-117[a]	n=48-58[a]	
CIMT (mm)	0.899±0.011	0.927±0.014	0.923±0.018	0.905±0.026	0.341
YEM (mmHg)	6260±247	6172±321	6448±404	9042±593¶	<0.001
SI	3.59±0.03	3.62±0.04	3.66±0.05	3.80±0.07§	0.038
CAC (%/10 mmHg)	1.03±0.03	0.93±0.04*	0.94±0.05	0.84±0.07*	0.031

Values are adjusted means ± SE. Adjustments for age, smoking, LDL cholesterol, BMI, SBP, triglycerides, and HDL cholesterol. [a] = variation in n is caused by missing elasticity data in some subjects. There were also few missing covariate values. BMI= body mass index, SBP= systolic blood pressure, NGT= normal glucose tolerance, IFG= impaired fasting glucose, IGT= impaired glucose tolerance, T2DM= type 2 diabetes, YEM = Young's elastic modulus, SI = beta stiffness index, CAC = carotid artery compliance.

*p <0.05, §p < 0.01, ¶p <0.001, pair wise comparison with the NGT group; Fisher's least significant difference test was used if a significant ANCOVA p value for glucose tolerance was observed.

significant differences between the groups were seen only in women after these adjustments. In women, a trend of worsening carotid artery elasticity across the glucose tolerance categories (with significant ANCOVA p values) was seen even in this model. In the T2DM group all the elasticity indices were significantly worse compared to the NGT group and even the IFG group had significantly lower CAC than the NGT group. The weakening of the observed associations after adjusting for CVD risk factors leads to the conclusion that these associations are partly mediated by the overall risk factor profile. There was indeed a gradual deterioration especially in the metabolic risk factors across the glucose tolerance groups (Tables 1 and 2).

The amount of studies reporting the results separately for men and women has been limited concerning the association of glucose tolerance and early carotid atherosclerosis. In a study by Kawamoto et al., T2DM had a stronger association with increased CIMT in women than in men [18]. On the other hand, other studies have not confirmed this observation [10,26,27]. In these studies, however, only subjects with NGT or T2DM were included. Two studies evaluated the association between glucose tolerance and CIMT in subjects with NGT, IGT or T2DM and no significant sex-related differences were found [9,19]. In a study by Salomaa et al. fasting blood glucose had a stronger association with carotid artery elasticity in women than in men [11]. On the other hand, no sex-related differences were observed in two other studies investigating the association of glucose tolerance and stiffness of the carotid arteries [12,14]. These studies by van Popele et al. [14] and Henry et al. [12] included subjects with NGT, IFG or T2DM and NGT, impaired glucose metabolism (i.e. IFG or IGT) or T2DM, respectively. All in all, previous results

regarding sex-related differences are limited and somewhat controversial. Our results support the hypothesis that there might be a stronger association between glucose intolerance and early carotid atherosclerosis in women than in men, particularly in relation to carotid artery elasticity.

Although it is beyond the scope of this investigation to evaluate the exact mechanisms behind the observed sex-related differences, we may speculate some possible explanations. Menopause and oestrogenic status are possible and commonly suggested mediators for sex-related differences in cardiovascular risk. Women in the present study were mostly postmenopausal and it is reasonable to speculate that it might have increased their vulnerability to such risk factors as glucose intolerance and T2DM. It has also been shown that the deteriorating tendency in other cardiovascular risk factors such as central obesity, adverse lipid profile, and hypertension is stronger in diabetic women than in their male counterparts [28]. These risk factors are associated with insulin resistance, which has also been associated with early atherosclerotic changes [29]. It has been suggested that insulin resistance is the key element in reversing the usually favourable female risk profile in diabetic women [28]. These previous reports are in agreement with our observation that the association between glucose tolerance and early carotid atherosclerosis was markedly weakened after the adjustments for metabolic and other CVD risk factors, although this phenomenon was seen in both sexes.

There are some limitations to the present study. Our study was cross-sectional, and the causality of the associations cannot be assessed. Our population consisted of middle-aged and elderly Caucasians, and the results may not apply to other ethnicities. On the other hand, as the female subjects were primarily post menopausal in the present study, the oestrogen status is not a major confounding factor when possible sex differences are analysed. Although the size of the overall population was quite large, the subgroups of different stages of glucose intolerance were substantially smaller. The results were, however, logical and consistent across the glucose tolerance groups.

In conclusion, our results suggest that women with glucose intolerance may be at greater risk for early atherosclerotic changes than men. There is a trend towards increasing carotid atherosclerosis with the worsening of glucose tolerance in both sexes. Overall, a deteriorated CVD risk factor profile in subjects with impaired glucose metabolism plays an important role in mediating the observed associations.

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