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METABOLIC SYNDROME IN YOUNG ADULTS

PREVALENCE, CHILDHOOD PREDICTORS AND ASSOCIATION WITH SUBCLINICAL ATHEROSCLEROSIS

The Cardiovascular Risk in Young Finns Study

by

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To Sebastian, Carlos and Vincent

ABSTRACT

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Metabolic syndrome in young adults – prevalence, childhood predictors and association with subclinical atherosclerosis. Departments of Medicine and Clinical Physiology, Turku University Hospital, Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku; and the National Graduate School of Clinical Investigation (CLIGS). Annales Universitatis Turkuensis, Medica-Odontologia, Turku, Finland, 2012.

Background: Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors including central obesity, insulin resistance, impaired glucose tolerance, hypertension and dyslipidemia. The prevalence of MetS is increasing worldwide in all age groups. MetS is associated with increased risk of cardiovascular disease and type 2 diabetes mellitus.

Aims: The aim of the present study was to investigate the prevalence, secular trends and childhood predictors of MetS in young adults. Furthermore, the relations between MetS and subclinical atherosclerosis were studied and whether apolipoproteins (apo) B and A-I, C-reactive protein (CRP) and type II secretory phospholipase A2 (sPLA2) were associated with MetS, and to what extent the atherogenicity of MetS was explained by these factors.

Participants and Methods: The present thesis is part of the large scale population-based, prospective study, the Cardiovascular Risk in Young Finns Study. The first cross-sectional study was conducted in 1980 and included 3,596 participants aged 3-18 years. Carotid and brachial ultrasound studies were performed for 2,283 of these participants in 2001 and 2,200 of these participants in 2007.

Results: The overall prevalence of MetS in young adults aged 24-39 years in 2001 was 10-15 % and 6 years later in 30-45 year-old adults it was 15-23 % depending on the MetS definition used. Between the years 1986 and 2001, MetS prevalence increased from 1.0 % to 7.5 % ($p<0.0001$) in 24-year-old participants that was mostly driven by the increased central obesity. Participants with MetS had increased carotid intima-media thickness (cIMT) and decreased carotid elasticity compared to those without the syndrome. Impaired brachial flow-mediated dilatation (FMD) was not related to MetS but it modified the relationship between MetS and cIMT (P for interaction 0.023). High levels of apoB, CRP, sPLA2 and low levels of apoA-I associated with MetS in young adults. In prospective analysis both MetS and high apoB predicted ($P<0.0001$) incident high cIMT, defined as cIMT>90th percentile and/or plaque. The association between MetS and incident high cIMT was attenuated by ~40 % after adjustment with apoB.

Conclusions: MetS is common in young adults and increases with age. Screening for risk factors, especially obesity, at an early life stage could help identify children and adolescents at increased risk of developing MetS and cardiovascular disease later in life. MetS identifies a population of young adults with evidence of increased subclinical atherosclerosis. Impaired brachial endothelial response is not a hallmark of MetS in young adults, but the status of endothelial function modifies the association between metabolic risk factors and atherosclerosis. In addition, the atherogenicity of MetS in this population assessed by incident high cIMT appears to be substantially mediated by elevated apoB.

Key words: apolipoproteins, atherosclerosis, inflammatory markers, metabolic syndrome, obesity

TIIVISTELMÄ

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Metabolinen oireyhtymä nuorilla aikuisilla – esiintyvyys, lapsuusiän ennustekijät ja yhteys varhaisiin valtimomuutoksiin. Departments of Medicine and Clinical Physiology, Turku University Hospital, Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku; and the National Graduate School of Clinical Investigation (CLIGS). Annales Universitatis Turkuensis, Medica-Odontologia, Turku, Finland.

Tausta: Metabolinen oireyhtymä (MBO) on sydän- ja verisuonitautien riskitekijäkasauma, johon kuuluvat olennaisena osana keskivartalolihavuus, insuliiniresistenssi, kohonnut verenpaine, sekä sokeri- ja rasva-aineenvaihdunnan häiriöt. MBO:n esiintyvyys kasvaa maailmanlaajuisesti kaikissa ikäryhmissä. MBO on yhteydessä lisääntyneeseen sydän- ja verisuonitautien riskiin ja tyypin 2 diabetekseen.

Tavoite: Tarkoituksena oli selvittää MBO:n esiintyvyyttä, metabolisten riskitekijöiden muutoksia ja MBO:ta ennustavia lapsuudenaikaisia riskitekijöitä suomalaisilla nuorilla aikuisilla. Lisäksi tutkittiin MBO:n yhteyttä ultraäänellä mitattuihin varhaisiin valtimomuutoksiin (kaulavaltimon seinämäpaksuuteen, joustavuuteen ja olkavaltimon sisäkalvon toimintaan) ja ovatko apoB, apoA-I, CRP ja sPLA2 yhteydessä MBO:n kehittymiselle ja kuinka paljon ne selittävät MBO:n yhteyttä valtimonkovettumatautiin.

Menetelmät: Tämä väitöskirjatutkimus on osa laajaa väestöpohjaista, pitkittäistä Lasten Sepelvaltimotaudin Riskitekijät –tutkimusta. Ensimmäinen poikkileikkaustutkimus toteutettiin vuonna 1980 ja siihen osallistui 3596 lasta ja nuorta (iältään 3-18 vuotiaita). Kaulavaltimon ja olkavaltimon ultraäänitutkimukset toteutettiin 2283 tutkittavalle vuonna 2001 ja 2200 tutkittavalle vuonna 2007.

Tulokset: MBO:n esiintyvyys nuorilla aikuisilla (24-39 vuotiailla) vuonna 2001 oli 10-15 % ja kuusi vuotta myöhemmin 15-23 % riippuen käytetystä MBO:n määritelmästä. Vuosien 1986 ja 2001 välisenä aikana MBO:n esiintyvyys kasvoi merkittävästi 1.0 %:sta 7.5 %:iin ($P<0.0001$) 24-vuotiailla pääosin lisääntyneen lihavuuden takia. MBO oli myös yhteydessä suurempaan kaulavaltimon paksuuteen ja kaulavaltimon alentuneeseen joustavuuteen. Vaikka alentunut olkavaltimon sisäkalvon toiminta ei ollut yhteydessä MBO:ään, sillä oli vaikutusta MBO:n ja kaulavaltimon paksuuden väliseen yhteyteen. Korkeat apoB, CRP, sPLA2 ja matalat apoA-I tasot olivat yhteydessä MBO:ään. Kuuden vuoden seurannassa sekä MBO että apoB ennustivat korkeaa kaulavaltimon paksuutta. MBO:n ja korkean kaulavaltimon paksuuden yhteys laimeni 40 %:lla vakioitaessa apoB:llä.

Johtopäätökset: MBO on yleinen nuorilla aikuisilla ja kasvaa voimakkaasti iän mukana. Havaitsemalla MBO:ta ennustavat riskitekijät ja puuttumalla niihin varhain, on mahdollista ehkäistä MBO:n ja sydän- ja verisuonitautien kehittymistä myöhemmin elämässä. MBO on suoraan yhteydessä varhaisiin valtimomuutoksiin (suurempaan kaulavaltimon paksuuteen ja kaulavaltimon alentuneeseen joustavuuteen). Olkavaltimon sisäkalvon toiminta ei ollut yhteydessä MBO:ään nuorilla aikuisilla, mutta hyvin toimiva sisäkalvo voi estää valtimomuutosten kehittymistä henkilöillä, joilla on MBO. Lisäksi apoB saattaa säädellä MBO:ään liittyvää valtimotaudin riskiä.

Avainsanat: apolipoproteiinit, lihavuus, metabolinen oireyhtymä, tulehdusmerkkiaineet, valtimonkovettumatauti

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ABBREVIATIONS

apoA-I	apolipoprotein A-I
apoB	apolipoprotein B-100
BMI	body mass index
CAC	carotid artery compliance
CAD	coronary artery disease
cIMT	carotid intima-media thickness
CL	confidence limit
CV	coefficient of variation
CVD	cardiovascular disease
CRP	C-reactive protein
EGIR	European Group for the Insulin Resistance
FFA	free fatty acid
FMD	flow-mediated dilatation
HDL	high-density lipoprotein
HOMA	homeostasis assessment index
IDF	International Diabetes Federation
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IL	interleukin
LDL	low-density lipoprotein
MetS	metabolic syndrome
NAFLD	non-alcoholic fatty liver disease
NCEP	National Cholesterol Education Panel
NHANES	National Health and Nutrition Examination Survey
OD	odds ratio
PAI-1	plasminogen activator inhibitor-1
PPAR-γ	peroxisome proliferator-activated receptor γ
SAS	Statistical analysis system
SD	standard deviation
sPLA2	secretory phospholipase A2
TNF-α	tumor necrosis factor-alpha
VLDL	very low density lipoprotein
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by roman numerals I-IV. In addition, previously unpublished data is presented.

I Mattsson N, Rönnemaa T, Juonala M, Viikari JSA, Raitakari OT. The prevalence of the metabolic syndrome in young adults. The Cardiovascular Risk in Young Finns Study**.** *J Intern Med*. 2007;261:159-169.

II Mattsson N, Rönnemaa T, Juonala M, Viikari JSA, Raitakari OT. Childhood predictors of the metabolic syndrome in adulthood. The Cardiovascular Risk in Young Finns Study. *Ann Med.* 2008;40:542-552.

III Mattsson N, Rönnemaa T, Juonala M, Viikari JSA, Jokinen E, Hutki-Kähönen N, Kähönen M, Laitinen T, Raitakari OT. Arterial structure and function in young adults with the metabolic syndrome: the Cardiovascular Risk in Young Finns Study. *Eur Heart J.* 2008;29:784-791.

IV Mattsson N, Magnussen CG, Rönnemaa T, Mallat Z, Benessiano J, Jula A, Taittonen L, Kähönen M, Juonala M, Viikari JSA, Raitakari OT. Metabolic Syndrome and Carotid Intima-Media Thickness in Young Adults: Roles of Apolipoprotein B, Apolipoprotein A-I, C-Reactive Protein, and Secretory Phospholipase A2: The Cardiovascular Risk in Young Finns Study. *ATVB.* 2010; 30:1861-1866.

1. INTRODUCTION

Lifestyle and behavioural changes that have occurred during the last century, such as an increasing obesity, sedentary lifestyle and excessively energy-rich nutrition, have contributed to a dramatic increase in the prevalence of metabolic syndrome (MetS) and type 2 diabetes (Zimmet *et al.* 2001). As obesity is becoming more common, the impact of MetS on public health will also increase. For all age groups, the global prevalence of type 2 diabetes was estimated to be 177 million (2.8 %) in 2000, and is expected to double to 366 million (4.4 %) by 2030 (Wild *et al.* 2004). There is evidence that the risk of developing type 2 diabetes or cardiovascular disease (CVD) begins long before the signs of clinical disease (Berenson *et al.* 1998). Therefore, various tools have been developed for the identification of healthy people at elevated risk of future clinical outcomes.

MetS is a multiplex risk factor for CVD consisting of independent, common risk factors: central obesity, insulin resistance, glucose intolerance, hypertension and dyslipidemia, that all increase the likelihood of vascular disease (Reaven 1988;Alberti and Zimmet 1998). The presence of MetS also increases the risk of death from all causes as well as from CVD (Lakka *et al.* 2002;Hu *et al.* 2004). The clustering of metabolic risk factors begins already in childhood (Smoak *et al.* 1987;Bao *et al.* 1994;Raitakari *et al.* 1995a;Chen *et al.* 2000) and these multiple risk factors tend to persist from childhood into adulthood (Ford *et al.* 2004). The pathophysiological mechanisms underlying MetS are not fully known (Eckel *et al.* 2005) but the major underlying cause has been considered to be either insulin resistance or visceral obesity. Moreover, genetic and environmental factors such as lack of physical activity and consumption of high-caloric food are involved. Of these risk factors, some can be modified whereas others, such as genetic predisposition, cannot.

The worldwide epidemic of obesity is related to the increase in the prevalence of MetS, which is currently highly prevalent in the general population. In most countries 20-30 % of the adult population can be characterized as having MetS (Grundy 2008). Different expert panels have provided various definitions for MetS to enable a clinical diagnosis and treatment of patients at risk of CVD. However, MetS has proved to have only a limited practical utility as it is a pre-morbid condition rather than a clinical diagnosis (Simmons *et al.* 2010). During the past 5 years, there has been increasing controversy regarding the concept of MetS and especially its usefulness as a diagnostic tool. Recent evidence has shown that MetS does not predict CVD events or disease progression any better than the sum of its components (Wannamethee *et al.* 2005;Koskinen *et al.* 2009). However, MetS as concept of risk factor clustering can be observed both in childhood and in adulthood, and exposure to metabolic risk factors, especially those related to obesity is associated with increased risk of atherosclerosis which can be measured by increased carotid intima-media thickness (cIMT). A better understanding of the pathophysiology of MetS and identification of individuals with MetS early in the life course could be

important for initiating interventions such as lifestyle modification, which forms the basis for prevention and treatment of MetS and related comorbidities, to those that may benefit most.

The Cardiovascular Risk in Young Finns Study, an ongoing, multi-centre follow-up study was originally designed to assess biological and lifestyle factors underlying cardiovascular disease in children, adolescents and young adults. In the present thesis, the main objectives were to study 1) the prevalence and secular trends of MetS, 2) childhood predictors of MetS and 3) the relation of MetS to the early vascular changes in young adults.

2. REVIEW OF THE LITERATURE

Metabolic syndrome (MetS) is characterized by mild and varying degrees of abnormalities of insulin, glucose and lipid metabolism, hypertension and overweight (Reaven 1988;Balkau and Charles 1999). These conditions are interrelated and share underlying mediators, mechanisms and pathways (Grundy *et al.* 2005). This thesis focuses on MetS in young adults. Special emphasis is put on prevalence, secular trends and childhood predictors of MetS. In addition, this study concentrates on the relations between MetS and subclinical atherosclerosis and whether apolipoprotein B (apoB), A-I (apoA-I), C-reactive protein (CRP) and type II secretory phospholipase A2 (sPLA2) are associated with MetS, and to what extent the atherogenicity of MetS is explained by these factors. (Figure 1)

2.1 Evolution of the concept of metabolic syndrome

The clustering of metabolic risk factors has been recognized for more than 80 years. Kylin, a Swedish physician, described a clustering of hypertension, hyperglycemia and gout (Kylin 1923). MetS has been in the scientific spotlight since Reaven reintroduced the concept of Syndrome X in 1988 and proposed that insulin resistance is the primary underlying reason behind MetS (Reaven 1988), as it strongly associates with other risk factors (Ferrannini 2006) and correlates with cardiovascular risk (Gami *et al.* 2007). Obesity was not originally included in the definition (Reaven 1988). First of all, Syndrome X was a pathophysiological construct, attempting to explain why minor degrees of cardiometabolic abnormalities tend to cluster in the same individual (Reaven 2011b).

Despite the vast number of publications devoted to MetS, pathophysiological mechanisms underlying it are still uncertain. The pathogenesis is likely heterogenous with central obesity, sedentary lifestyle, unhealthy diet and largely unknown genetic factors acting together to produce it. Evidence now indicates that MetS generally begins due to excess visceral obesity (Cameron *et al.* 2008a) and thereafter insulin resistance explains most of the syndrome, as no other mechanisms have come close to justifying the individual components of their clustering (Eckel *et al.* 2010). However, several studies have emphasized that MetS is also a prothrombotic (Juhan-Vague *et al.* 1991;Abbasi *et al.* 1999) and a proinflammatory condition (Ridker *et al.* 2003). The terminology used to describe MetS has varied. In addition to metabolic syndrome and Syndrome X, the insulin resistance syndrome, the deadly quartet and the dysmetabolic syndrome have been used to describe the cluster of different cardiometabolic abnormalities. During the last decade, many highly recognized scientific organizations have developed their own definitions for MetS.

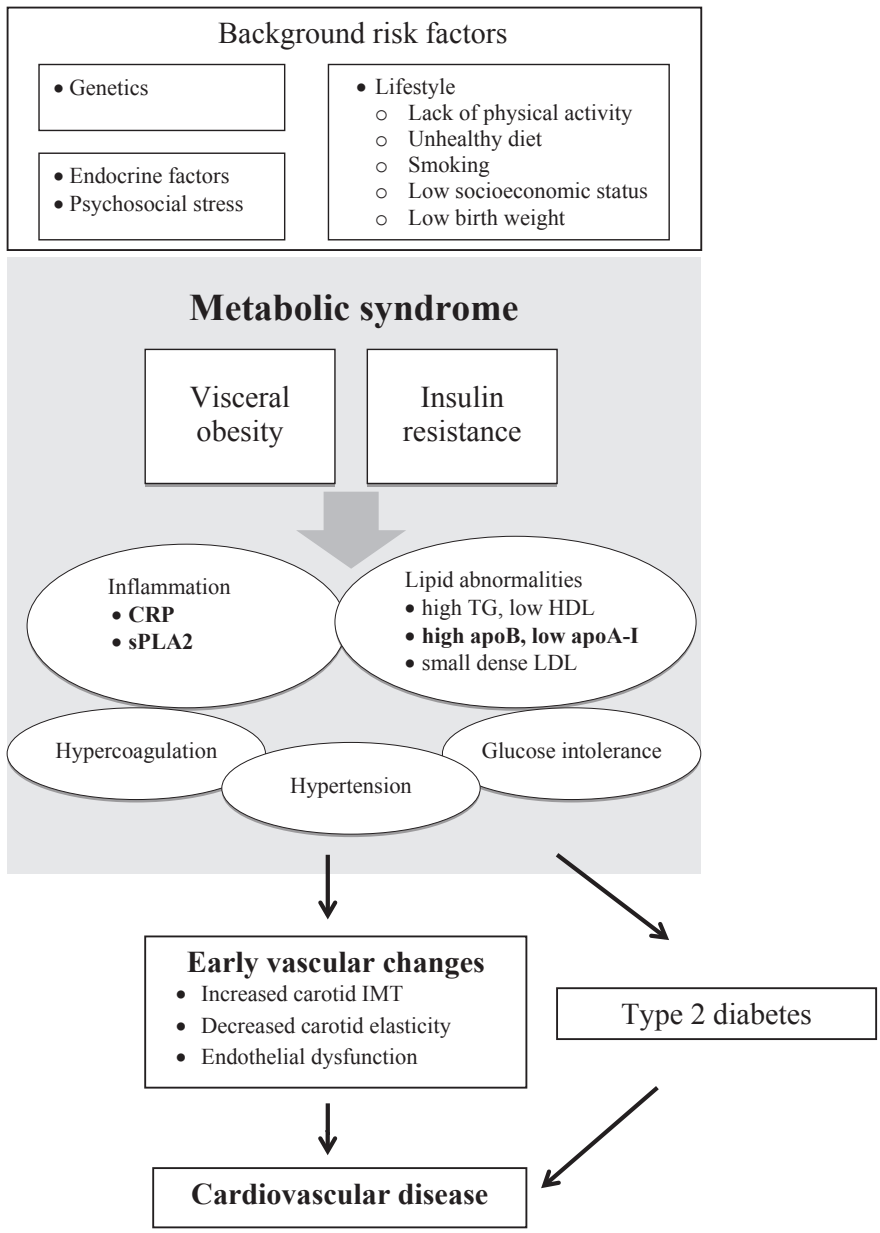

Figure 1. Risk factors, components and comorbidities of metabolic syndrome.

2.1.1 Definitions of metabolic syndrome

Table 1 summarizes four of the most commonly used definitions of MetS. The World Health Organization (WHO) was the first to specify the diagnostic criteria for MetS in 1998 (Alberti and Zimmet 1998). The WHO definition emphasized insulin resistance and therefore required the individual to be insulin resistant (indicated by euglycaemic clamp test) or having type 2 diabetes, or impaired glucose tolerance (indicated by oral glucose tolerance test). The practical difficulties in meeting the insulin criteria limited the use of the WHO definition. In addition to this absolute requirement of insulin resistance, two additional criteria have to be met. These included obesity (measured as elevated waist-hip ratio or body mass index (BMI)), dyslipidemia (high triglycerides or low high density lipoprotein (HDL) -cholesterol), hypertension and microalbuminuria. Inclusion of microalbuminuria as a core component has been criticized, mainly because it is quite uncommon in non-diabetic individuals.

In 1999, the European Group for the Study of Insulin Resistance (EGIR) proposed a modification of the WHO definition (Balkau and Charles 1999). The WHO criteria was modified for better practical approach and the EGIR proposed their own definition relying on fasting insulin instead of the euglycaemic clamp to measure insulin resistance. Therefore, participants with type 2 diabetes were excluded since fasting insulin may not be a useful measure of insulin resistance in such persons. The obesity criterion was simplified to waist circumference. Moreover, microalbuminuria was eliminated from the criteria of MetS.

In 2001, the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) introduced an alternative clinical definition for MetS. The purpose of the NCEP definition was to identify people at higher long-term risk for CVD who would need clinical lifestyle intervention to reduce risk (Grundy *et al.* 2005). Clustering of metabolic risk factors was recognized, but it did not draw conclusions on mechanistic pathogenesis. NCEP took a less glucose-centric approach by treating all MetS components with equal importance. The cut points for abdominal obesity were somewhat higher than in the EGIR definition, as they were derived from the definition used in the 1998 National Institutes of Health obesity clinical guidelines (1998), which identified approximately the highest quartile of the US population. An updated NCEP definition was proposed by the American Heart Association (AHA) and the National Heart Lung and Blood Institute (NHLBI) in 2005 (Grundy *et al.* 2005). This definition retained most of the NCEP ATP III criteria but suggested a lower threshold for elevated fasting glucose. The NCEP definition is one of the most widely used MetS definition because it is practical and easy to apply. However, it does not include the measurement of insulin and therefore it may fail to take insulin resistance into account.

In 2005, the International Diabetes Federation (IDF) proposed a new definition for MetS, which was quite similar to the NCEP definition except that central obesity was required (Alberti *et al.* 2005). The threshold of abdominal obesity differs according to ethnicity taking into account that different populations, ethnicities and nationalities have different distributions of obesity.

Table 1. Four commonly used MetS definitions.

	WHO **(Alberti and Zimmet 1998)**	**EGIR** **(Balkau and Charles 1999)**	**Updated NCEP** **(Grundy et al. 2005)**	**IDF** **(Alberti et al. 2005)**
	Glucose intolerance or type 2 diabetes and/or insulin resistance (glucose uptake below lowest quartile) and ≥ 2 of the following:	Hyperinsulinemia (highest quartile of fasting insulin in non-diabetic population) and ≥ 2 of the following:	≥ 3 of the following:	Central obesity (ethnicity specific) and ≥ 2 of the following:
Obesity	waist-hip ratio > 0.90 in men > 0.85 in women and/or BMI > 30 kg/m^2	waist ≥ 94 cm in men ≥ 80 cm in women	waist ≥ 102 cm in men ≥ 88 cm in women	waist ≥ 94 cm in men ≥ 80 cm in women
Blood pressure	≥ 140/90 mmHg	≥ 140/90 mmHg *	≥ 130/85 mmHg *	≥ 130/85 mmHg *
Triglycerides	≥ 1.7 mmol/L	≥ 2.0 mmol/L	≥ 1.7 mmol/L*	≥ 1.7 mmol/L*
HDL-cholesterol	< 0.9 mmol/L in men < 1.0 mmol/L in women	< 1.0 mmol/L	< 1.03 mmol/L in men* < 1.29 mmol/L in women*	< 1.03 mmol/L in men* < 1.29 mmol/L in women*
Fasting plasma glucose		≥ 6.1 mmol/L	≥ 5.6 mmol/L*	> 5.6 mmol/L or type 2 diabetes
Microalbuminuria	Urine albumin excretion rate ≥ 20 ug/min or albumin:creatinine ratio ≥ 30mg/g			

WHO = World Health Organization
EGIR = European Group for the Study of Insulin Resistance
NCEP = National Cholesterol Education Program
IDF = International Diabetes Federation
Waist is given using European cut-points
*Drug treatment is an alternative

During the last ten years, a substantial number of studies have been published comparing the prevalence and the ability of the different MetS criteria to predict different outcomes. The lack of a unifying definition of MetS has been of concern to many investigators. Therefore, NCEP and IDF, joined by several other organizations, introduced the most recent definition, a harmonized definition of MetS in 2009 (Alberti *et al.* 2009). A general consensus was that there should not be an obligatory component in the definition, but waist circumference, using population-specific values, continued to be a useful preliminary screening tool.

From the theoretical point of view, it would be important to have a definition that is easy to use, reliably predicts CVD risk, and can be used clinically to detect individuals at high risk of future CVD events. Irrespective of the many attempts to unify the definition of MetS, there is increasing evidence that although MetS may be useful as an educational concept, it has limited practical utility as a diagnostic or management tool (Simmons *et al.* 2010).

2.1.2 Pathophysiology of metabolic syndrome

The pathophysiology of MetS remains controversial. No single central underlying mechanism has been accepted, although Reaven (Reaven 1988) and later many other investigators (Ferrannini *et al.* 1991) have proposed that insulin resistance may be the primary underlying reason behind MetS because it strongly associates with other metabolic risk factors and correlates with CVD risk. MetS is also considered to be strongly related to central obesity, especially intra-abdominal adiposity (Lemieux *et al.* 2000;Carr *et al.* 2004). Possible underlying mechanisms include release of free fatty acids from adipocytes (Adiels *et al.* 2006), chronic activation of the immune system (Ridker *et al.* 2003), cellular processes such as endoplasmic reticulum stress and mitochondrial dysfunction leading to the formation of reactive oxygen species (Furukawa *et al.* 2004;Kim *et al.* 2008), disorders of the hypothalamic-pituitary-adrenal axis (Björntorp and Rosmond 2000), altered glucocorticoid hormone activation (Whorwood *et al.* 2002) and the contributions of cytokines, hormones and other molecules produced by adipocytes (Apridonidze *et al.* 2005). Moreover, prenatal and early-life influences (Fernandez-Twinn and Ozanne 2006), chronic psychosocial stress (Vitaliano *et al.* 2002) as well as gene combinations (Sjögren *et al.* 2008) may have a role in the development of MetS. Other associated conditions may be physical inactivity (Gustat *et al.* 2002), poor diet, and aging.

2.1.3 Prevalence of metabolic syndrome

A considerable amount of research has been conducted in attempt to define the epidemiology of MetS (Grundy 2008). The existence of several different definitions has led to confusion especially when comparing MetS prevalences. The prevalence of MetS varies widely among countries and ethnic groups, ranging from approximately 1 % to 40 % (Cameron *et al.* 2004). However, in all countries and ethnic groups, the prevalence of MetS increases rapidly with age (Cameron *et al.* 2004). Analysis of data from the National Health and Nutrition Examination Survey (NHANES) III on 8814 men and women aged 20 to 70 years showed that 24 % of the U.S. adults have MetS, with the prevalence rising from 6.7 % among 20-29 year old to 43.5 % among 60-69 year old subjects (Ford *et al.* 2002). Among U.S. adults the prevalence of MetS did not differ significantly between sexes (24.0 % in men and 23.7 % in women) (Ford *et al.* 2002). However, a majority of epidemiological studies have demonstrated that the prevalence of MetS is higher in men compared to women (Cameron *et al.* 2004). The highest prevalence of MetS among U.S.

ethnic groups was found in Hispanics (32 % of the population) compared to Whites (22 %) and African Americans (22 %) (Ford *et al.* 2002). Increase in the prevalence of MetS was observed from 24 % in NHANES III (1988-1994) to 27 % in NHANES carried out during 1999-2000 (Ford *et al.* 2004). Increase in the prevalence of obesity and aging of population seem to be responsible for the secular increase in the prevalence of MetS (Grundy 2008). Results from the San Antonio Heart and Framingham Offspring studies were consistent with the data from Ford and co-workers considering U.S. adults (Meigs *et al.* 2003).

Similar prevalences have been detected in European populations. Approximately one-fourth of the European adult population can be defined to have MetS (Grundy 2008). Consistent with the U.S. data, the prevalence of MetS varies in Europe by age group and characteristics of the population studied, geographic location and criteria used. Previously in Finland, prevalence of MetS in the FINRISK cohort (aged 45 to 64 years) was significantly higher in men than in women (39 % vs. 22 %) (Ilanne-Parikka *et al.* 2004). The prevalence tended to increase with age and associate with abnormalities in glucose metabolism (Ilanne-Parikka *et al.* 2004). Abnormal glucose tolerance is shown to be closely related to MetS. In the Botnia study, MetS was diagnosed in 10 % of women and 15 % of men with normal glucose tolerance, 42 % and 64 % of those with increased fasting glucose or impaired glucose tolerance, and 78 % and 84 % of those with type 2 diabetes (Isomaa *et al.* 2001). In the Kuopio Ischemic Heart Disease Risk Factor Study, using the data collected in 1980s, MetS was measured from participants aged 42-60 years, without any prevalent CVD, using the WHO and the NCEP definitions (Lakka *et al.* 2002). The prevalence of MetS ranged from 8.8 % to 14.3 % depending on the definition. Although there are several studies, which have investigated the prevalence of MetS, few studies have specifically evaluated MetS in adolescents and young adults, the part of population that is rapidly becoming more overweight (Ogden *et al.* 2002a).

2.1.4 Metabolic syndrome and clinical outcomes

Several prospective studies have indicated that MetS predicts future CVD (Isomaa *et al.* 2001;Lakka *et al.* 2002) and type 2 diabetes (Laaksonen *et al.* 2002a;Ford *et al.* 2008). Moreover, individuals with MetS are susceptible to other conditions such as fatty liver, gallstones, asthma, obstructive sleep apnea, hypogonadism in men and polycystic ovarian syndrome in women, a clinical syndrome including anovulation, androgen excess and insulin resistance.

MetS is shown to be a strong predictor of incident type 2 diabetes. According to previous studies, the relative risk of incident type 2 diabetes in participants with MetS ranges from 2 to 5 (Meigs *et al.* 2007;Wang *et al.* 2007b;Ford *et al.* 2008). Impaired fasting glucose has tended to be the abnormality most strongly associated with incident type 2 diabetes (Sattar *et al.* 2003;Wang *et al.* 2007b;Cameron *et al.* 2008b). However, in the EPIC-Potsdam study, abdominal obesity was more strongly associated with incident type 2

diabetes than impaired fasting glucose (IFG) (Ford *et al.* 2008). Recently, several studies have demonstrated that MetS is not a significant predictor of type 2 diabetes when its components are taken into account (Wilson *et al.* 2005;Cameron *et al.* 2008b). Wilson et al. reported that fasting glucose is a better predictor of type 2 diabetes than any of the combinations of metabolic risk factors (Wilson *et al.* 2005). In the San Antonio Heart Study, MetS predicted incident type 2 diabetes independently of other risk factors such as age, sex, ethnicity, family risk of type 2 diabetes, impaired glucose tolerance (IGT) and fasting insulin (Lorenzo *et al.* 2003). MetS was not superior to IGT for detecting participants at high risk for type 2 diabetes, but the combination of the IGT and the NCEP definition detected over 70 % of participants with increased risk for type 2 diabetes (Lorenzo *et al.* 2003). In the same study cohort, Stern et al. showed that MetS as defined by the NCEP criteria predicted the future development of type 2 diabetes less effectively than the Diabetes Risk Score (Stern *et al.* 2004).

Findings from different studies have been concordant in showing that CVD morbidity, mortality and all-cause mortality are increased in participants with MetS (Lakka *et al.* 2002;Hu *et al.* 2004;Hunt *et al.* 2004). A report from the Botnia study showed, that middle-aged adults with MetS have an approximately 3-fold increased risk of incident coronary heart disease and more than a 2-fold increased risk of stroke (Isomaa *et al.* 2001). The cardiovascular mortality rate was also higher in those with MetS than those without (Isomaa *et al.* 2001). In the NHANES III study, with a source population of over 10,000 patients, MetS was associated with prevalent myocardial infarction and stroke (Ninomiya *et al.* 2004). In addition, the risk of incident CVD was shown to increase with the number of components of MetS (Klein *et al.* 2002). Recently, two large meta-analyses demonstrated that participants with MetS have nearly 2-fold risk of CVD events and death compared to those without (Gami *et al.* 2007;Mottillo *et al.* 2010). The cardiovascular risk related to MetS was a one-third higher in women than in men (Gami *et al.* 2007).

The mechanisms explaining why MetS in women is associated with higher CVD risk is unclear. Central obesity is more pronounced in postmenopausal women than in men of the same age and thus may be linked to a higher CVD risk (Donato *et al.* 2006). Furthermore, in postmenopausal women, high-density lipoprotein (HDL) -cholesterol decreases, low-density lipoprotein (LDL) -cholesterol increases and LDL particles become smaller and denser and more atherogenic (Blake *et al.* 2002). There is also evidence that elevated serum triglycerides associate more strongly with coronary artery disease in women than in men. A previous meta-analysis showed that an increase in triglycerides of 1 mmol/l was associated with a 76 % increased risk of CVD in women compared with a 32 % increased risk in men (Hokanson and Austin 1996). The interaction of sex on the effects of MetS on CVD risk may be different in young adults compared with older adults. Additionally, conditions, such as polycystic ovary syndrome (Legro 2003) and gestational diabetes (Carr *et al.* 2006) may predispose young women to develop MetS later in life.

The role of MetS in predicting future risk of CVD is not surprising, as all MetS components by themselves are recognized risk factors for CVD and type 2 diabetes. The prognostic importance of MetS has been recently debated by many investigators. There is now increasing evidence that the diagnosis of MetS does not increase CVD morbidity and mortality over and above its individual components (Wang *et al.* 2007a;Koskinen *et al.* 2009). Data on studies that have investigated the risk associated with MetS independently of the risk of its individual components is still limited. Regardless of the recent controversy over the concept of MetS, the cardio-metabolic abnormalities tend to coexist together more often than might be expected by chance alone (Wilson *et al.* 1999;Hanley *et al.* 2002) and this condition associates with an increased risk of developing type 2 diabetes or CVD later in life (Gami *et al.* 2007). Furthermore, MetS and the components of MetS are shown to associate with the early markers of the atherosclerosis (Tzou *et al.* 2005).

2.1.5 Metabolic syndrome in the pediatric and adolescent populations

Children and adolescents are becoming more overweight and the first traits of MetS can be found in these obese children (Ogden *et al.* 2002a). There are no widely accepted criteria available to diagnose MetS in childhood. Most commonly, modifications of the adult definitions with age- and sex-specific percentiles have been used in pediatric research. Several study groups have investigated the prevalence of MetS in childhood and adolescence, although it is known that the clinical utility of the diagnosis may be reduced especially in young people because of changing growth patterns and the effects of puberty on insulin sensitivity and lipid profile (Zimmet *et al.* 2007).

Generally it can be concluded that MetS is common worldwide even in children and adolescents and particularly in overweight and obese children (De Ferranti and Osganian 2007;Tailor *et al.* 2010). The overall prevalence of MetS among U.S adolescents aged 12 to 19 years has varied from 4.2 % (Cook *et al.* 2003) to 9.2 % (De Ferranti *et al.* 2004) depending on the definition used. The prevalence of MetS increased and each element of the syndrome worsened directly with the degree of obesity among children and adolescents (Weiss *et al.* 2004). Cook et al. showed that MetS was present in 27.8 % of overweight (BMI $\geq 95^{th}$ percentile) participants, 6.8 % of at-risk participants (BMI, 85^{th} to $<95^{th}$ percentile) and 0.1 % of those with a BMI below the 85^{th} percentile. In a report by Jolliffe and Janssen, the age- and sex-specific adolescent criteria for MetS, using Lambda Mu Sigma (LMS) growth curve modelling for each MetS component, were used. The prevalence of MetS was 7.6 % according to the adolescent NCEP criteria and 9.6 % according to the adolescent IDF criteria (Jolliffe and Janssen 2007).

There is evidence that the clustering of metabolic risk factors begins already in childhood (Smoak *et al.* 1987;Bao *et al.* 1994;Chen *et al.* 2000) and multiple risk factors tend to persist from childhood into adulthood (Ford *et al.* 2004). However, cluster-tracking may not be particularly strong. Previously, in the Young Finns cohort, Raitakari et al showed that only about 25 % of children and young adults initially at high-risk (with

all three risk factors, serum total cholesterol, HDL-cholesterol and diastolic blood pressure in the extreme tertile) remained there after 6 years (Raitakari *et al.* 1994). In the 3-year follow-up of the Princeton School District Study, approximately half of the adolescents with MetS at baseline lost the diagnosis by follow-up (Goodman *et al.* 2007). These and several other observations indicate that there may be substantial instability in the diagnosis of MetS leading to limited clinical utility of the syndrome in the pediatric population (Goodman *et al.* 2007;Steinberger *et al.* 2009;Gustafson *et al.* 2009). Zimmet et al. have suggested that MetS should not be diagnosed in children under the age of 10 years, but instead weight reduction should be targeted for those children with abdominal obesity (Zimmet *et al.* 2007). A recent Scientific Statement from the American Heart Association has outlined the strengths and weaknesses of the concept of MetS in pediatric patients (Steinberger *et al.* 2009). Recently, Magnussen et al demonstrated that despite instability of diagnosis, MetS diagnosed in adolescence predicted adult outcomes such as MetS, high cIMT and type 2 diabetes in adulthood (Magnussen *et al.* 2010). Interestingly, high BMI itself predicted each outcome as well as or better than the categorical MetS definition (Magnussen *et al.* 2010). This finding emphasizes the benefit of screening for high BMI or overweight and obesity in pediatric populations and questions the clinical utility of the dichotomized MetS definition.

2.1.6 Controversy over the concept of metabolic syndrome

Strong criticism has been directed against the concept of MetS and especially its pathophysiological basis and clinical utility (Kahn *et al.* 2005;Reaven 2011b). The many definitions used to define MetS and its role and value in clinical practise has also been confused. Kahn, et al. have questioned whether the condition can be labelled a syndrome, since the cause is unknown (Kahn *et al.* 2005). However, the pathogenesis of MetS is widely considered to be multifactorial. Cluster analyses confirm that metabolic abnormalities occur simultaneously to a greater extent than would be expected by chance alone (Wilson *et al.* 1999;Hanley *et al.* 2002). When the concept of MetS was introduced for clinical use, it was thought that the risk associated with the syndrome is greater than the sum of risk derived from its components. Although MetS has been associated with an increased risk of type 2 diabetes and CVD (Lakka *et al.* 2002;Gami *et al.* 2007), there is increasing evidence that MetS does not predict CVD risk to a greater magnitude than the additive effects of the individual MetS components (Wang *et al.* 2007a;Koskinen *et al.* 2009). Recently, in the Young Finns Study, Koskinen et al. observed that the association between MetS and cIMT progression was independent of the main cardiovascular risk factors, but the inclusion of the dichotomous MetS components in the multivariable model, did not improve the overall predictive value (Koskinen *et al.* 2009). Furthermore, the results from a number of studies have shown that fasting glucose is as good, if not better marker than MetS in predicting type 2 diabetes (Wilson *et al.* 2005;Sattar *et al.* 2008;Cameron *et al.* 2008b) and the Framingham Risk Score is superior to MetS in predicting CVD risk (Stern *et al.* 2004;McNeill *et al.* 2005). However, there is also

evidence that MetS remained a significant predictor of CVD morbidity after adjusting for Framingham Risk Score (Dekker *et al.* 2005;Sipilä *et al.* 2009).

Moreover, the definition of MetS has undergone significant debate, especially focusing on which components and which component cut points should be part of the definition. The dichotomization of MetS risk factors has been particularly criticized. Report of a WHO Expert Consultation have recently discussed potential limitations of MetS in detail and highlighted many important issues (Simmons *et al.* 2010). The use of discrete thresholds for defining the components of MetS is more or less artificial and crucial information about the magnitude of risk factors may be lost (Eddy *et al.* 2008). Moreover, according to the concept of MetS, all five components are considered of equal value in contributing to the risk of developing type 2 diabetes or CVD and the presence of a combination of any three components indicates increased risk (Reaven 2011b). However, there is an increasing trend in risk with increasing number of MetS components (Sattar *et al.* 2003;Ford 2005b;Sattar *et al.* 2008); individuals with 5 MetS risk factors are at greater risk of CVD than individuals with 4 MetS risk factors, and so on. Furthermore, different risk factors have different effects and simply counting them ignores this fact (Eddy *et al.* 2008). MetS does not take account of other important cardiovascular risk factors such as age, sex, family history, smoking or physical activity. From the clinical point of view, all possible cardiovascular risk factors have to be taken into account when an individual's total risk for CVD events is defined.

2.2 Metabolic risk factors

Certain CVD risk factors tend to occur together. MetS is a cluster of central obesity, insulin resistance, hyperglycemia, hypertension and dyslipidemia. These conditions are interrelated and share underlying mediators, mechanisms and pathways (Grundy *et al.* 2005).

2.2.1 Central obesity

MetS and the components of MetS are strongly linked to the presence of obesity and, in particular, to waist circumference as a measure of intra-abdominal obesity (Carr *et al.* 2004). This relation may not be due to obesity itself, but rather excess adiposity causes a decrease in insulin-mediated glucose uptake and increases likelihood of insulin resistance (Abbasi *et al.* 2002;McLaughlin *et al.* 2003). Nevertheless, not all overweight/obese individuals are insulin resistant and carry metabolic abnormalities. For example, a substantial portion of these overweight/obese persons are insulin sensitive, without any of the metabolic abnormalities (McLaughlin *et al.* 2004;Wildman *et al.* 2008). Alternatively, insulin resistance and other metabolic abnormalities are not uncommon in normal-weight persons (McLaughlin *et al.* 2004;Wildman *et al.* 2008) especially in persons of South Asian ethnicity (Lee *et al.* 2007). Approximately 5,000 participants from the NHANES (1999-2004) were divided using BMI into normal

weight, overweight and obese categories and NCEP criteria, HOMA-IR (90th percentile) and CRP (90th percentile) were used to define participants as metabolically normal (≤1 abnormality) or metabolically abnormal (≥2 abnormalities). Based on these parameters 51 % of overweight and 32 % of obese participants were classified as metabolic healthy and on the contrary 24 % of normal-weight participants were metabolically abnormal (Wildman *et al.* 2008).

Although persons with MetS need not be clinically obese, they commonly have an abnormal fat distribution that is characterized by excess upper body fat, which can be accumulated either intraperitoneally (visceral fat) or subcutaneously (Grundy *et al.* 2005). There is substantial evidence that visceral fat is more strongly associated with insulin resistance and MetS than other adipose tissue compartments in obese participants (Carr *et al.* 2004;Després 2006). Measuring waist circumference has been considered to provide additional information over BMI concerning abdominal obesity and patients risk of CVD (Canoy *et al.* 2007) and type 2 diabetes (Balkau *et al.* 2007). However, some investigators have claimed that because these two indices of adiposity are highly correlated, the ability of BMI to predict risk is comparable to that achieved with measurement of waist circumference (Reaven 2011b). Alternatively, although waist circumference correlates well with the amount of total abdominal fat, it cannot distinguish between visceral adiposity, an important correlate of metabolic abnormalities, and subcutaneous abdominal fat (Després *et al.* 2008).

Figure 2 shows contributions of excess adiposity and insulin resistance to the development of MetS. Although abdominal obesity is one of the main features of MetS, the underlying mechanisms related to MetS are not fully understood (Després *et al.* 2008). Three possible explanations have been proposed to account for the relation of visceral adiposity to MetS (Després and Lemieux 2006). In the normal state, there is a balance between adipose tissue lipolysis and triglyceride synthesis. A decrease in lipoprotein lipase activity and an increase in hormone sensitive lipase activity leads to increased release of nonesterified fatty acids from adipose tissue (Eckel *et al.* 2005). The hyperlipolytic state of the visceral adipose tissue contributes to the development of insulin resistance and because, in insulin resistance, the inhibitory effect of insulin on lipolysis is suppressed, it causes the increased free fatty acid (FFA) flux into portal circulation. Impaired insulin action also leads to compensatory increased insulin production leading to hyperinsulinemia (decreased insulin clearance), glucose intolerance (increased hepatic glucose production) and hypertriglyceridemia (increased triglyceride rich $VLDL_1$-apoB secretion). (Després *et al.* 2008)

Alternatively, the adipose tissue which is also a metabolically active organ, is a source of adipokines including adiponectin and leptin; inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins (ILs); angiotensinogen and plasminogen activator inhibitor-1 (PAI-1) (Gustafson *et al.* 2007). Visceral obesity is related to the overproduction of these bioactive molecules (primarily inflammatory cytokines TNF-α and IL-6) and reduction of potentially protective adiponectin which may contribute

to the development of insulin resistance, proinflammatory, prothrombotic and prohypertensive state of visceral obesity and modify risk for atherosclerosis (Després and Lemieux 2006).

Thirdly, the excess visceral adiposity may also prevent subcutaneous adipose tissue to act as a protective fat deposit distracting its ability to expand (lipodystrophy) or predisposing adipose cells to enlarge (hypertrophy) and causes ectopic lipid formation at undesired sites such as in muscles, heart, liver and pancreas further increasing insulin resistance in these sites (Després and Lemieux 2006).

Free fatty acid accumulation to liver induces VLDL overproduction due to hepatic insulin resistance and may lead to non-alcoholic fatty liver disease (NAFLD) and even proceed to steatohepatitis and further to cirrhosis and liver failure (Day 2005). The liver fat content reflects the balance between FFA flux, fatty acid oxidation, de novo lipogenesis and VLDL secretion (Adiels *et al.* 2006). Moreover, fatty liver disease is characterized by an impaired ability of insulin to suppress hepatic glucose production resulting in mild hyperglycemia and stimulation of insulin secreation, leading to hyperinsulinemia (Angulo 2002). Inceasing prevalence of obesity and MetS are srongly associated with an increase in the prevalence of NAFLD (Kotronen and Yki-Järvinen 2008). NAFLD may also be involved in the pathogenesis of CVD through its contribution to insulin resistance and atherogenic dyslipidemia (Targher *et al.* 2010).

2.2.2 Insulin resistance and glucose intolerance

The most accepted hypothesis to describe the pathophysiology of MetS (clustering of metabolic abnormalities) is insulin resistance. The defects in insulin action directly affect glucose metabolism defined as an insufficient ability of insulin to suppress glucose production by the liver, and to mediate glucose uptake and metabolism in skeletal muscle and adipose tissue (Eckel *et al.* 2005). Insulin sensitivity is determined not only by the number and affinity of insulin receptors but also by the functional state of the intracellular signaling pathways that transduce insulin binding to the various effectors (e.g. glucose transport, phosphorylation and oxidation, glycogen synthesis, lipolysis and ion exchange) (Ferrannini 2006). Therefore, a massive reduction in the number of insulin receptors (or the presence of high titers of circulating anti-insulin or anti-insulin-receptor autoantibodies) is associated with insulin resistance (Ferrannini 2006;Kashyap and Defronzo 2007). Signaling through phosphatidylinositol 3-kinase is crucial for insulin-mediated glucose transport and activity of this enzyme is reduced in insulin resistant states. Stimulation of mitogen-activated protein kinase (MAPK) pathway is intact in participants with insulin resistance leading to smooth muscle cell proliferation, increased collagen formation and increased production of growth factors and inflammatory cytokines that may contribute to the development of atherosclerosis (Cusi *et al.* 2000). In addition, not all tissues share the defect in insulin action. For example, many manifestations of the insulin resistance syndrome result from the actions of compensatory hyperinsulinemia on tissues that remain normally insulin sensitive (Reaven 2011a).

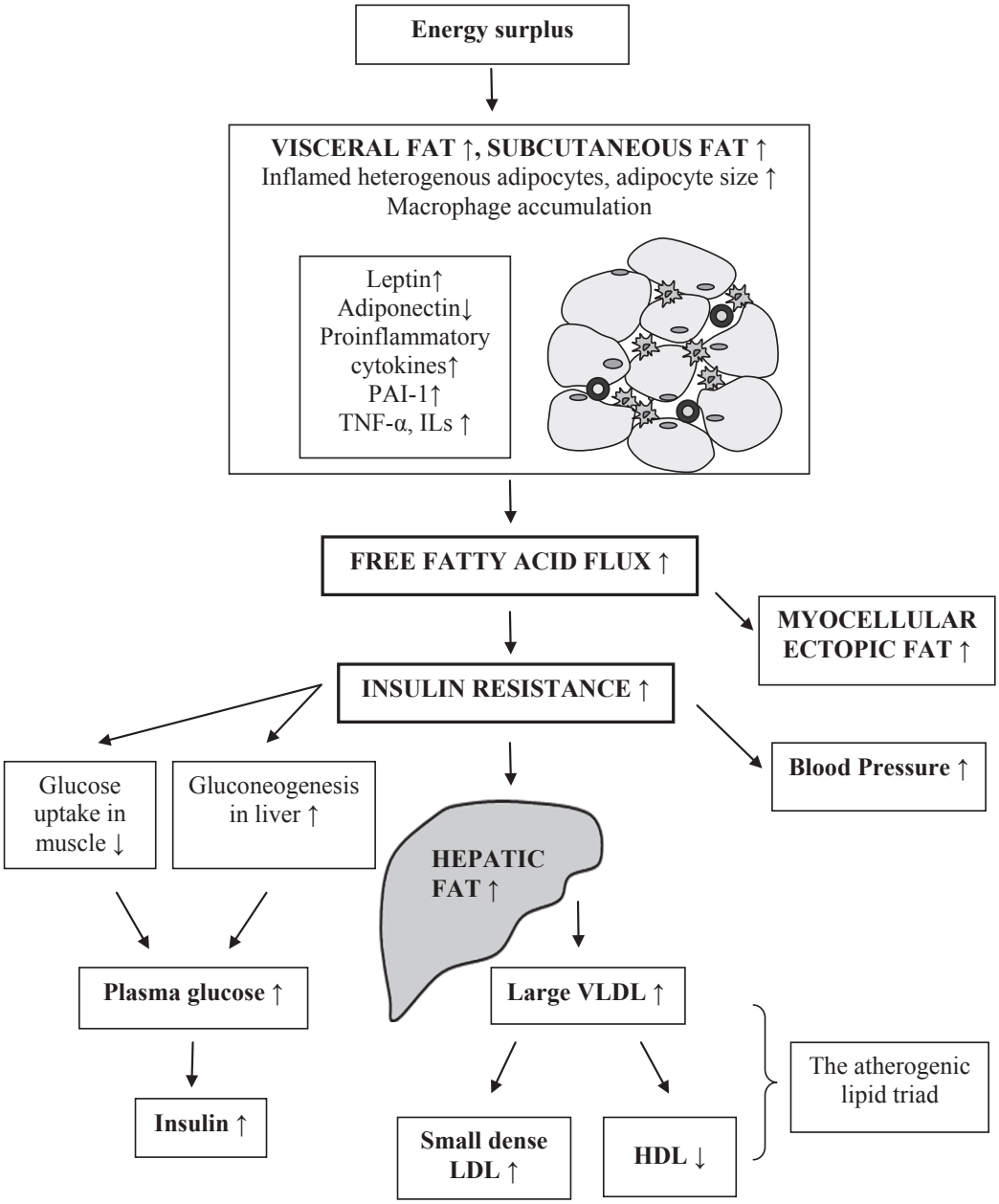

Figure 2. Effect of energy surplus on the adipose tissue and the liver.

Sensitivity to insulin-mediated glucose uptake by muscles varies six to eight-fold in apparently healthy non-diabetic adults (Ferrannini *et al.* 1988;Yeni-Komshian *et al.* 2000). Approximately 50 % of the variability in insulin action results from the degree of adiposity and physical fitness and the other 50 % is probably of genetic origin (Reaven 2011a). There is no absolute criteria that classifies participants to insulin-resistant or insulin sensitive. Despite substantial variability from person to person, enough insulin is secreted in these insulin-resistant individuals to prevent frank decompensation of glucose homeostasis (Reaven 1988). Type 2 diabetes occurs when genetically predisposed insulin-resistant individuals are no longer able to maintain the degree of compensatory hyperinsulinemia needed to maintain normal glucose homeostasis (Ferrannini *et al.* 1988). Most individuals are able to sustain the level of compensatory hyperinsulinemia needed to maintain normal fasting and postprandial glycemia for years.

2.2.3 Hypertension

The relation between insulin resistance and hypertension is well established and is related to several different mechanisms (Ferrannini *et al.* 1987). In an insulin-resistant, non-diabetic individual not all tissues are equally insulin-resistant. For example the kidney is not resistant to the ability of insulin to promote the renal reabsorption of sodium and altering cell electrolyte composition. Similarly, compensatory hyperinsulinemia may raise blood pressure by increasing sympathetic nervous system activation (Rowe *et al.* 1981) and stimulation of vascular smooth muscle cell growth (Defronzo 1992).

2.2.4 Lipid risk factors

Low HDL-cholesterol, elevated triglycerides, and high levels of small, dense LDL are considered to be the major lipid abnormalities in MetS (Howard 1999). This abnormal lipid profile is also called atherogenic lipid triad. Recent data also shows that individuals with MetS may have smaller HDL mean particle size and higher serum apo E concentration (Söderlund *et al.* 2010).

There are several mechanisms that could explain the cause and effect relation between insulin and clustering of metabolic risk factors. Free fatty acid concentrations released from adipose tissue are suggested to be the critical link between insulin resistance and dyslipidemia (Reynisdottir *et al.* 1997). In patients with insulin resistance, the suppression of FFA release from adipocytes is impaired, providing increase in the hepatic production of apoB containing VLDL particles, especially triglyceride-rich $VLDL_1$ particles, leading to hypertriglyceridemia (Reynisdottir *et al.* 1997). The defect in lipoprotein lipase activity in insulin resistance also contributes to the decrease in plasma HDL-cholesterol, and the LDL-cholesterol pool may become enriched with small dense, highly atherogenic LDL particles (Ginsberg 2002). Importantly, the increased function of cholesterol ester transfer protein (CETP) and hepatic lipase are

associated with the generation of low HDL levels and small, dense LDL (Murakami *et al.* 1995).

In addition, atherogenic dyslipidemia may activate several pathways that can lead to endothelial dysfunction (Deedwania 2003). For example, small dense LDL particles have low affinity to the LDL receptor and long retention time in the circulation (Nigon *et al.* 1991). Therefore, they can more easily penetrate the arterial endothelium and have increased capacity for binding to the intimal proteoglycans. In addition, small, dense LDL particles contain relatively smaller amounts of antioxidants compared to LDL cholesterol and therefore are more prone to oxidation (de Graaf *et al.* 1991).

Apolipoprotein B and A-I

Apolipoproteins regulate the synthesis and metabolism of cholesterol and triglyceride containing lipoprotein particles and moreover stabilize their structure (Sierra-Johnson *et al.* 2008). ApoB exists in two isoforms, apoB-100, a dominating protein in plasma, which is produced in the liver and apoB-48, found in plasma only in very low concentrations, which is synthesized in the small intestine (Powell *et al.* 1987). ApoB is the main stuctural protein on the surface of chylomicrons, VLDL and LDL particles and each of these lipoprotein particles contains only one apoB molecule (Elovson *et al.* 1988). Therefore, total apoB indicates the total number of potentially atherogenic particles.

ApoA, subfamily of apolipoproteins, has two major proteins, apoA-I and apoA-II. ApoA-I is synthesized in the gut and the liver and apoA-II only in the liver. ApoA-I is the main structural protein (accounting for 80-90 % of the total protein) in HDL particles and one major atheroprotective function is to transport excess cholesterol from the peripheral cells back to the liver for excretion (Rader 2006). Liver is the crucial site where cholesterol can be actively secreted out from the body. ApoA-I has also anti-inflammatory, antioxidant and anti-thrombotic effects (Barter and Rye 2006;Oslakovic *et al.* 2009;Oslakovic *et al.* 2010). Its plasma concentration correlates strongly with the concentration of HDL-cholesterol. The balance between apoB and apoA-I, apoB/apoA-I ratio may indicate cardiovascular risk, as it represents the balance between proatherogenic and antiatherogenic lipoproteins (Walldius and Jungner 2005). The higher the value of apoB/apoA-I ratio, the higher the likelihood that cholesterol is deposited in the arterial wall, thus accelerating atherogenesis (Walldius and Jungner 2005).

Avogaro and co-workers first showed the clinical importance of apoA-I and apoB in predicting coronary heart disease (Avogaro *et al.* 1979). Since then many others have observed the relation between apolipoproteins and CVD morbidity and mortality. In the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study, apolipoproteins have been associated with post-mortem arterial lesions (Rainwater *et al.* 1999). Several studies have reported that the apoB/apoA-I ratio is strongly associated with MetS and its components (Sung and Hwang 2005;Sierra-Johnson *et al.* 2006). In NHANES III,

Sierra-Johnson et al. described the relation between the apoB/apoA-I ratio and MetS and its components (Sierra-Johnson *et al.* 2006). In addition, the apoB/ApoA-I ratio was associated with insulin resistance (defined using the HOMA-index) independent of traditional risk factors, MetS components and inflammatory markers (Sierra-Johnson *et al.* 2007). Several other clinical studies, such as the Apolipoprotein-Related Mortality Risk (AMORIS) study (Sniderman *et al.* 2006), the INTERHEART study (Yusuf *et al.* 2004) and the UPPSALA longitudinal Study of Adult Men (ULSAM) study (Dunder *et al.* 2004) have shown that the apoB/apoA-I ratio predicts the risk of both nonfatal and fatal myocardial infarction. Serum levels of apoB and apoA-I may be even better predictors of vascular disease compared with their cholesterol concentrations (Walldius *et al.* 2001;Williams *et al.* 2003).

Recently, both apoB and apoA-I have been included in ESC/EAS clinical guidelines for the management of dyslipidaemias especially to follow the efficacy of drug treatment, however, not that much as a risk predictor (2011). According to the statement, apoB should be considered at least as an alternative risk marker, especially in combined hyperlipidemias, diabetes, MetS and chronic kidney disease.

2.2.5 Inflammatory markers

C-reactive protein

Atherosclerosis is characterized by a nonspecific local inflammatory process followed by a systemic response (Ross 1999). Slightly elevated levels of C-reactive protein (CRP), certain cytokines and blood white cell count have been associated with cardiovascular morbidity and mortality (Koenig *et al.* 1999;Ridker *et al.* 2002). CRP is a non-specific biochemical marker of inflammation that is synthesized in the liver in the response to IL-6 secretion, but also in the endothelium of atherosclerotic plaques, smooth muscle cells and macrophages (De Ferranti and Rifai 2007). CRP is the best characterized of the currently available inflammatory biomarkers. CRP is shown to be produced in smooth muscle cells within human coronary arteries and is expressed preferentially in diseased vessels (Calabro *et al.* 2003). CRP may stimulate the expression of adhesion molecules, impact fibrinolysis, activate leucocytes and the complement system (Ridker *et al.* 2004;De Ferranti and Rifai 2007). Moreover, CRP may affect the arteries of healthy children by promoting intima-media thickening and disturbing endothelial function supporting the hypothesis that CRP plays a role in the pathogenesis of early atherosclerosis (Järvisalo *et al.* 2002). MetS and its components have also been shown to associate with measures of inflammation and CRP (Festa *et al.* 2000;Frohlich *et al.* 2000;Laaksonen *et al.* 2004). In addition, CRP may have pathophysiological role in the atherogenicity of MetS (Ridker *et al.* 2003;Sattar *et al.* 2003). In a large prospective cohort study among 27,111 women, MetS was associated with an increased risk of future peripheral artery disease and the risk appeared to be mediated by the effects of inflammation measured by elevated CRP (Conen *et al.* 2009).

Secretory phospolipase A2

Type II secretory phospholipase A2 (sPLA2), is a member of a family of phospholipases that hydrolyze phospholipids at the *sn*-2 position to generate lysophospholipids and free fatty acids (Hurt-Camejo *et al.* 2001). This action may affect the functions and properties of vascular endothelial and smooth muscle cells, and of macrophages at sites of LDL accumulation leading to the activation of inflammatory processes related to atherosclerosis (Divchev and Schieffer 2008). Increased sPLA2 enzyme activity, which encompasses several types of sPLA2 (types IIA, V and X), is considered atherogenic (Mallat *et al.* 2007). High enzyme activity has been found in human and mouse atherosclerotic lesions (Rosenson 2009) and has been shown to predict CVD events independently of traditional risk factors (Boekholdt *et al.* 2005;Mallat *et al.* 2007). Previously, sPLA2 was shown to associate independently with insulin resistance (Leinonen *et al.* 2003). In another cross-sectional study, sPLA2 was not a determinant of cIMT in the diabetic patients (Leinonen *et al.* 2004). The role of sPLA2 contributing to the atherogenicity of MetS, however, has not been studied.

2.2.6 Risk factors associated with lifestyle

Sedentary lifestyle is associated with several features (insulin resistance, obesity, dyslipidemia, hypertension, elevation in ROS species etc.) of MetS (Lakka *et al.* 2003). Individuals with low levels of leisure-time physical activity and cardiorespiratory fitness are more likely to have MetS (Lakka *et al.* 2003). In contrast, a physically active lifestyle across the lifespan may prevent or delay the onset of MetS in young adults (Yang *et al.* 2008).

There is no consensus as to what type of diet may be optimal for those with MetS. According to the recent systematic review, dairy intake was inversely associated with incidence or prevalence of MetS in seven out of 13 studies, suggesting that dairy consumption may have a beneficial effect on MetS (Crichton *et al.* 2011). A large Finnish population based cohort of middle-aged subjects showed that increased daily sodium intake was an independent dietary indicator of MetS in middle-aged subjects (Räisänen *et al.* 2011). The Mediterranean diet that is moderately lower in carbohydrate (45 %), and moderately higher in fat (35-40 %), with less than 10 % of saturated fat, may be beneficial for improving features of MetS, including effects on insulin sensitivity, serum lipids and liver function (Esposito *et al.* 2004).

The relationship between alcohol intake and MetS remains controversial (Goude *et al.* 2002;Djousse *et al.* 2004;Yoon *et al.* 2004). In some cross-sectional studies, moderate alcohol consumption has been associated with a lower prevalence of MetS (Djousse *et al.* 2004;Freiberg *et al.* 2004). On the contrary, a large Korean study showed an increasing dose-response relation between alcohol consumption and MetS (Yoon *et al.* 2004) and another population-based study of clinically healthy men showed that there was no difference in alcohol intake across the groups of men with none of the components of

the MetS with one or more components and men fulfilling all components (Goude *et al.* 2002). However, there was an independent relation between insulin sensitivity, as measured by the clamp technique, and alcohol intake (Goude *et al.* 2002). Smoking is known to be a strong risk factor for atherosclerosis and CVD and it may also have dose dependent association with MetS (Oh *et al.* 2005).

2.2.7 Other risk factors and mechanisms

Insulin resistance has been shown to be related to many other alterations that are not included in the diagnostic criteria of MetS such as increased uric acid, fibrinogen, PAI-1, serum viscosity, microalbuminuria, asymmetric dimethylarginine and homocysteine (Eckel *et al.* 2005). Moreover, increasing evidence indicates that vitamin D deficiency may be associated with risk of CVD. Results from the NHANES 2001-2004 survey demonstrated that low concentrations of vitamin D were strongly associated with overweight and abdominal obesity and also with MetS (Reis *et al.* 2009).

Suboptimal intrauterine conditions, such as low nutrient or oxygen supply or fetal exposure to glucocorticoid or sex steroid excess may program the effects on the development of metabolic diseases (Seckl 2001;Fowden *et al.* 2006). According to the Barker´s hypothesis, children born small for gestational age are particularly vulnerable to develop reduced insulin sensitivity and increased risk of CVD later in adulthood, especially if they experience a rapid catch-up growth during the first years of life (Barker *et al.* 1993). In contrast, overnutrition of the fetus may also lead to permanent changes in appetite control and insulin metabolism, which leads to increased risk of obesity and other metabolic abnormalities later in life (Whitaker and Dietz 1998;Lawlor and Chaturvedi 2006). Moreover, children who are large-for-gestational-age at birth and exposed to an intrauterine environment of either maternal diabetes or maternal obesity are at increased risk of developing MetS (Boney *et al.* 2005).

2.2.8 Genetics of metabolic syndrome

Substantial effort has been made toward defining the precise genetic abnormalities related to MetS (Nestel 2003). Several candidate genes have been proposed that include genes regulating peroxisome proliferator-activated receptor γ (PPAR-γ), leptin, adiponectin, glucocorticoids and lipoprotein lipase. Several studies have attempted to identify underlying genetic risk factors but no study has yet been able to identify genetic variants shared by more than two of the components (Benyamin *et al.* 2007;Sjögren *et al.* 2008). Reports from the Malmö Preventive Project, a large prospective study of 16,143 non-diabetic individuals showed that polymorphisms in susceptibility genes for type 2 diabetes (TCF7L2, WFS1, IGF2BP2) and obesity (FTO) predispose to MetS by increasing the risk of one or two specific components of MetS. However, the findings argue against a common genetic background of MetS (Sjögren *et al.* 2008). Similar findings have been obtained using genome-wide association studies for MetS (Kraja

et al. 2011). MetS is likely to be polygenic disorder that still requires environmental influences to manifest (Nestel 2003).

2.3 Treatment of metabolic syndrome

MetS is a constellation of several interrelated cardiometabolic abnormalities which are associated with an increased risk of developing type 2 diabetes and CVD. Treatment of coexisting risk factors in participants with MetS may prevent the onset of these clinical outcomes. The clinical significance of MetS is still controversial. However, the importance of identifying these patients as early as possible to initiate effective treatment for individual risk factors when needed and follow-up patients at regular intervals is recognised. Lifestyle modifications, healthy diet, regular physical activity, cessation of smoking and weight loss/control, are the key treatment elements in the management of persons with MetS (Grundy *et al.* 2005;Ilanne-Parikka *et al.* 2008).

2.3.1 Lifestyle modifications

It is well established that lifestyle changes that induce weight loss are the first-line therapeutic targets of MetS. Weight loss is beneficial for treating all of the components of MetS including obesity, dyslipidemia, hypertension, insulin resistance and hyperglycemia. The Finnish Diabetes Prevention Study showed that lifestyle intervention reduced the occurrence of abdominal obesity and the overall prevalence of MetS in the long term (Ilanne-Parikka *et al.* 2008). Even a modest weight loss as 5-10 % of body weight can significantly reduce triglycerides and increase HDL-cholesterol (VanGaal *et al.* 1997).

Dietary intake is shown to have an impact on all of the components of the MetS. Each patient should be treated individually but it is reasonable to recomment, beyond weight control and reduction of total calories, a diet low in saturated fat, higher in unsaturated fats, high in complex carbohydrates (whole grains), and low in sodium (Grundy *et al.* 2005;Cornier *et al.* 2008). Carbohydrate restriction in diet, especially in the form of added sugars, may have favourable impacts on the MetS and particularly on serum lipid levels. Previous studies have demonstrated that the low-carbohydrate diet produced a greater weight loss than did the conventional diet for the first six months, but the difference was not significant at one year (Foster *et al.* 2003;Shai *et al.* 2008). This may be due to greater weight regain in the low-carbohydrate group suggesting that long-term adherence to the low-carbohydrate diet may be difficult. Low-carbohydrate diet was associated with greater decreases in serum triglycerides and greater increases in HDL cholesterol than was the conventional diet (Foster *et al.* 2003). However, an important health concern of consuming unrestricted amounts of saturated fat is the potential increase of LDL cholesterol. It is also possible that the large amount of saturated fats and small amounts of fruits, vegetables, and fiber consumed during the low-carbohydrate diet can independently increase the risk of coronary heart disease (Foster *et al.* 2003). Longer

and larger studies are required to determine the long-term safety and efficacy of low-carbohydrate, high-protein, high-fat diets. In contrast, Sacks et al. have recently shown that reduced-calorie diets result in clinically meaningful weight loss regardless of which macronutrients they emphasize (Sacks *et al.* 2009).

Data from randomized controlled trials indicate that exercise training has at least modest effects on individual features of the MetS, much it is still uncertain whether exercise training can prevent or treat the MetS itself (Lakka and Laaksonen 2007). In a population-based study of 612 middle-aged Finnish men who engaged in moderate- or high-intensity leisure-time physical activity at least 3 h/week were half as likely as sedentary men to develop the MetS during the 4 year follow-up period (Laaksonen *et al.* 2002b). Results from the Diabetes Prevention Study showed that counselling of middle-aged, overweight participants to reduce weight and increase physical activity reduced the cumulative incidence of diabetes over 4 years by 58 % compared with a control group (Tuomilehto *et al.* 2001). Furthermore, increased participation in moderate-to-vigorous aerobic physical activity and regular long-term participation in resistance training improved MetS status among adults with impaired glucose tolerance (Ilanne-Parikka *et al.* 2010). Even in the absence of weight loss, long term physical activity, may prevent MetS (LaMonte *et al.* 2005). These results suggest that lifestyle interventions of individuals with MetS are important for reducing the risk of type 2 diabetes as well as CVD.

2.3.2 Pharmacological intervention

Participants with MetS may also benefit from pharmacological intervention to further modify CVD risk factors. However, there is no appropriate pharmacological treatment for MetS. The idea of reducing multiple risk factors with a single drug is attractive but fairly unrealistic. There have been several attempts to develop single drugs, that have multiple targets or modulate targets that affect several risk factors (Grundy 2006). Nevertheless, concern over severe side effects has led to recent withdrawals of two weight-loss drugs, sibutramine and rimonabant, from the market. One therapeutic class of drugs that has been shown to reduce the incidence of new-onset diabetes is hypertensives, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), which lowers blood pressure through their action on the renin–angiotensin system and also improves dysglycemia via the PPAR-γ signaling pathway (Grundy 2006;Haffner 2006). According to current guidelines, the focus should be to assess all well-known CVD risk factors and to treat these risk factors separately with one or multiple drugs.

2.4 Ultrasonic examination of arteries in healthy young adults

Measurements of carotid artery intima-media thickness (IMT), carotid artery elasticity and brachial artery flow-mediated dilatation (FMD) are markers of arterial health that can be measured non-invasively, reliably and reproducibly by ultrasound.

2.4.1 Arterial wall thickness

Measurement of arterial wall IMT was first presented in 1986 by Pignoli et al. showing strong correlation between aortic arterial IMT measured by B-mode ultrasound and histology (Pignoli *et al.* 1986). Carotid IMT (cIMT) can be measured relatively simply and noninvasively, therefore it is well suited for use in large population studies and has been recognized as a surrogate measure of atherosclerosis (De Groot *et al.* 2004). However, the addition of cIMT assessment to prevention guidelines is still under discussion.

Increased cIMT correlates well with childhood and current vascular risk factors (Davis *et al.* 2001;Raitakari *et al.* 2003), and relates to the severity and extent of coronary artery disease (Burke *et al.* 1995). Furthermore, several longitudinal studies have shown that increased cIMT predicts future cardiac (myocardial infarction, angina pectoris and coronary intervention) and cerebrovascular (stroke or transient ischemic attack) events (Burke *et al.* 1995;O'Leary *et al.* 1999). In a meta-analysis, including 37,197 subjects, a small increase of 0.1 mm in mean cIMT was associated with an increase in relative risk for myocardial infarction by 10-15 % and stroke by 13-18 % (Lorenz *et al.* 2007). Recently, mean cIMT and plaque score have shown to predict the presence of complex coronary artery lesions and correlate with the degree of lesion complexity (Ikeda *et al.* 2012).

Several cross-sectional studies have shown an association between MetS and cIMT (Scuteri *et al.* 2004;Tzou *et al.* 2005;Ahluwalia *et al.* 2006). There is still limited data available on the association between metabolic risk factors and cIMT especially in young adults. Recently, in the Young Finns Study, it was shown in young adults that conventional risk factors and MetS were associated with accelerated cIMT progression and that spontaneous recovery from MetS is associated with reduced cIMT progression (Koskinen *et al.* 2010).

2.4.2 Arterial elasticity

The elasticity of the proximal large arteries is the result of the proportion of elastin to collagen in their walls (O'Rourke *et al.* 2002). Aging and exposure to cardiovascular risk factors leads to increased collagen, decreased quantities of elastin, unorganized and dysfunctional fiber distribution and infiltration of vascular smooth muscle cells into the intima and elevated smooth muscle tone (Zieman *et al.* 2005). The elastic properties of large arteries can be measured non-invasively by using measurements of ultrasound-

based distensibility (the arterial pressure-diameter) or recording of the pulse wave velocity.

Decreased carotid artery elasticity is associated with risk factors (Raitakari 1999) and has been implicated as a predictor for cardiovascular events (Haluska *et al.* 2010). A recent report from the Atherosclerosis Risk in Communities (ARIC) study, however, showed that after adjusting for cardiovascular risk factors, ultrasound measures of carotid arterial stiffness are associated with incident ischemic stroke but not CVD events (Yang *et al.* 2012). Measurement of arterial function may also add incremental benefit to Framingham risk scores in participants with intermediate cardiovascular risk (Haluska *et al.* 2010).

Moreover, several cross-sectional studies have demonstrated decreased arterial elasticity in individuals with MetS or with increasing number of traits of MetS (Scuteri *et al.* 2004;Urbina *et al.* 2004;Li *et al.* 2005). Prospective studies have shown that the increase in arterial stiffness with age is greater in individuals with MetS than without (Safar *et al.* 2006). Individuals whose MetS status has regressed or remained negative over time show lower rates of increase in arterial stiffness (Tomiyama *et al.* 2006;Koskinen *et al.* 2010).

2.4.3 Endothelial function

The vascular endothelium is a monolayer of cells and lies between the lumen and the vascular smooth muscle. Normal endothelial function has a central role in vascular homeostasis including control over thrombosis and thrombolysis, platelet and leukocyte interactions with the vessel wall and regulation of vascular tone and growth (Celermajer 1997). Endothelial dysfunction indicates inadequate vasodilatation and/or paradoxical vasoconstriction in coronary and peripheral arteries in response to stimuli of nitric oxide (Celermajer 1997;Mullen *et al.* 2001). Reduction in the bioavailability of endothelial-derived nitric oxide is considered an early event of atherosclerosis, which may appear long before structural atherosclerotic changes (Mullen *et al.* 2001).

The noninvasive assessment of endothelial function by ultrasound was first introduced in 1992 (Celermajer *et al.* 1992). Flow-mediated dilatation (FMD) of the brachial artery can be determined by inflating to suprasystolic pressure a pneumatic cuff around the forearm for 4 minutes 30 seconds and release of the cuff (sudden increase in blood flow) causing the endothelium-dependent vasorelaxation which can be measured with the ultrasound (Celermajer *et al.* 1992). Since then, brachial FMD has become widely used marker of systemic arterial endothelial function (Adams and Celermajer 1999). Brachial FMD also correlates well with epicardial coronary dilatation in response to intracoronary infusion of acetylcholine (Anderson *et al.* 1995;Takase *et al.* 1998).

Several studies have demonstrated association between brachial FMD and conventional risk factors, including type 2 diabetes, hypertension, dyslipidemia, obesity and smoking

both in children and adults (Celermajer *et al.* 1992;Järvisalo *et al.* 2004;Kallio *et al.* 2007;Kawano *et al.* 2012). The status of endothelial function may also modify the association between risk factors and atherosclerosis (Järvisalo *et al.* 2004;Juonala *et al.* 2004b). Endothelial dysfunction is also considered to be often present in patients with type 2 diabetes or MetS, although the precise mechanisms remain unclear (Deedwania 2003). Despite the wide use of brachial FMD as a research tool, limitations on its reproducibility have impaired its applicability as a screening tool in clinical practice (Bonetti *et al.* 2003).

3. AIMS OF THE STUDY

The present thesis is based on the findings from the Cardiovascular Risk in Young Finns Study and specifically from the 21-year follow-up study performed in 2001 and the 27-year follow-up study performed in 2007. The purpose was to examine the prevalence, secular trends, childhood predictors of MetS and relation to the early vascular changes.

The major aims of this thesis were as follows:

1. To study the prevalence of metabolic syndrome in Finnish young adults and to study the secular trend in metabolic syndrome among 24-year-old adults during the time period 1986-2001. (I)

2. To study the childhood predictors of metabolic syndrome and especially the contribution of childhood obesity to the prediction of risk of developing metabolic syndrome in adulthood. (II)

3. To study the relations between metabolic syndrome and subclinical atherosclerosis (carotid IMT, carotid elasticity, brachial FMD) in young adults and whether apoB, apoA-I, CRP and sPLA2 are associated with metabolic syndrome, and to what extent the atherogenicity of metabolic syndrome is explained by these apolipoproteins and inflammatory markers. (III,IV)

4. PARTICIPANTS AND METHODS

4.1 Description of the Cardiovascular Risk in Young Finns Study

The present thesis is based on the Cardiovascular Risk in Young Finns Study, which is an ongoing, multi-centre follow-up study of atherosclerotic risk factors of Finnish children, adolescents and young adults (Raitakari *et al.* 2008). Participants were randomly chosen from each of the participating centres (cities of Helsinki, Turku, Tampere, Oulu and Kuopio) and from nearby rural communities. Two pilot studies were conducted in 1978 and 1979. The original sample size was 4,320 healthy children and adolescents aged 3, 6, 9, 12, 15 and 18 years and a total of 3,596 (83.2 %) individuals participated in the first cross-sectional study in 1980 (Åkerblom *et al.* 1985). The follow-up studies were performed for the whole study group in 1983, 1986, 2001 and 2007 when 2,991 (83.2 %), 2,779 (77.3 %), 2,283 (63.5 %) and 2,204 (61.3 %) participants from the original cohort participated.

In 2001 and 2007 ultrasound examinations were performed to study early structural and functional atherosclerotic vascular changes. The protocol included measurements of cIMT, carotid artery elasticity and brachial FMD. Pregnant women (in 2001, n=62 and in 2007, n=37) and participants with type 1 diabetes (in 2001 and 2007, n=16) were excluded. In 2001, amongs 24-39-year-old participants no one had prevalent CVD, 45 participants (1.6 %) had antihypertensive and 7 participants (0.3 %) had lipid-lowering medication. In 2007, amongs 30-39-year-old participants 152 (6.9 %) received antihypertensive medication and 46 (2.1 %) statins.

Table 2. The design of the Cardiovascular Risk in Young Finns Study

Year	Participants	Age													
1980	3596	**3**	**6**	**9**	**12**	**15**	**18**								
1983	2991		6	9	12	15	18	21							
1986	2779			9	12	15	18	21	**24**						
1989	350				12	15	18	21	24	27					
1992	572					15	18	21	24	27	30				
1995	-						18	21	24	27	30	33			
1998	-							21	24	27	30	33	36		
2001	2283								**24**	**27**	**30**	**33**	**36**	**39**	
2007	2204										**30**	**33**	**36**	**39**	**42**	**45**

Highlighted age groups were included in analyses of secular trends.

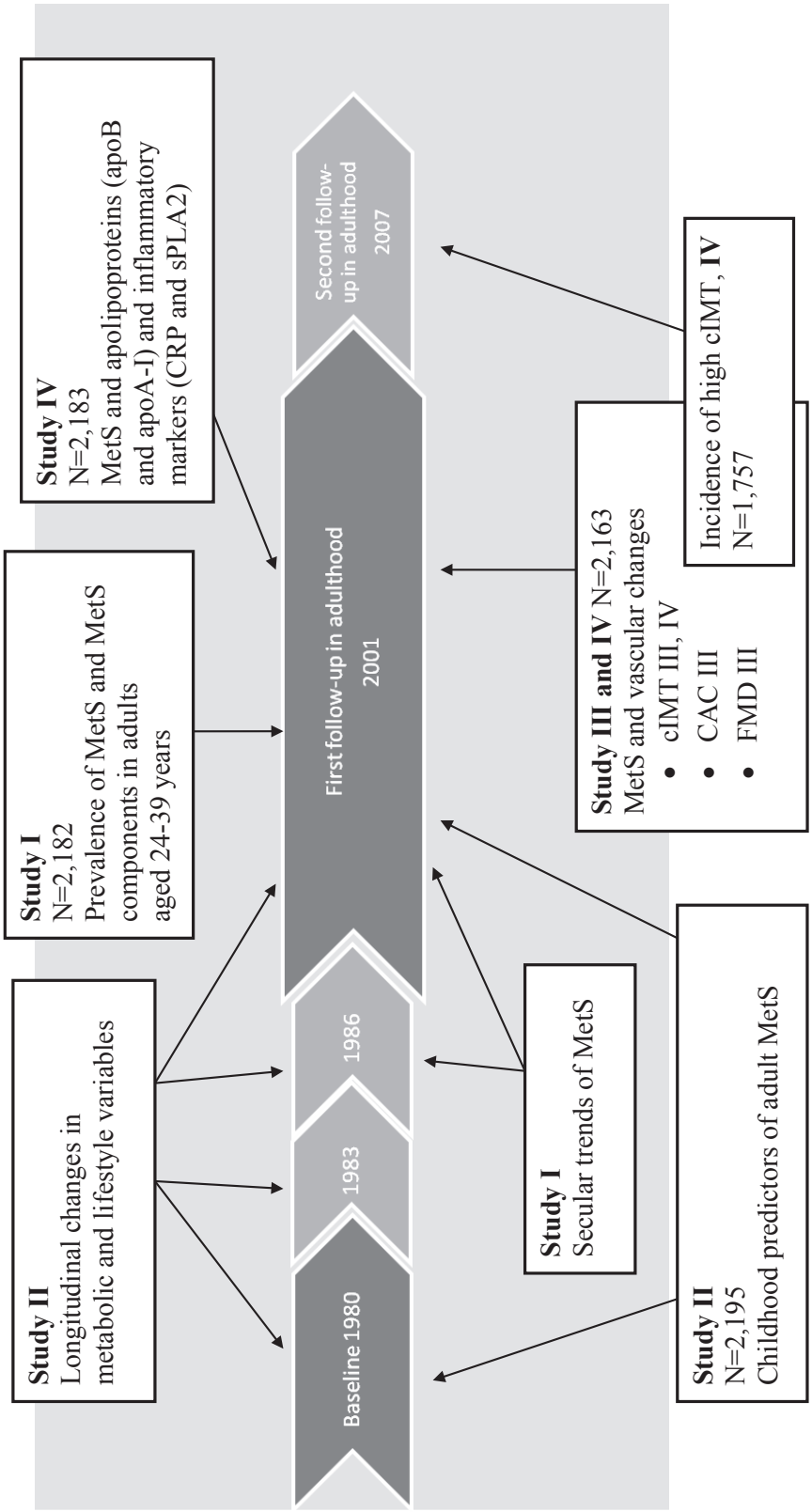

Figure 3. Participants and data assessed in studies I-IV.

4.2 Study design and participants

Design of the studies I-IV and participant number and study years are shown in Figure 3. In study I, the main objective was to examine the prevalence of MetS and its components in 2001 amongst 2,182 young Finnish adults (aged 24-39 years) using 3 applied MetS definitions: NCEP, EGIR and IDF. In this thesis, the prevalence of MetS was also reported 6 years later in 2007. In addition, the secular trends of MetS were studied amongst 24-year-old adults from 1986 to 2001 and amongst 30-39-year-old adults from 2001 to 2007.

In study II, longitudinal data from the Cardiovascular Risk in Young Finns Study were used to examine the association of childhood and adolescent risk variables and adulthood MetS. The primary aim was to examine childhood determinants of the adulthood MetS. In addition, to gain insight of the changes in risk profile during maturation in obese individuals who develop adulthood MetS, we compared serial changes in metabolic and lifestyle variables and from childhood to young adulthood between obese individuals, who developed and those who did not develop MetS in adulthood. In these analyses, the study population with complete data included 2,195 participants (1,014 men and 1,181 women) aged 3 to 18 years in 1980 and 24 to 39 years in 2001. In this thesis, a child is considered to be a person aged 3 to 9 years and an adolescent is considered to be a person aged 12 to 18 years.

In study III, the associations between MetS and cIMT, carotid compliance and brachial FMD were studied in 2,163 participants. In addition, it was investigated whether the relation between MetS and cIMT is modified by the magnitude of the FMD response.

Study IV focused on the question whether the association between MetS and cIMT in a population of young adults is modified by apolipoproteins and/or inflammatory markers. For cross-sectional analyses, 2,183 participants who had full risk factor data measured in 2001 were included. For longitudinal analyses, data from 1,757 participants, who had participated in both the 2001 and 2007 studies, were used.

4.3 Physical examination and lifestyle risk factors

Physical examination included measurements of height, weight, waist and hip circumferences and systolic and diastolic blood pressure. Height was measured with a Seca anthropometer and weight with Seca weighing scales. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist (midway between lowest rib at midaxillary line and iliac crest) and hip (at the greater trochanters) circumferences were calculated as the average of two measurements. In 1980, 1983 and 1986 biceps, triceps and subscapular skinfold thicknesses were measured in triplicate from the nondominating arm with the Harpenden-calipers (Holtain and Bull, British Indicators Ltd., Luton, Beds., UK) to the nearest 0.2 mm readings. Their sum variable was used in statistical analysis.

In 1980 and 1983, blood pressure was measured with a standard mercury sphygmomanometer except in 3-year-old children whose systolic blood pressure was

measured by an ultrasound device (Arteriosonde 1020, Roche). In 1986, 2001 and 2007, blood pressure was measured using a random zero sphygmomanometer in sitting position after 5 minutes rest. Korotkoff´s fifth sound was used as the sign of diastolic blood pressure and first sound as the sign of systolic blood pressure. In the 3-year-old children systolic blood pressure was recorded as the pressure in the cuff at the point when the pulse sound was first heard (Korotkoff´s first sound) and the change in sound was used as the sign of diastolic blood pressure (Korotkoff´s fourth sound). Readings to the nearest even number of mmHg were performed. The average of the three readings of systolic and diastolic sound was used as the measure of blood pressure in analyses. We considered the current use of antihypertensive medication as an indication of high blood pressure.

Data on lifestyle risk factors such as smoking, alcohol consumption, physical activity, socioeconomic status and family history of coronary heart disease, hypertension or type 2 diabetes were obtained from questionnaires. Data on breastfeeding and birth measurements (height and weight) were obtained from the parents by a questionnaire in 1983 when participants were 6-21 years old. A positive family history was defined as mother and/or father having hypertension, type 2 diabetes mellitus or coronary heart disease at or before the age of 55 years. The information on smoking habits was collected in participants aged 12 years or older. Adolescents (aged 12 to 18 years in 1980) smoking on weekly basis or more often were considered as smokers, and those reporting consuming beer, wine or liquor once a week or more often, were classified as regular users of alcohol. In 2001 and 2007, participants smoking daily were considered as smokers.

In 1980, physical activity and participation in sports were collected using sef-report questionnaires from participants at 9 years or older. Physical activity index was calculated by assessing the frequency and intensity of leisure-time physical activity, participation in sport club training, participation in competitive sport events and common activity during leisure time using a modification from a method described by Telama et al (Telama *et al.* 1985). The answers were coded from 1 to 3 (participation in competitive sport events from 1 to 2) with a total score ranging from 5 to 14. In 2001, the physical activity questionnaire consisted of questions concerning frequency and intensity of physical activity, hours spent on vigorous physical activity, average duration of a physical avtivity session and participation in organized physical activity. Physical activity index was calculated in the same way as for the adolescent groups.

Childhood socioeconomic status (SES) was determined using information on parental education, occupation and family's income. Parental education was determined on the basis of school years completed, and it was classified into three groups: less than 9 years, 9-12 years and more than 12 years. Occupation status was coded by using the scale presented by the Central Statistical Office of Finland in 1979. The 14 original subgroups were combined to form five occupational classes: F, farmers; I, lower manual; II, upper manual; III, lower non-manual; and IV, upper non-manual. The annual income of the household was classified into three income groups: family´s income level being low, medium or high. The group of low SES included participants having parents with manual occupations (classes I or II), less

than 9 school years and low income level. The high SES group consisted of participants having parents with non-manual occupations (classes III or IV) with more than 12 school years, and whose income level was high. The medium SES group consisted of those who were not classified into the low or high SES. The participants from farming families were excluded from the classification. (Leino *et al.* 2000)

4.4 Biochemical analyses

All venous blood samples were taken from the right antecubital vein of recumbent participants after 12 hours fast. Serum or plasma was separated and stored at -70 ºC until analysis. Venous blood was sampled for the measurement of plasma concentrations of glucose, and serum concentrations of total cholesterol, HDL-cholesterol, triglycerides, apoA-I, apoB, insulin, CRP and sPLA2. All analyses were performed in the laboratory of the Research and Development Unit of the Social Insurance Institution, Turku, in 2001 and in the laboratory for the Population Research of the National Institute for Health and Welfare, Turku, in 2007.

4.4.1 Lipid and apolipoprotein measurements

All lipid determinations were done in duplicate in the same laboratory. Standard enzymatic methods (Olympus System Reagent; Germany) were used for serum cholesterol and triglycerides. Serum HDL-cholesterol concentration was measured from the serum supernatant after precipitation of VLDL and LDL with dextrane sulphate-$MgCl_2$. LDL-cholesterol concentration was calculated by using the Friedewald formula in samples where triglyceride level was below 4 mmol/l (Friedewald *et al.* 1972). ApoB and apoA-I were analyzed immunoturbidimetrically (Orion Diagnostica, Espoo, Finland). Interassay coefficient of variation (CV) was 2.19 % for total cholesterol, 2.28 % for HDL-cholesterol, 3.76 % for triglycerides, 2.8 % for apoB and 3.2 % for apoA-I.

Because of changes in determination methods and reagents, lipid levels from 1980, 1983, 1986 and triglycerides from 2007 were corrected to correspond to the samples taken in 2001 by using correction factor equations which were determined with linear regression analysis utilizing standardized principal components adjustments (Juonala *et al.* 2004a). No correction equations were needed for the 2007 total cholesterol, LDL-cholesterol and HDL-cholesterol levels.

Total cholesterol = 1.091 * total cholesterol (1980-1986) – 0.271 mmol/l.
HDL-cholesterol = 1.068 * HDL-cholesterol (1980-1986) – 0.0277 mmol/l.
Triglycerides = 1.00756 * triglycerides (1980-1986) + 0.0582 mmol/l.
Triglycerides = (triglycerides (2007) + 0.03226)/0.9811

4.4.2 Glucose and insulin measurements

In 2001 and 2007, glucose concentrations were analyzed enzymatically and serum insulin was measured by microparticle enzyme immunoassay kit (Abbott Laboratories,

Diagnostic Division, Dainabot). In 1986, serum glucose was measured with the β-D-glucose: NAD oxidoreductase method and in 1980, 1983 and 1986 serum insulin using a modification of the immunoassay method of Herbert et al (Herbert *et al.* 1965). Due to changes in methods or reagents from 2001 to 2007 glucose and insulin levels were corrected by using the following correction factor equations.

Glucose = (glucose (2007) – 0.0235)/ 0.9471
Insulin = insulin (2007) x 1.3728 – 0.8795

4.4.3 C-reactive protein and secretory phospholipase A2 measurements

In 2001, high-sensitivity serum CRP was measured by an automated analyzer (Olympus AU400) using a turbidimetric immunoassay kit (CRP-UL- assay, Wako Chemicals, Neuss, Germany). Childhood serum samples taken in 1980 were stored at -20 ºC. In 2005, they were analyzed using the same method as in 2001. During the storage, the samples were not thawed or refrozen. Details have been presented elsewhere (Juonala *et al.* 2006c). In analyses, participants with CRP levels more than 10mg/l (N=67) were excluded (Juonala *et al.* 2006c). Exclusion of these participants from the analyses did not affect the conclusions made from the data.

Serum sPLA2 enzyme activity was measured in 2006 from samples taken in 2001 and stored at -80°C by a selective fluorometric assay by using fluorescent substrate 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3 phosphomethanol, sodium salt (Interchim, Montluçon, France), as previously described (Mallat *et al.* 2005). One-hundred percent hydrolysis of the fluorescent substrate was measured using 0.1 U sPLA2 from bee venom (Sigma Chemical Co). The hydrolysis of substrate in the absence of plasma was used as negative control and deduced from sPLA2 activity. All samples were tested in duplicate and plasma activity was expressed as nmol/min/ml. The minimum detectable activity was 0.10 nmol/min/ml and the intra- and inter-assay CV was <10 %. The measurement of sPLA2 activity encompasses several types of sPLA2, including types IIA, V, and X (Mallat *et al.* 2007), that have been shown to be expressed in human and mouse atherosclerotic lesions and secreted to general circulation (Rosenson 2009).

4.5 Defining metabolic syndrome

In the present thesis, 3 definitions for MetS were used: the updated NCEP, EGIR and IDF. The definitions are presented in Table 1 and paragraph 2.1.1 Definitions of metabolic syndrome

4.6 Ultrasound studies (III, IV)

4.6.1 Carotid artery intima-media thickness

Carotid ultrasound studies were performed for 2,264 participants in 2001 and for 2,197 participants in 2007. A high-resolution ultrasound system (Sequoia 512, Acuson, CA,

USA) with 13.0 MHz linear array transducer was used. Physicians and ultrasound technicians performed all studies simultaneously in five centres. Carotid artery intima-media thickness (cIMT) was measured approximately 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 (Woo *et al.* 1999;Toikka *et al.* 2000). At least four measurements were taken at each scan of the common carotid artery incident with the R-wave of the continuously monitored electrocardiogram to derive mean carotid IMT (Figure 3). The scans were analyzed by a reader blinded to participants' details. The between visit (two visits three months apart) coefficient of variation (CV) of cIMT measurements was 6.4 % (Raitakari *et al.* 2003).

4.6.2 Carotid artery elasticity

To assess carotid artery elasticity, the best quality cardiac cycle was selected from the 5-second clip images. The common carotid diameter was measured from the B-mode images at least twice in end-diastole and end-systole (Figure 4). Ultrasound and concomitant brachial blood pressure measurements were used to calculate the carotid artery compliance, CAC =[(D_s-D_d) /D_d]/(P_s –P_d) (recently in the Young Finns publications, the term distensibility has been used interchangeably). In the CAC formula D_s stands for systolic diameter, D_d for diastolic diameter, P_s, for systolic blood pressure and $P_{d,}$ for diastolic blood pressure. CAC measures the ability of the arteries to expand as the response to pulse pressure caused by cardiac contraction and relaxation (Juonala *et al.* 2005). The between-visit coefficient of variation was 16.3 % for CAC and intra-observer CV was 13.6 %.

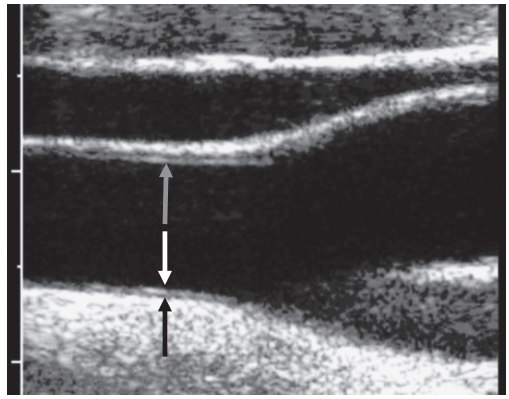

Figure 4. cIMT and CAC were measured from B-mode ultrasound scans. Area between the white (lumen-intima interface, far wall) and the black arrow (media-adventitia interface) indicates intima-media layer in the ultrasound image. The distance between near wall (grey arrow) and far wall (white arrow) of lumen- intima interface in diastole and systole was used to calculate the CAC.

4.6.3 Brachial flow-mediated dilatation

To assess brachial FMD, the left brachial artery diameter was measured both at rest and during the reactive hyperemia in 2,109 participants in 2001 (Figure 5). Due to insufficient image quality 147 scans were excluded. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 minutes, followed by release. 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 average of three measurements at each time point was used to derive the maximum FMD (the greatest value between 40 to 80 seconds). (Juonala *et al.* 2004b)

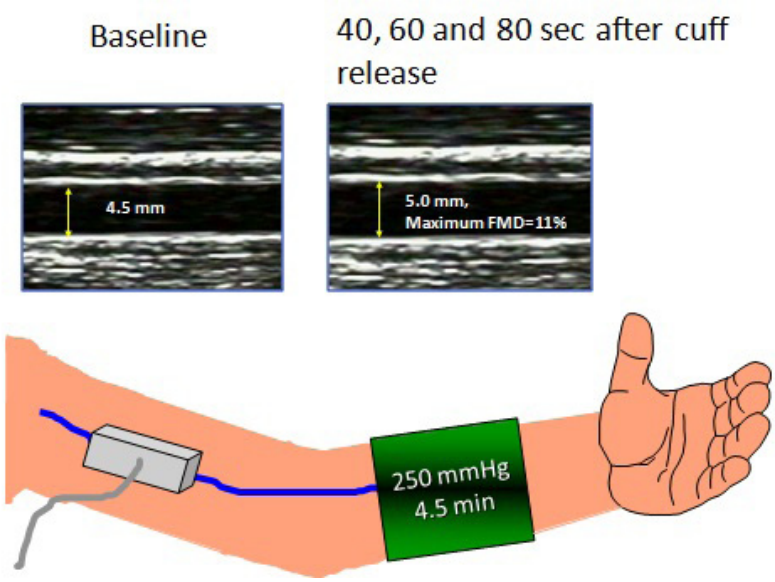

Figure 5. The brachial artery diameter was measured from ultrasound scans both at rest (baseline) and during the reactive hyperaemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the left forearm to a pressure of 250 mmHg for 4.5 minutes, followed by a release. Flow-mediated dilatation (FMD) is the increase in vessel diameter after reactive hyperemia as the percentage relative to the resting scan.

4.7 Statistical analyses

Values are expressed as mean and standard deviation (SD) for continuous variables and as proportions for categorical variables unless stated otherwise. Group comparisons were performed using *t* test for continuous variables and χ^2 test for categorical variables. Serum concentrations of triglycerides, insulin and CRP were log-transformed and sPLA2 square root (sqrt) transformed because of skewness before analyses. All data analyses

were carried out using SAS software (version 8.0, SAS Institute, Cary, NC, USA) and differences were considered statistically significant at 2-tailed P-value <0.05.

Study I:

The effect of age on MetS and its components was examined using logistic regression. The analysis of secular trend was made for participants aged 24-years in 1986 and in 2001 and, in addition in this thesis, for participants aged 30-39 years in 2001 and 2007 (Table 2). As waist circumference was not measured in 1986, it was estimated from the regression line between waist and body mass index in 2001. In men the regression equation was waist (cm) = 23.7+2.514*BMI (kg/m^2) (correlation r=0.931), and in women waist (cm) = 23.8+2.214*BMI (kg/m^2) (correlation r=0.901). The corresponding BMI cut-off point for a waist of 102 cm in men was 31.1 kg/m2, and for a waist of 88 cm in women 29.0 kg/m2. In order to analyse the secular trend of MetS between 2001 and 2007, the age cohort of 30-39-year-old participants in 2001 was compared with its counterpart in the 2007 follow-up. Because some of the participants belonged to both groups, statistical analyses were done separately for participants aged 30-33-years and 36-39 years.

Study II:

Stepwise logistic regression analysis was performed to establish the independent childhood and adolescent determinants of the adulthood MetS. In multivariable models, risk factors were defined as values at or above the age- and sex-specific 80th percentile for BMI, systolic blood pressure, triglycerides, insulin, CRP and at or below the age- and sex-specific 20th percentile for HDL-cholesterol. The longitudinal association of childhood obesity status with risk variables was assessed by repeated measures analysis of variance (adjusted for age, sex and Tanner's pubertal stage) to study childhood risk factors in obese participants with and without MetS in adulthood and to compare these groups at different time points. All statistical analyses were performed stratified by two groups according to the baseline age when the risk factor assessments were completed, in 3-9 and 12-18 year-olds. This age stratification paralleled pubertal staging, as 85 % of 12-18 year-olds at baseline were classified as having puberty on-going or completed (Raitakari *et al.* 2003).

Study III:

Mean cIMT, CAC and brachial FMD for each MetS definition was compared between groups using two-way analysis of variance (ANOVA). Linear regression models were used to examine the association of MetS and its components to ultrasound variables. There was no interaction between sexes in MetS definitions and ultrasound variables, indicating that the effects of MetS definitions on ultrasound markers were similar between sexes. Therefore, analyses were performed sexes combined and including sex as a covariate in regression models. All analyses were repeated after excluding participants taking lipid-lowering or antihypertensive medications, with essentially similar results.

Study IV

Linear regression was used to examine the associations between MetS components and apoB, apoA-I, (log)CRP, and (sqrt)sPLA2. These models included age and sex as covariates in addition to all MetS components. To assess the degree to which increased cIMT associated with MetS is mediated by apolipoproteins and inflammation, separate models, adjusted for each of apoB, apoA-I, (log)CRP, and (sqrt)sPLA2 in addition to age and sex, were created and subsequently all of these variables were added to the same model. To assist in interpretation, the percent change in MetS regression coefficient was presented for each model using the age and sex adjusted model as reference. These analyses were performed using linear regression for the continuous outcomes of 2001 and 2007 cIMT, and using logistic regression for the dichotomous outcome of incident high cIMT (0=those without high cIMT or plaque in 2001 who did not have high cIMT or plaque in 2007; 1=those without high cIMT or plaque in 2001 who developed high cIMT or plaque in 2007). To study the combined effects of MetS and high apoB, we estimated the relative risks of incident high IMT according to 4 groups: MetS(+)/high apoB (>90th percentile), MetS(+)/normal apoB (≤90th percentile), MetS(-)/high apoB with MetS(-)/normal apoB as the reference group.

4.8 Ethics

The Cardiovascular Young Finns Study was approved by the Joint Commission on Ethics of Turku University and Turku University Hospital. The participants gave written informed consent in 2001 and 2007 and their parents gave it in 1980.

5. RESULTS

5.1 Characteristics of participants

This thesis examined MetS and its relation to CVD risk factors and subclinical atherosclerosis (cIMT, CAC, brachial FMD) of more than 2000 young adults. Significant differences (P<0.0003) between participants with MetS and those without MetS were found for the components of MetS (waist circumference, blood pressure, HDL-cholesterol, triglycerides, glucose and insulin), as well as for other risk factors: BMI, total cholesterol, LDL-cholesterol, apoB, apoA-I, CRP and sPLAII enzyme activity (Table 3). Smoking habits were similar in participants with and without MetS. There was a significant sex-interaction in HDL-cholesterol (P=0.02), indicating that the association with MetS was dissimilar between sexes. Participants with MetS had lower HDL-cholesterol than those without the syndrome, but the relation was slightly stronger in women than in men (mean±SD in participants without MetS vs in participants with MetS, 1.42±0.28 vs. 1.12±0.26, P<0.0001 in women and 1.20±0.27 vs. 0.97±0.23, P<0.0001 in men). No other significant sex-interactions were observed. Therefore further analyses were performed mainly sexes combined.

Table 3. Clinical characteristics of study participants in the 2001 follow-up of the Young Finns study.

Variable	No MetS by any criteria	Updated NCEP	EGIR	IDF
N	1781	283	214	326
Sex (% men)	43.1	60.3	59.8	56.1
Age (years)	31.4±5.0	33.0±4.7	32.6±4.9	33.4±4.6
BMI (kg/m^2)	23.9±3.5	30.8±4.9	31.4±4.9	30.7±4.5
Waist (mm)	808±100	1001±113	1021±115	1001±109
Syst. blood pressure (mmHg)	115±12	129±14	128±15	129±14
Diast. blood pressure (mmHg)	69±9	82±12	81±12	80±12
Total cholesterol (mmol/l)	5.0±0.9	5.7±1.1	5.7±1.1	5.6±1.1
LDL-cholesterol (mmol/l)	3.2±0.8	3.6±0.9	3.6±0.9	3.6±0.9
HDL-cholesterol (mmol/l)	1.34±0.30	1.00±0.23	1.02±0.25	1.04±0.25
Triglycerides (mmol/l)	1.11±0.50	2.35±1.20	2.46±1.46	2.25±1.19
Glucose (mmol/l)	4.9±0.4	5.5±0.8	5.4±0.7	5.4±0.8
Insulin (mU/l)	6.5±3.5	14.1±9.0	16.9±9.1	13.4±8.8
ApoA-I (g/L)	1.51±0.25	1.36±0.20	1.38±0.23	1.38±0.22
ApoB (g/L)	1.00±0.22	1.33±0.26	1.35±0.26	1.32±0.26
ApoB/ApoA-I	0.68±0.19	0.99±0.20	0.99±0.20	0.97±0.21
C-reactive protein (mg/l)	1.6±3.7	3.2±4.7	3.6±5.1	3.2±4.7
sPLA2 activity (nmol/min/ml)	1.61±0.61	1.64±0.57	1.63±0.62	1.66±0.59
Smoking (%)	24.6	23.7	21.7	24.9

Data are presented as the mean value±SD or percentage of participants. All comparisons between NCEP-No MetS, EGIR-No MetS, and IDF-No MetS P<0.0001 except for age between EGIR-No MetSdr P<0.01 and for smoking all P=NS.

5.2 Metabolic syndrome and apolipoproteins and inflammatory markers

Aberrations in lipoprotein metabolism and increased systemic inflammation associate with MetS and may contribute to its atherogenicity. Therefore, it was specifically examined whether apoB, apoA-I, CRP and sPLA2 are associated with MetS in young adults. ApoB, CRP and sPLA2 enzyme activity levels were significantly higher and apoA-I levels lower in participants with MetS by any definition, when compared with those participants without the syndrome (all P≤0.002). The apoB, apoB/apo-I ratio and CRP levels increased and apoA-I levels decreased significantly with the number of components of MetS (P<0.0001).

Partial correlations between the components of MetS using the IDF definition and apoB, apoA-I, CRP and sPLA2, adjusted for age and sex, are shown in Table 4. ApoB, apoA-I and CRP correlated significantly with each component of MetS. Triglycerides and waist circumference showed a strong positive correlation (r=0.32-0.66, p<0.0001) with apoB and CRP. As expected, apoA-I correlated very strongly with HDL-cholesterol (r=0.85, P<0.0001) but there was only a weak relation between other components of MetS and apoA-I. sPLA2 correlated weakly, yet significantly, (r=0.06-0.09, P<0.006), with waist circumference and triglycerides, but not with the other components.

Table 4. Partial correlations (Spearman) between serum concentration of apoB, apoA-I, CRP and MetS components defined by the IDF definition.

MetS components	apoB		apoA-I		CRP		sPLA2	
	r	P-value	r	P-value	r	P-value	r	P-value
Waist	0.41	<0.0001	-0.16	<0.0001	0.43	<0.0001	0.06	0.006
Syst BP	0.15	<0.0001	0.07	0.0007	0.17	<0.0001	-0.02	0.38
Diast BP	0.21	<0.0001	0.05	0.017	0.17	<0.0001	0.004	0.86
HDL-chol	-0.23	<0.0001	0.85	<0.0001	-0.10	<0.0001	0.01	0.59
Triglycerides	0.66	<0.0001	0.06	0.0035	0.32	<0.0001	0.09	<0.0001
Glucose	0.14	<0.0001	-0.06	0.0039	0.07	0.0007	0.02	0.34
Insulin (EGIR)	0.34	<0.0001	-0.11	<0.0001	0.28	<0.0001	-0.005	0.83

Correlation coefficients are age and sex specific.

Age and sex adjusted regression coefficients between each of the MetS components and apoB, apoA-I, (log)CRP and (sqrt)sPLA2 are shown in Table 5 (Study IV: Table 2). Obesity, high triglycerides and hyperinsulinemia associated significantly with apoB and (log)CRP. Hypertension associated with (log)CRP but not with apoB. High triglycerides and low HDL-cholesterol were significantly associated with apoA-I. High triglycerides was a multivariable predictor of (sqrt)sPLA2.

Table 5. Multivariable relations between each component of MetS and apoB, apoA-I, (log)CRP and (sqrt)sPLA2 adjusted for age, sex and other MetS components in 2,180 men and women aged 24 to 39 years. (Study IV, Table 2)

Metabolic syndrome components (IDF) (absent-present)	ApoB		ApoA-I		(log)CRP		(sqrt)sPLA2	
	Beta±SE	P-value	Beta±SE	P-value	Beta±SE	P-value	Beta±SE	P-value
Obesity	0.080±0.011	<0.0001	-0.022±0.009	0.06	0.661±0.057	<0.0001	0.012±0.012	0.30
High TG	0.296±0.011	<0.0001	0.140±0.010	<0.0001	0.342±0.063	<0.0001	0.042±0.013	0.001
High insulin (EGIR)	0.045±0.012	0.0001	0.010±0.010	0.30	0.381±0.065	<0.0001	-0.012±0.013	0.37
Hypertension	0.012±0.011	0.30	-0.007±0.010	0.47	0.156±0.062	0.01	-0.017±0.013	0.19
Low HDL-chol	0.007±0.009	0.44	-0.338±0.008	<0.0001	-0.015±0.051	0.76	-0.004±0.010	0.69
High glucose	0.017±0.014	0.24	0.002±0.012	0.76	0.011±0.078	0.89	0.022±0.016	0.16

Regression coefficients indicate the change in apoB (g/l), in apoA-I (g/l), in (log)CRP (mg/l) and in (sqrt)sPLA2 enzyme activity (nmol/min/ml) for absence or presence of individual MetS components. Values of CRP were log transformed and values of sPLA2 were square root transformed before analyses. EGIR= European Group for the Study of Insulin Resistance definition (Balkau and Charles 1999) of high insulin (highest population specific quartile; >9 mU/L).

5.3 The prevalence of metabolic syndrome in young adults

In 2001, the overall prevalence of MetS in young adults, aged 24 to 39 years (N=2,182), was 13.0 % using the updated NCEP criteria, 9.8 % using the EGIR criteria and 14.9 % using the IDF criteria. The syndrome was more common in men (17.1 % with the updated NCEP, 12.8 % with the EGIR, 18.1 % with the IDF) than among women (9.5 %, 7.2 %, 12.1 %). About 82 % of the participants did not fulfill the criteria of MetS by any definition. The three criteria, used in the present study, partly overlapped each another (Study I: Figure 1). However, only 38 % of participants with MetS by any definition had MetS by all definitions.

Six years later, in 2007, the overall prevalence of MetS in 30-45-year-old participants (N=2,132) was 16.2 % using the updated NCEP criteria, 15.3 % using the EGIR criteria and 23.3 % using the IDF criteria.

The prevalences of MetS across age groups using three different definitions are shown in Figure 6. The prevalence of MetS was mostly higher among young adult men compared to women. In men, the prevalence of MetS increased significantly by age with all definitions both in 2001 and 2007. For example, there was over a 6-fold increase in MetS from 4.0 % to 25.2 % ($p<0.0001$) in men in 2001 between ages 24 and 39 years using the IDF criteria. There was also a significant age trend in women with the updated NCEP and the IDF definitions. When using the EGIR definition there was also significant age trend in men but not in women.

5.3.1 The prevalences of metabolic syndrome components

The prevalences of each individual component of MetS using the updated NCEP criteria and hyperinsulinemia using the EGIR criteria in 2001 and 2007 across different age groups are summarized in Table 6a and 6b. In 2001 and 2007, in both sexes, the prevalence of obesity increased with age, but more clearly in 2007 among 36-45 year-old women. There were also significant increases in hypertension and glucose by age in both men and women. In 2007, over 50 % of 39-45 year-old men had hypertension defined by the updated NCEP criteria. Men had significantly increased serum triglycerides contrary to women in both years. A large proportion of participants had low HDL-cholesterol in all age groups.

There were 16 possible combinations of metabolic risk factors that fulfill the updated NCEP criteria (Study I: Table 3). Component frequencies differed between sexes. Isolated low HDL-cholesterol and obesity were frequent in women and isolated hypertension, high glucose and high triglycerides in men. The most common combination of MetS risk factors using the updated NCEP definition was hypertension + low HDL-cholesterol + hypertriglyceridemia in men and central obesity + low HDL-cholesterol + hypertriglyceridemia in women.

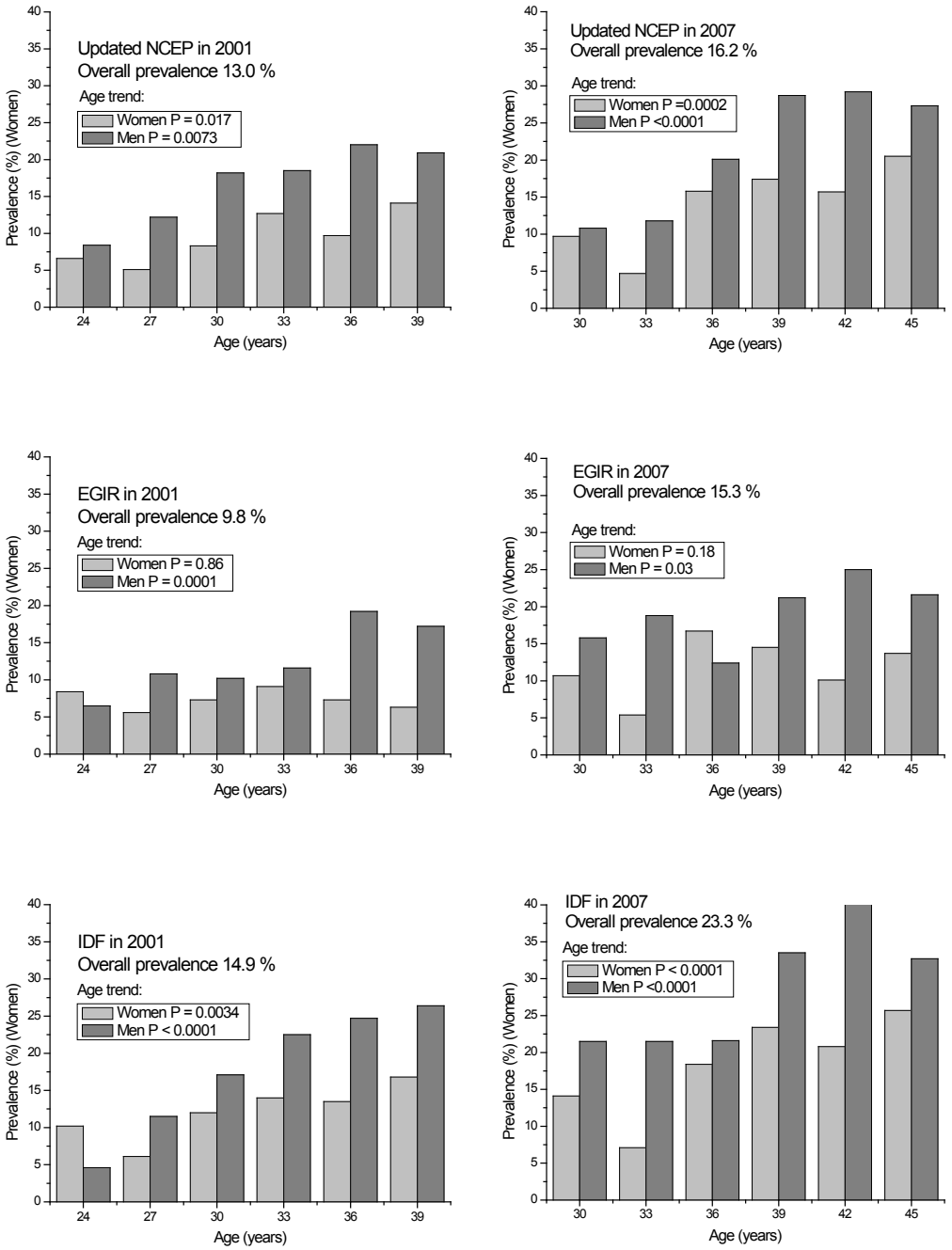

Figure 6. The prevalence of MetS in young adults in 2001 (N=2,182) and 2007 (N=2,132) using the updated NCEP; the EGIR and the IDF criteria across different age groups.

Table 6a. The prevalences of MetS components by the updated NCEP and hyperinsulinemia by the EGIR criteria in young adults (N=2,182) aged 24 to 39 years (year 2001).

		Age						
2001		24	29	30	33	36	39	Age trend
N	Men	154	148	187	173	182	163	
	Women	166	198	192	221	207	191	
Obesity, %	Men	6.5	8.8	12.3	16.2	13.7	22.7	<0.0001
	Women	14.5	14.7	19.8	24.4	17.4	24.6	0.0096
Hypertension, %	Men	20.3	22.3	30.0	28.9	34.6	36.8	<0.0001
	Women	7.2	10.6	9.9	17.2	12.1	18.3	0.0013
Low HDL-c, %	Men	29.4	40.5	39.6	37.0	36.3	28.2	0.48
	Women	41.0	39.4	43.8	39.4	40.6	40.8	0.96
High TG, %	Men	18.3	28.4	33.7	33.5	39.6	33.7	0.0004
	Women	20.5	13.1	15.1	14.9	12.6	13.6	0.12
High glucose, %	Men	7.2	11.5	17.7	17.9	19.2	25.8	<0.0001
	Women	2.4	3.0	6.3	6.8	9.2	11.0	<0.0001
High insulin, %	Men	24.8	20.3	24.1	22.0	26.4	21.5	0.99
(EGIR)	Women	29.1	19.7	22.9	20.8	18.4	22.5	0.28

Table 6b. The prevalences of MetS components by the updated NCEP and hyperinsulinemia by the EGIR criteria in young adults (N=2,132) aged 30 to 45 years (year 2007).

		Age						
2007		30	33	36	39	42	45	Age trend
N	Men	160	143	173	168	177	153	
	Women	157	192	184	225	213	187	
Obesity, %	Men	16.3	18.2	20.2	24.4	23.7	26.8	0.0078
	Women	21.0	24.0	32.6	36.4	31.5	35.8	0.0007
Hypertension, %	Men	36.5	35.0	32.4	53.0	55.4	52.9	<0.0001
	Women	16.6	11.5	14.7	22.2	25.8	32.1	<0.0001
Low HDL-c, %	Men	26.9	32.9	30.1	26.8	30.5	23.5	0.42
	Women	33.1	37.5	31.5	33.3	33.3	37.4	0.75
High TG, %	Men	24.4	29.4	27.8	39.3	40.1	36.0	0.0009
	Women	14.7	8.3	14.7	15.1	14.1	12.3	0.68
High glucose, %	Men	20.6	22.4	24.9	38.1	37.3	38.6	<0.0001
	Women	7.6	6.8	15.8	18.2	16.9	22.5	<0.0001
High insulin, %	Men	24.4	23.8	17.9	29.2	31.6	28.8	0.044
(EGIR)	Women	21.7	24.0	27.2	23.1	21.6	27.3	0.59

Updated NCEP definition for MetS is presented in Table 1. EGIR definition: hyperinsulinemia = fasting insulin level in the highest quartile (insulin >9.0 mU/l in 2001 and >10.8 mU/l in 2007).

5.4 Secular trends in metabolic syndrome

5.4.1 Changes between 1986 and 2001 among 24-year-old adults

The study design allowed examination of secular trends of MetS between 1986 and 2001 among 24-year-old adults and between 2001 and 2007 among 30-39-year-old adults (see Table 2). From 1986 to 2001, the prevalence of MetS, using the updated NCEP criteria, increased from 0.7 % to 7.5 % among adults aged 24-years (P<0.0001). When examined by sex, the prevalence increased from 1.6 % to 8.5 % in men and from 0.6 % to 6.6 % in women. Significant changes were observed in obesity, low HDL-cholesterol and hypertriglyceridemia. The prevalence of hypertension decreased from 30.5 % to 13.8 % (p<0.0001) between years 1986 and 2001. (Table 7 and Study I: Table 5)

Table 7. The prevalence of MetS and MetS components amongst 24-year-old adults in 1986 and 2001. (Study I: Table 5)

	1986 N=292	2001 N=320	P-value
Updated NCEP	1.0	7.5	<0.0001
Obesity	3.1	10.6	0.0003
Hypertension	30.5	13.8	<0.0001
Low HDL-cholesterol	8.2	35.6	<0.0001
High triglycerides	7.2	19.4	<0.0001
High glucose	1.0	5.0	0.0047
Hyperinsulinemia (EGIR)	28.8	26.3	0.49

Data are presented as percentages. Updated NCEP and EGIR definitions for MetS are presented in Table 1.

5.4.2 Changes between 2001 and 2007 among 30-39-year-old adults

Six-year changes of MetS and MetS components among 30-39-year old participants according to the updated NCEP definition are shown in Table 8. Between 2001 and 2007, MetS increased significantly among those aged 36-39 years, but not among those at 30-33 years. Among 36-39-year old individuals, there was also a significant increase in the prevalence of obesity, hypertension and high fasting glucose. Among 30-33-year-old participants, there were no increasing secular trends in the components of MetS. However, there was a significant decrease in the prevalence of high triglycerides in those at 30-33 years, but no change in 36-39-year-old adults. Between 2001 and 2007, the prevalence of low HDL-cholesterol decreased significantly in both age groups.

Table 8. The prevalence of MetS and MetS components in 30-39 year-old adults in 2001 and 2007.

MetS components	30-33 yr			36-39 yr		
	2001	2007	P-value	2001	2007	P-value
Updated NCEP	14.2	11.9	0.20	16.3	21.0	0.02
Obesity	18.4	21.7	0.10	19.9	30.7	<0.0001
Hypertension	21.0	24.0	0.17	25.2	30.4	0.03
Low HDL-cholesterol	38.8	32.1	0.009	36.2	30.9	0.03
High triglycerides	23.4	18.9	0.04	23.9	23.9	0.99
High glucose	11.7	14.0	0.18	15.6	23.6	<0.0001
Hyperinsulinemia (EGIR)	22.5	22.6	0.99	22.1	23.7	0.50

Data are presented as percentages. Updated NCEP and EGIR definitions for MetS are presented in Table 1.

5.5 Childhood predictors of metabolic syndrome

Childhood characteristics (year 1980 at baseline) of the study participants, stratified by baseline age group, with and without MetS in adulthood (year 2001) are shown in Table 9 (Study II: Table 1). Participants who had MetS in adulthood had higher skinfold thickness, BMI, systolic blood pressure, triglycerides, insulin, and lower HDL-cholesterol in childhood compared with participants without MetS. In males, participants with MetS were also older and had higher level of CRP compared to those participants without MetS. Moreover, participants with positive family history for hypertension and for type 2 diabetes were more prone to have MetS in adulthood.

Table 9. Childhood and adolescent characteristics of study participants with and without the IDF MetS in adulthood.

Characteristics in childhood and in adolescence	3-9 years at baseline				12-18 years at baseline			
	Men		Women		Men		Women	
	IDF -	IDF +	IDF –	IDF +	IDF -	IDF +	IDF -	IDF +
N	434	59	507	53	392	129	530	91
Age (years)	6.1±2.5	7.3±2.1‡	6.1±2.4	6.3±2.6	14.9±2.4	15.0±2.4	14.8±2.4	15.0±2.5
Skinfold thickness (mm) §	19.9±6.2	24.4±10.4†	23.4±7.9	30.4±13.3‡	22.4±10.0	28.8±13.2‡	33.2±11.7	38.8±14.9†
BMI (kg/m^2)	15.8±1.5	17.1±2.4‡	15.7±1.7	17.0±2.0‡	19.4±2.7	21.2±3.5‡	19.5±2.6	20.8±3.4‡
Syst. blood pressure (mmHg)	107±11	111±9†	107±10	110±11*	119±12	122±13†	116±10	118±9*
Diast. blood pressure (mmHg)	67±9	70±9*	67±9	69±9	70±9	71±11	69±9	71±9
Total cholesterol (mmol/l)	5.3±0.9	5.4±0.8	5.5±0.9	5.5±0.9	5.1±0.9	5.0±0.9	5.2±0.9	5.5±0.9*
LDL-cholesterol (mmol/l)	3.5±0.8	3.5±0.7	3.7±0.8	3.7±0.7	3.3±0.8	3.2±0.9	3.3±0.8	3.6±0.8†
HDL-cholesterol (mmol/l)	1.60±0.31	1.59±0.33	1.56±0.30	1.46±0.30	1.53±0.31	1.40±0.29‡	1.60±0.29	1.49±0.25†
Triglycerides (mmol/l)	0.57±0.25	0.65±0.39	0.61±0.23	0.75±0.31‡	0.68±0.30	0.80±0.41†	0.72±0.33	0.88±0.36‡
Insulin (mU/l)	6.0±4.0	8.0±4.4‡	6.5±3.7	7.7±5.3	11.0±5.2	13.3±6.7‡	13.4±5.5	15.4±6.3†
CRP (mg/l)	0.8±2.8	1.1±2.3*	0.8±1.4	1.0±1.8	0.6±1.4	0.8±1.1‡	0.5±1.1	0.5±0.7
Birth weight (g)	3560±570	3570±550	3450±530	3410±440	3540±530	3610±660	3440±530	3350±560
Birth height (cm)	50.6±2.3	50.7±2.3	49.9±2.2	50.1±1.7	50.6±2.0	50.9±2.4	50.0±2.4	49.7±2.2
Breast feeding (months)	4.0±3.6	3.6±3.6	3.8±4.2	3.4±3.0	3.6±4.0	4.2±3.8	3.8±3.7	4.1±3.5
Smoking (%)					17.3	20.3	15.8	8.0
Alcohol use (%)					6.9	6.5	3.7	2.3
Physical activity index					9.3±2.1	9.6±2.0	8.5±1.7	8.3±1.5
Family history of CHD (%)	14.1	15.3	14.0	18.9	30.1	34.9	33.2	35.2
Family history of hypertension (%)	40.2	55.4*	44.9	61.5*	51.8	68.9†	52.0	62.1
Family history of diabetes (%)	8.7	18.9*	13.0	20.0	20.7	30.0	18.2	33.3†
Socioeconomic status (%) low	3.4	8.0	5.9	13.0	9.9	13.1	9.8	4.5
medium	79.4	78.0	78.8	76.1	75.8	73.7	76.2	86.6
high	17.2	14.0	15.3	10.9	14.2	13.1	14.0	9.0

Values are mean±SD for continuous variables and as percentages for categorical variables. IDF definition for MetS is presented in Table 1. § Sum of biceps, triceps and subscapular. * P<0.05; † P<0.01; ‡ P<0.001

Mean levels of variables in childhood by number of MetS components present in adulthood are given in Table 10 (Study II: Table 2). Childhood risk factor levels showed significant positive trends in BMI, skinfold thickness, blood pressure, triglycerides, insulin and a negative trend in HDL-cholesterol with increasing number of MetS components.

Significant 21-year tracking correlations were observed between childhood/adolescent and adulthood metabolic risk variables. The highest Spearman's correlation coefficient was seen with HDL-cholesterol (r=0.45, P<0.0001), followed by BMI (r=0.41, P<0.0001), systolic blood pressure (r=0.30, P<0.0001), triglycerides (r=0.26, P<0.0001) and glucose (r=0.17, P<0.0001, 15-year correlation.

Table 10. Childhood and adolescent risk variables by number of IDF MetS components present in adulthood.

	Number of MetS components in adulthood (2001)					
Childhood risk variable	0	1	2	3	≥4	P for trend
N	724	687	408	237	139	
BMI (kg/m^2)	17.4	17.7	18.3	18.6	19.2	<0.0001
Skinfold thickness (mm)*	23.9	25.3	27.6	29.1	31.8	<0.0001
Syst. blood pressure (mmHg)	112	112	114	114	116	<0.0001
Diast. blood pressure (mmHg)	68	69	69	69	71	0.0018
Total cholesterol (mmol/l)	5.3	5.2	5.3	5.3	5.4	0.23
LDL-cholesterol (mmol/l)	3.4	3.4	3.5	3.5	3.6	0.095
HDL-cholesterol (mmol/l)	1.65	1.54	1.51	1.49	1.44	<0.0001
Triglycerides (mmol/l)	0.62	0.65	0.69	0.73	0.84	<0.0001
Insulin (mU/l)	9.0	9.5	10.3	11.1	12.5	<0.0001
CRP (mg/l)	0.6	0.6	0.7	0.8	0.7	0.0002

Values are adjusted for age and sex. IDF definition for MetS is presented in Table 1. * Sum of biceps, triceps and subscapular

5.5.1 Childhood and adolescent determinants of the adulthood metabolic syndrome

Multivariable logistic regression models were constructed to examine the independent contributions of childhood and adolescent risk variables to the development of adulthood MetS defined by the IDF criteria (Table 11, Study II: Table 3). In children (aged 3 to 9 years at baseline), childhood obesity, high triglycerides, family history of hypertension and age were independently associated with adulthood MetS (Table 11, model I). In adolescents (aged 12 to 18 years at baseline), the independent determinants of adulthood MetS included, obesity, male sex, high insulin level, family history of hypertension, high triglycerides, family history of type 2 diabetes and high CRP level (Table 11, model II).

The effect of all potential risk factors on association between childhood risk variables and adulthood MetS were taken into consideration in multivariable analysis. Risk variables that were not associated with adulthood MetS included elevated blood pressure,

low HDL-cholesterol, smoking, alcohol use, physical activity index, family history of coronary heart disease, birth size, breast feeding and parental socioeconomic status. Similar results were seen when adult MetS was defined by using the updated NCEP or the EGIR criteria.

Table 11. Childhood (Model I) and adolescent (Model II) characteristics that predict adulthood IDF MetS (N=1955). (Study II: Table 3)

Model I **3-9 years at baseline (N=958)**	**OR**	**95 % CL**	**P-value**
Obesity (BMI >80th ~17.0 kg/m^2)	3.0	2.0-4.7	<0.0001
High TG (>80th ~0.76 mmol/l)	2.3	1.5-3.6	0.0002
Family history of hypertension	1.8	1.1-2.7	0.009
Age	1.1	1.0-1.2	0.028
Model II **12-18 years at baseline (N=996)**	**OR**	**95 % CL**	**P-value**
Obesity (BMI >80th ~21.5 kg/m^2)	2.1	1.4-3.1	0.0002
Male sex	1.7	1.2-2.4	0.002
High insulin (>80th ~16.8 mU/l)	1.7	1.2-2.5	0.006
High TG (>80th ~0.95 mmol/l)	1.6	1.1-2.3	0.019
CRP (>80th ~0.7mg/l)	1.6	1.1-2.4	0.019
Family history of type 2 diabetes	1.6	1.1-2.3	0.021
Family history of hypertension	1.5	1.1-2.2	0.015

Initial stepwise logistic regression models also included childhood high systolic blood pressure, low HDL-cholesterol, smoking, alcohol use, physical activity, family history of coronary heart disease, birth size, socioeconomic status. OR = odds ratio, CL = confidence limit

To examine in detail the relation between early adiposity and MetS, the prevalence of MetS in young adults according to overweight/obesity status in childhood (at ages 3, 6 and 9 years) and in adolescence (at ages 12, 15 and 18 years) is provided in Table 12. The prevalence of adulthood MetS using the IDF definition was 14.3 % in participants who had been overweight in childhood and 31.3 % in those who had been obese in childhood. The prevalence of adulthood MetS was 40.3 % in participants who had been overweight in adolescence and 63.6 % in those who had been obese in adolescence.

Table 12. Prevalence of MetS defined by the IDF definition in adulthood (2001) according to the obesity status in childhood and in adolescence (1980).

Obesity status in 1980	MetS in adulthood	
	Childhood ages 3, 6 and 9 years in 1980	Adolescence ages 12,15 and 18 years in 1980
All	8.3 % (87)	12.7 % (144)
Lean	7.5 % (71)	10.3 % (108)
Overweight	14.1 % (10)	40.3 % (29)
Obese	31.3 % (5)	63.6 % (7)
Overall P-value	P=0.0006	P<0.0001

Data is presented as percentages (N). Obesity status is defined using the Cole's criteria (Cole *et al.* 2000).

5.5.2 Serial changes in metabolic and lifestyle variables from childhood to young adulthood

To examine which factors are associated with the development of adulthood MetS in obese children and adolescents, the longitudinal changes in risk variables from childhood to young adulthood were examined in initially obese participants with respect to adulthood MetS. The longitudinal serial changes in study variables in initially obese participants who developed and did not develop adulthood MetS are shown in Study II: Figures 1-6 along with the data in all study participants. At baseline, among 3 to 9 year-olds, there were 169 obese children, and 38 (22 %) of these participants were diagnosed with MetS in adulthood. At baseline, among 12 to 18 year-olds, there were 125 obese adolescents, and 38 (30 %) of these participants were diagnosed with MetS in adulthood.

Obese children and adolescents who developed MetS in adulthood had significantly higher BMI already during childhood and adolescence than those who did not develop MetS. They also increased their BMI more rapidly, especially between 1986 and 2001, i.e. during the transition into adulthood (Study II: Figure 1). Regarding insulin, the physiological state of insulin resistance during puberty was reflected as high insulin levels during adolescence in all participants as well as in obese participants (Study II: Figure 2). Normally insulin levels decrease after puberty and this normalization was seen in obese adolescents who did not develop MetS. In contrast, those individuals who developed MetS continued presenting with high insulin levels in adulthood (Study II: Figure 2).

The trends in triglycerides levels resembled those seen in BMI: a rapid increase especially during the transition from adolescence/early adulthood to adulthood (Study II: Figure 3). Obese children and adolescents who developed MetS had consistently lower HDL-cholesterol compared to those who did not develop the syndrome (Study II: Figure 5).

Development of systolic blood pressure levels is shown in Study II: Figure 4. Obese children and adolescents who developed MetS had a steady rise in systolic blood pressure by age. In comparison, obese individuals who did not develop MetS showed no change by age in systolic blood pressure. Their blood pressure changes over time resembled the trend seen in all participants. No significant differences were observed in the levels of physical activity indices between obese adolescents who developed and did not develop MetS (Study II: Figure 6). The smoking rates were also similar between obese participants who developed and did not develop MetS, and resembled the rates in all participants.

5.6 Metabolic syndrome and subclinical atherosclerosis

5.6.1 Metabolic syndrome and cIMT

MetS was cross-sectionally associated with higher cIMT in both sexes using any of MetS definitions. Study III: Figure I, Panel A shows the age- and sex-adjusted mean values of cIMT among those with and without MetS according to the IDF, the updated NCEP, and the EGIR definitions in 2001. The cIMT was lowest in participants without MetS by any criteria (0.576±0.088mm). Higher cIMT values were measured in individuals with the IDF (0.615±0.102mm), the updated NCEP (0.617±0.104mm) or the EGIR (0.607±0.097mm) criteria. There was a linearly increasing trend in cIMT across groups as the number of MetS components increased ($P<0.0001$). All definitions were significantly associated with cIMT in regression models adjusted for age and sex (Study III: Table 2).

In the IDF and the updated NCEP definitions, all the individual components, except low HDL-cholesterol, correlated significantly with cIMT. In addition, hyperinsulinemia, which is the key component of the EGIR definition, did not correlate with cIMT. Multiple regression models were constructed to the three criteria that included all the MetS components (Study III: Table 4). Obesity and hypertension were significantly associated with cIMT in all models.

5.6.2 Metabolic syndrome and carotid compliance

All MetS definitions correlated equally well with CAC (Study III: Figure 1, Panel B). In the univariate analysis, all MetS components correlated significantly with CAC. The multiple regression models for the 3 criteria, exploring the independent effects of the individual components on CAC are shown in Study III: Table 4. Obesity, hypertension and hyperinsulinemia were inversely correlated with CAC.

5.6.3 Metabolic syndrome and brachial FMD

Participants with MetS had slightly higher average FMD responses than participants without the syndrome, irrespective of the definition of MetS used (Study III: Figure 1, Panel C). Obesity associated directly with FMD both in univariate and multivariate models (Study III: Table 3 and 4). Hypertension defined by the EGIR criteria associated inversely with FMD. Other MetS components were not related to FMD.

5.6.4 Interrelations between brachial FMD and cIMT

Previous reports from the population of the Cardiovascular in Young Finns Study have shown that brachial FMD response may modify the association between conventional risk factors and cIMT (Juonala *et al.* 2004b). Therefore, it was decided to analyze whether the relation between MetS and cIMT is modified by the magnitude of the FMD response. Figure 7 (Study III: Figure 3) shows cIMT values in participants with and without IDF

categorized MetS according to their FMD response defined in 3 groups (Juonala *et al.* 2004b), impaired (lowest age and sex specific decile, n=206, FMD=1.1±1.4 %, mean±SD), intermediate (values between the 10th and 90th percentiles n=1601, FMD=7.8±2.9 %), and enhanced (highest age and sex specific decile n=206, FMD=15.9±2.8 %). MetS was associated with higher cIMT values in participants with impaired and intermediate FMD response. In contrast, participants with MetS and enhanced FMD response had cIMT values corresponding to the population mean (Figure 7). FMD response status modified the relation between MetS and cIMT (P for interaction 0.023). This significant interaction indicates that the strength of the association between MetS and cIMT is different across the categories of the FMD response. Similar results were seen using any of the diffrerent MetS definitions.

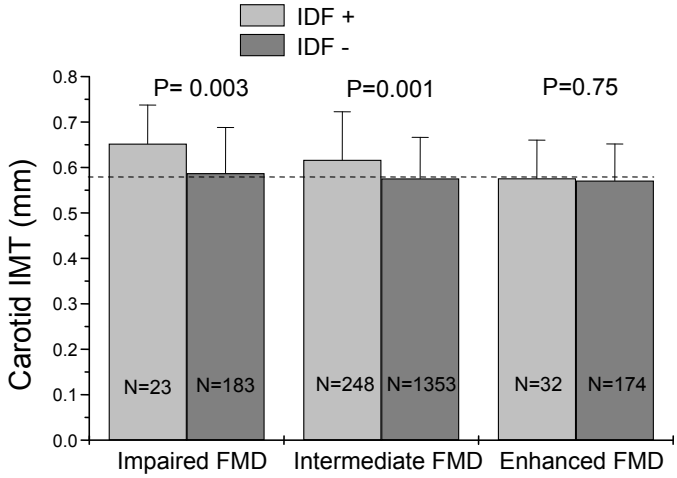

Figure 7. Association between brachial FMD (impaired, intermediate or enhanced FMD response) and cIMT (mean±SD) in young adults with or without MetS by IDF definition. Dashed line indicates the population mean. FMD response status modified the relation between the MetS and cIMT (P for interaction 0.023).

5.6.5 The effects of apo A-I and B and inflammatory markers CRP and sPLA2 on cIMT

To analyze whether the relations between MetS and cIMT are modified by apolipoproteins or inflammatory markers, multivariable analyses were performed with cIMT as dependent variable entering sequentially MetS, apoB, apoA-I, CRP, and sPLA2 enzyme activity as explanatory variables in the same model (Study IV: Table 3). In cross-sectional analysis of cIMT in 2001, the inclusion of apoB reduced the effect of MetS on cIMT by 18 %, followed by (log)CRP (14 %) and both apoA-I (1 %) and (sqrt)sPLA2 (1 %). When all variables were added to the same model, the association was attenuated by 32 %. Nevertheless, MetS remained a significant independent predictor of cIMT (P always <0.003). Similar findings were found when cIMT in 2007 was used as the outcome.

Figure 8 (Study IV: Figure 1) shows the effect of apolipoproteins A-I and B and inflammatory markers CRP and sPLA2 on the association between MetS in 2001 and incident high cIMT. The addition of apoB to the model reduced the association by 41 % such that the effect of MetS on incident high cIMT was attenuated to borderline significant (P=0.055). Otherwise, the inclusion of apoA-I, (log)CRP, or (sqrt)sPLA2 individually had more modest effects on the association between MetS and incident high cIMT and at no stage was the association attenuated to a level that was not statistically significant.

In the final multivariable models presented in Figure 8 (Study IV: Table 3 and Figure 1), apoB remained a borderline significant predictor of baseline cIMT (regression coefficient=15.8, P=0.06), and a strong independent predictor of cIMT in 2007 (regression coefficient=36.3, P<0.001) and incident high cIMT (standardized odds ratio, 95 % CI for apoB=1.33, 1.11 to 1.60, P=0.002). No evidence was found of an interaction between MetS and apoB in these models (all P>0.31).

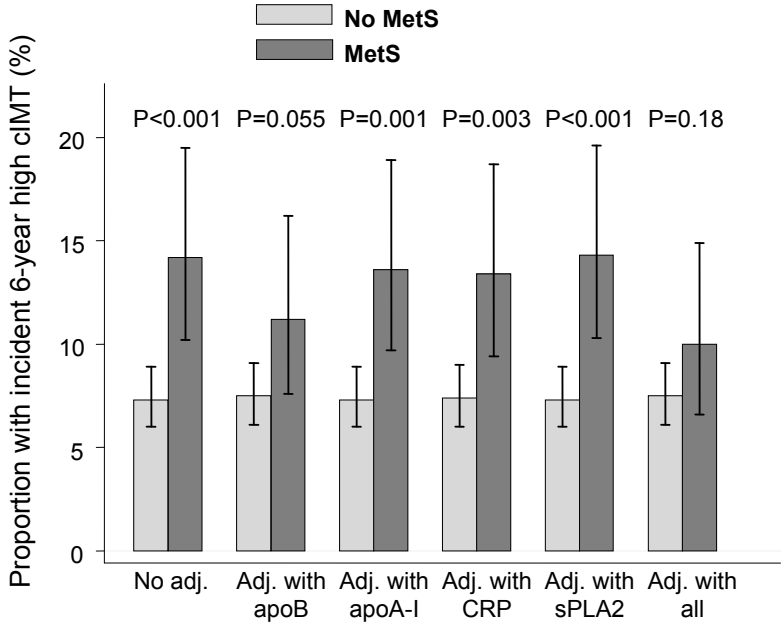

Figure 8. Proportion of participants with incident high carotid IMT after 6-years according to MetS status using the IDF criteria in 2001. All models are adjusted for age and sex. Additional models adjusted separately for apoB, apoA-I, (log)CRP, (sqrt)sPLA2, or all of these. Error bars indicate 95 % confidence intervals. Number of participants do not differ between models (N=1587). P-value expressed for absence/presence of MetS from logistic regression model. Incident high cIMT was defined as cIMT > 90th percentile and/or plaque. (Study IV: Figure 1)

To examine the contributions of Mets and high apoB to the incident high cIMT, the relative risk (RR) of incident high cIMT was calculated for the participants in 3 different groups: MetS(+)/high apoB, MetS(+)/normal apoB, and MetS(-)/high apoB groups compared to controls MetS(-)/normal apoB. The RRs were 3.41 (95 % CI 2.21-5.24) for participants with MetS(+)/high apoB, 2.30 (95 % CI 1.55-3.42) for participants with MetS(+)/normal apoB and 2.41 (95 % CI 1.45-4.01) for participants with MetS(-)/high apoB (Figure 9).

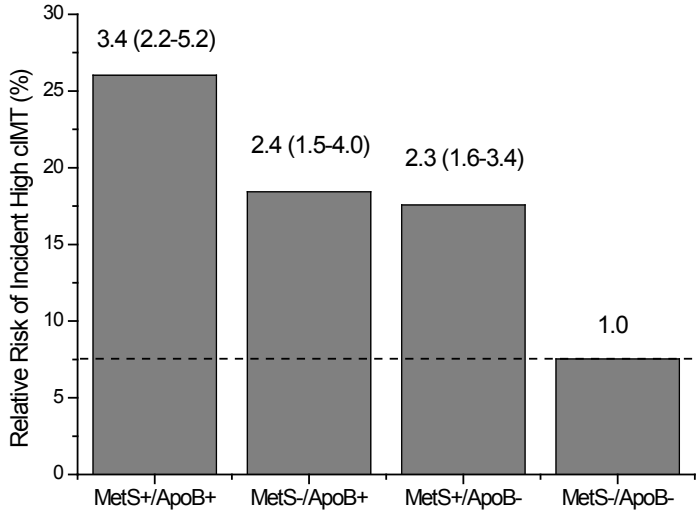

Figure 9. The relative risk of incident high cIMT according to different MetS defined by the IDF definition and apoB groups. IDF definition for MetS is presented in Table 1.

6. DISCUSSION

6.1 Participants

The present thesis is based on the Cardiovascular Risk in Young Finns Study, which is an on-going epidemiological study of CVD risk factors and precursors in children, adolescents and young adults. Originally, 4,320 participants aged 3, 6, 9, 12, 15 and 18 years, equal numbers from both genders, were invited to the first cross-sectional study in 1980. They were randomly selected from five different cities and from nearby rural areas, both eastern and western Finland, to represent Finnish children and adolescents as closely as possible. A total of 3,596 participants, 83.2 % of those invited, participated in the first follow-up and were considered to be representative of the total random sample (Åkerblom *et al.* 1985). The follow-up studies were performed for the whole study group in 1983, 1986, 2001 and 2007.

In a longitudinal study, it is inevitable that some participants are lost to follow-up. A total of 2,283 individuals (63.5 %) in 2001 and 2,204 individuals (61.3 %) in 2007 of the original study cohort, participated in the follow-up study. The analysis of loss to follow-up has been performed earlier and the main findings were that over half of those lost-to follow-up have taken part in at least some of the later follow-ups (Juonala *et al.* 2004a). Those participants who were lost to follow-up between 1980 and 2001 were more likely to be men and younger than those who participated at both time-points. When comparing non-participants and participants using age-adjusted analysis there were no significant differences in childhood risk factors between groups. Between 2001 and 2007, 223 men and 232 women were lost to follow-up. In both sexes, significant differences were found in systolic blood pressure and in the prevalence of smoking between non-participants and participants (Raiko *et al.* 2010). Female non-participants were also younger and had higher waist circumference than participants. According to these findings, selection bias may exist in the cohort. However, mean levels of subclinical atherosclerosis and the prevalence of MetS were similar in participants and non-participants suggesting that major biases are unlikely to affect the data interpretation.

Using data from different follow-up studies in analyzing secular trends in metabolic risk factors can be a source of error. When studying the changes between 1986 and 2001, the healthy participant effect may complicate interpretation of results. In the 2001 follow-up, 24-year-old participants had been in the study 15 years longer than those participants who were 24 years old in 1986. Because less healthy participants would be expected to be more likely to drop out, this might have affected the observed secular trends in MetS components. In particular, the observed significant decreasing trend in the prevalence of hypertension may actually be slower. However, all other MetS components increased significantly between 1986 and 2001 suggesting that this potential bias, related to

secular trends, did not have a remarkable effect on the findings. In the analyses of the secular trend between 2001 and 2007, statistical analyses were performed separately for participants aged 30-33 years and 36-39 years to avoid using data from the same participants in different study years. In conclusion, the original study population has been randomly selected, the sample size is sufficient for the statistical analyses and the selection bias does not seem to have substantially affected the findings.

6.2 Ultrasound methods

Carotid intima-media thickness (cIMT) is a method for detecting subclinical atherosclerosis and predicting cardiovascular risk in large-scale population studies. In addition, it is a safe, quick and reproducible measurement that correlates well with vascular risk factors (Davis *et al.* 2001;Raitakari *et al.* 2003), relates to the severity and extent of coronary artery disease (Burke *et al.* 1995) and predicts future cardiovascular events (O'Leary *et al.* 1999).

Between studies there is heterogeneity with respect to ultrasound methods, namely which carotid segments are investigated, should measurements be taken on one or both left and right carotid arteries, whether to use near or far wall, or if the mean or maximal cIMT measurements are to be used (Lorenz *et al.* 2007). There is no standardized protocol despite recent attempts (Touboul *et al.* 2007). In the present thesis, the anatomic landmark used as reference point was the beginning of the bulb widening. cIMT was measured approximately 10 mm from the bifurcation on the left common carotid artery and at least four measurements were used to derive mean cIMT. Nonetheless, cIMT was measured only from the common carotid artery, which may be less sensitive to local atherosclerosis than carotid bifurcation or the internal carotid segments (Solberg and Eggen 1971). Therefore, the present study may underestimate the relationships between MetS and carotid atherosclerosis.

The prognostic value of cIMT measurements to predict future cardiovascular events may increase when data from all 3 segments are combined (O'Leary *et al.* 1999). Results from the Framingham Offspring Study cohort showed that the maximum internal and mean common cIMT both predicted cardiovascular outcomes, but only the maximum cIMT of (and presence of plaque in) the internal carotid artery significantly (albeit modestly) improved the classification of risk of CVD (Polak *et al.* 2011). More recently, the Atherosclerosis Risk in Communities (ARIC) study showed that measuring common cIMT alone in concert with plaque information improves CHD risk prediction and is comparable to using all cIMT segments (Nambi *et al.* 2012). Moreover, the implementation of edge-detection wall-tracking software for the analysis of cIMT can reduce reader variation and improve reproducibility (Bartoli *et al.* 2008). The scans of the present study (both in 2001 and 2007) were analyzed by a reader blinded to participants' details (Raitakari *et al.* 2003). The reproducibility of cIMT measurements (the 3-month

between-visit coefficient of variation) in this study was 6.4 % which can be considered acceptable and is in agreement with other reports (Johnson *et al.* 2007).

Decreased arterial elasticity is considered an independent risk factor for CVD and has been implicated as a predictor for cardiovascular events (Haluska *et al.* 2010). Although the aging process itself affects arterial elasticity, other CVD risk factors including MetS are also important determinants. MetS has been reported to be associated with decreased elasticity in young, middle-aged and older participants (Scuteri *et al.* 2004;Li *et al.* 2005). Arterial elasticity can be noninvasively measured using pulse wave velocity (regional) or ultrasound-based distensibility (local) measurements. In this study, the carotid compliance, which measures the ability of the arteries to expand as a response to pulse pressure caused by cardiac contraction and relaxation, was used. A potential limitation of the study was that the pulse pressure was measured from the brachial artery to calculate carotid artery compliance. Because of pulse pressure amplification, this may not represent the carotid artery pulse pressure. It would be more ideal to measure pulse pressure directly from the carotid artery, because the use of brachial blood pressure can overestimate the pulse pressure in central arteries (Karamanoglu *et al.* 1993). Nevertheless, previous reports have shown excellent correlation between blood pressure measured invasively from the ascending aorta and noninvasively from the brachial artery (Borow and Newburger 1982) suggesting that the brachial pulse pressure can be used in calculating CAC. The long term variation for CAC was somewhat high (CV= 14.3 %), but comparable with earlier reports (Arnett *et al.* 1999). However, the CV of end-systolic carotid diameter was only 2.7 % supporting the assumption that most of the long-term variation in CAC is caused by physiological fluctuation in arteries and not measurement errors.

Brachial FMD is a widely used marker of systemic arterial endothelial function (Adams and Celermajer 1999). Endothelial integrity depends on the balance of all cardiovascular risk factors and in parallel vasculoprotective elements, including genetic predisposition and possible unknown factors (Bonetti *et al.* 2003). Despite its wide use as a research tool, problems with its reproducibility have limited its clinical use. Protocols for assessing brachial FMD may vary among different laboratories and are operator dependent. Ultrasound resolution, artefacts, and patient movements are the major sources of variation in FMD studies (Bartoli *et al.* 2008). Moreover, endothelial function is sensitive to several factors that change diurnally and even hourly, such as temperature, diurnal pattern in vascular tone, sympathetic nerve stimuli, menstrual cycle, fat-rich meal as well as reading variation (Corretti *et al.* 2002). In this study, there was a large long-term variation in FMD measurements, as the 3 month between-visit CV was 26 %. Nevertheless, it is comparable with values from other reports (Lind *et al.* 2000). However, it is quite obvious that the observed associations for FMD might have been stronger if the reproducibility of the measurements had been better.

6.3 The prevalence of metabolic syndrome (I)

MetS, using any of the three applied criteria (updated NCEP; EGIR; IDF), is common among young adults. There was a substantial increase in the prevalence of MetS in healthy young adults with age driven mostly by the increase in obesity. The prevalence was significantly higher among young adult men compared to women. In addition, participants with MetS had worse cardiovascular risk factor profile than those without the syndrome.

This study reinforces previous findings that MetS is increasing in Finland and throughout the western world (Ford *et al.* 2004;Grundy 2008). Lifestyle and behavioural changes during the last century, such as increasing obesity, sedentary lifestyle and excessively rich nutrition are mainly responsible. In the U.S, MetS typically affects 20-30 % of middle-aged adults (Ford *et al.* 2002;Meigs *et al.* 2003). Similar prevalences have been obtained from other populations (Cameron *et al.* 2004). Previously in Finland, in the FINRISK cohort, MetS was present in 38.8 % men and 22.2 % women (aged 45 to 64 years) and the prevalence increased in both sexes with age and abnormalities in glucose metabolism (Ilanne-Parikka *et al.* 2004). In the Kuopio Ischemic Heart Disease Risk Factor Study, the prevalence of MetS in participants aged 42-60 years was substantially lower (8.8 % to 14.3 % depending on the definition) compared to the FINRISK cohort (Lakka *et al.* 2002). This difference in prevalences is due to the data from the Kuopio Ischemic Heart Disease Risk Factor Study being collected already in 1980s and none of the participants having diabetes or CVD. Although there are several studies that have investigated the prevalence of MetS, few studies have specifically evaluated MetS in young adults; a population that is becoming more overweight (Ogden *et al.* 2006). More importantly, this study extends these findings to young adults.

In this study, the overall prevalence of MetS was 10-15 % among 24-39 year old adults (in 2001) and 15-23 % among 30-45 year-old adults (in 2007) depending on the definition used. There was a substantial increase in the prevalence of MetS with age. Previously, the Amsterdam Growth and Health Longitudinal Study demonstrated that the prevalence of MetS at the age of 36 years was 10.4 % (Ferreira *et al.* 2005). In addition, there was quite a large difference in prevalences between sexes (18.3 % in men vs. 3.2 % in women). Our findings confirm that the clustering of risk factors is stronger in men than in women among young adults.

Epidemiological studies have shown that the prevalence of obesity has increased widely from the 1990s to 2000 in many western countries, especially among U.S. adults (Flegal *et al.* 2010). Our findings confirm that obesity is becoming and almost is a major problem also in Finland. The prevalence of central obesity increased from 7 % to 23 % in men and from 15 % to 25 % in women between the ages of 24 and 39 years using the updated NCEP definition. The prevalence of obesity with the IDF and the EGIR definitions were 2-fold higher across all age groups than with the updated NCEP definition. For example, at the age of 39 years, 41 % of men and 45 % of women fulfilled the obesity criteria

by the IDF and the EGIR. This is a consequence of the different component thresholds for waist circumference, which is 94 cm for men and 80 cm for women using the IDF and the EGIR definitions and 102 cm and 88 cm using the updated NCEP definition. Furthermore, significant age trends were seen in the prevalence of hypertension and hyperglycemia in both sexes. Interestingly, the prevalence of hypertriglyceridemia increased with age in men but not in women. The prevalence of low HDL-cholesterol was high in Finnish young adults (approximately 30-40 %) in all age groups across both sexes. There was no age trend in low HDL-cholesterol levels.

There has been substantial debate concerning the issue of how to define MetS. Several organizations have proposed their own definitions for MetS using different components and cut-off points (Balkau and Charles 1999;Grundy *et al.* 2005;Alberti *et al.* 2005;Alberti *et al.* 2009). In addition, due to the lack of a unifying definition, reported prevalences of MetS have varied substantially. In this thesis, the updated NCEP, the IDF and the EGIR criteria were used. The components of the updated NCEP and the IDF criteria are easily ascertainable, as they do not require the measurement of insulin, which is an obligatory element using the EGIR definition. The IDF has lower criteria in some component cut-points such as waist-circumference and it emphasizes the role of visceral fat and waist circumference in diagnosing MetS. The threshold for impaired fasting plasma glucose has also been reduced from 6.1 to 5.6 mmol/l and all other definitions, except the EGIR, include the lower value in the definition of MetS. Although different definitions of MetS have minor differences in component cut points, different criteria identify different participants to meet the definition of MetS as showed in Study I: Figure 2. Component thresholds of the different criteria and the presence or absence of hyperinsulinemia are largely responsible for this. Among U.S. adults the use of the IDF definition of MetS was found to lead to a higher prevalence estimate than that based on the NCEP definition (Ford 2005a). The two definitions similarly classified approximately 93 % of the participants as having or not having MetS (Ford 2005a). In the present study there were more marked differences in the point estimates between these two definitions as the IDF and the updated NCEP definitions identified together approximately 66 % of the participants with MetS.

During recent years, strong criticism has been directed against the concept of MetS and especially its clinical utility (Kahn *et al.* 2005;Reaven 2011b). All metabolic risk factors defined by any criteria of MetS are not used as continuous variables but measured as presence or absence of an abnormality. This dichotomization may exclude too much information concerning the magnitude of risk factors (Eddy *et al.* 2008). Furthermore, there are 16 possible combinations of metabolic risk factors that fulfill, for example, the NCEP criteria. It is very unlikely that all of these combinations carry the same risk to develop CVD or type 2 diabetes, but it is more likely that risk can vary according to which components are present. It is at least as important to take into consideration other non-metabolic risk factors when stratifying an individual's total risk for future CVD events. However, there is increasing evidence that MetS is not a significant predictor

of CVD or type 2 diabetes after adjusting for the components of MetS suggesting that MetS does not increase CVD morbidity and mortality over and above its individual components (Koskinen *et al.* 2009).

In addition, MetS is inferior to the Framingham Risk Score and established type 2 diabetes prediction models (Stern *et al.* 2004). One likely reason may be that the diagnosis of MetS does not contain other well-established risk factors such as age, sex and smoking. Furthermore, the Framingham Risk Score and Diabetes Risk Score were specifically designed to be used as global risk assessment tools, whereas the main purpose of MetS is to identify individuals at future health risk (McNeill *et al.* 2005). Above all, regardless of controversy surrounding the MetS, identification of individuals with MetS may be useful from the clinical point of view, as the syndrome is highly prevalent in a population of young adults and it can be postulated that these participants would benefit from interventions aimed at reducing the risk of type 2 diabetes and CVD.

6.4 Secular trends in metabolic syndrome and its components (I)

The prevalence of MetS is increasing worldwide in all age groups. Ford et al. have previously reported that the prevalence of MetS has increased among U.S. Adults from 23.1 % (NHANES III, 1988-1994) to 26.7 % (NHANES 1999-2000) (Ford *et al.* 2004). In the present study, the prevalence of MetS at the age of 24-year-old adults increased substantially from 1986 to 2001 from 1.0 % to 7.5 %. The increase in MetS prevalence in 15 years was mostly caused by the increase in obesity. Increasing trends in hypertriglyceridemia and low HDL-cholesterol may also have contributed, although it is unclear if the changes are secondary to obesity.

The worldwide increase in the prevalence of obesity during the past decades is well documented (Mokdad *et al.* 2003;Berg *et al.* 2005;Flegal *et al.* 2010). In Finland, nationwide epidemiological health surveys, including the FINNRISK and the Mini-Finland Health Survey have shown the prevalence of obesity to have increased across all age groups but especially in men from the late 1980s to the early 2000s (Lahti-Koski *et al.* 2010). However, in the year 2000 obesity was still more common in women than in men (Lahti-Koski *et al.* 2010). On the other hand, in this study, the prevalence of hypertension decreased from 1986 to 2001. This finding is in line with previous epidemiological studies that have observed decreasing blood pressure levels over the last decades (Vartiainen *et al.* 2000;Gregg *et al.* 2005).

When the 6-year changes of MetS prevalence between 2001 and 2007 were investigated separately among 30-33-year-old and 36-39-year-old participants MetS increased significantly only in the age group of 36-39 year. This suggests that the increase in prevalence of MetS as well as the prevalence of obesity may have slowed down. Among 36-39-year-old participants, there was, however, a significant increase in obesity, hypertension and high fasting glucose whereas there was a significant decrease in low

HDL-cholesterol profile. It was remarkable that, the prevalence of high fasting glucose (glucose ≥5.6 mmol/l) increased between 2001 and 2007 substantially, from 16 % to 24 %, in 36- to 39-year old adults. In 30-33 year old individuals, there were no increasing trends in the components of MetS, but the prevalence of low HDL-cholesterol and high triglycerides decreased between 2001 and 2007.

6.5 Childhood predictors of metabolic syndrome (II)

Childhood obesity was the strongest predicting risk factor for the development of adulthood MetS. These data confirm the results of previous studies demonstrating that childhood obesity predicts the development of MetS in adulthood (Vanhala *et al.* 1999;Srinivasan *et al.* 2002). In addition to obesity, male sex, hyperinsulinemia, high triglycerides, family risk for hypertension, family risk for type 2 diabetes and elevated CRP values measured in childhood were independent predictors of adulthood MetS.

Obesity is strongly associated with insulin resistance and it increases the risk of type 2 diabetes and to a lesser extent CVD (DiPietro *et al.* 1994). Previously, the Harvard Alumni Health Study has shown a direct correlation between body weight and mortality (Lee *et al.* 1993). The prevalence of obesity is increasing worldwide in all age groups, including children and adolescents (Daniels *et al.* 2005). Overall, 17.1 % of 2-19 year-old U.S children are overweight (Ogden *et al.* 2006) and during the past two decades there has been a 3-fold increase in the prevalence of overweight in U.S. children and adolescents (Ogden *et al.* 2002b). Moreover, overweight children are at increased risk for adult obesity (Freedman *et al.* 2001;Juonala *et al.* 2006a). A previous report from the Young Finns Study showed that the risk of being obese in adulthood (BMI>30 kg/m^2) was increased three-fold among overweight or obese (BMI>80th percentile) children (ages 3-9 years) and four-fold among overweight or obese adolecents (ages 12-18 years) (Juonala *et al.* 2006a). Alarmingly, the incidence of type 2 diabetes and other morbidities reported in obese children has increased markedly in the past 20 years (Ogden *et al.* 2002a). A recent analysis of data from four prospective cohort studies clearly demonstrated that childhood overweight or obesity was predictive of type 2 diabetes, hypertension, dyslipidemia and increased cIMT in adulthood (Juonala *et al.* 2011). The data also showed that participants who were overweight or obese as children but who grew up to be nonobese as adults had a similar cardiovascular risk profile than participants who were never obese, suggesting that cardiovascular risk may be substantially reduced if childhood obesity is successfully treated (Juonala *et al.* 2011).

There is evidence that obesity is causally related to MetS. The effects of varying degrees of obesity on the prevalence of MetS was examined in a large cohort of children and adolescents, observing that the prevalence of MetS increased and each element of the syndrome worsened directly with the degree of obesity (Weiss *et al.* 2004). However, not all obese individuals will go on to develop MetS later in life. The pathophysiological mechanisms that predispose some obese individuals to develop MetS are not fully

understood. Potential underlying mechanisms may be inflamed adipose tissue, spillover of free fatty acids from adipose tissue etc. Actually, a substantial portion of overweight or obese persons are insulin sensitive without any of the metabolic abnormalities associated with insulin resistance (Reaven 2011b).

Because not all overweight and obese individuals appear to be at equal risk of developing MetS, the longitudinal serial changes of risk factor levels were studied in initially obese participants with and without MetS later in young adulthood. The serial data demonstrated that those obese children and adolecents who developed MetS had significantly higher BMI already during adolescence than those who did not develop the syndrome. They also gained weight more rapidly during transition into adulthood. In addition to obesity, elevated level of triglycerides in childhood was another individual component of MetS that independently predicted the risk of developing MetS in adulthood. The serial trends in triglyceride levels in obese individuals who developed adulthood MetS demonstrated a constant rise during childhood and adolescence, and a rapid increase during the transition into young adulthood. Elevation in triglyceride levels may thus be a specific marker of the early metabolic events related to the pathogenesis of MetS. In adults, the simultaneous occurrence of hypertriglyceridemia and obesity has been suggested as a high risk phenotype with atherogenic and diabetogenic profiles (Lemieux *et al.* 2000).

High insulin level in adolescence was also an independent risk factor for adulthood MetS. Previously, Raitakari et al. have shown in the Young Finns cohort that high fasting insulin measured in children predicted the clustering of high triglycerides, low HDL-cholesterol and elevated systolic blood pressure in a six-year follow-up (Raitakari *et al.* 1995b). The current observation with considerably longer follow-up provide more evidence for the idea that hyperinsulinemia may be causally related with the deterioration of lipid and blood pressure profile. In the serial data, the deviation in insulin metabolism was clearly seen in obese individuals who developed adulthood MetS. Normally insulin levels decrease after puberty, but this normalization was not observed among those individuals who continued to have elevated insulin levels throughout follow-up. These observations are in line with the reports from Bogalusa Heart Study showing that when insulin concentrations are increased in childhood they tend to remain elevated in adulthood, and those adults with consistently elevated insulin levels also tend to have a combination of increased obesity, hypertension and dyslipidemia (Bao *et al.* 1994)

In the present study, elevated blood pressure in childhood or adolescence was not an independent predictor of MetS in adulthood in models adjusted for several other metabolic risk factors. Recently, in the Fels Longitudinal Study, a direct relation between childhood blood pressure and MetS in adulthood was shown (Sun *et al.* 2007). The effect of childhood BMI on adulthood MetS was mediated by childhood blood pressure. These observations could not be confirmed in the present study. However, children and adolescents who eventually developed adulthood MetS had higher blood pressure levels at baseline compared to others. In addition, obese adolescents who developed MetS had a steady rise in systolic blood pressure by age.

Regarding inflammation and MetS, increased CRP is shown to associate with obesity (Wisse 2004) and is an independent predictor of cardiovascular events (Willerson and Ridker 2004). In the present study, elevated CRP level in adolescence was an independent predictor of adulthood MetS. Previous studies have suggested that children and adolescents with MetS show evidence of low-grade inflammation (Ford *et al.* 2005;De Ferranti *et al.* 2006). In the present study, the relation between elevated CRP and adulthood MetS remained independent in a multivariable model after adjustment for other confounding factors including obesity. Together, these observations point to the possibility that inflammation may play a role in the pathogenesis of MetS.

Other independent predictors of MetS in adulthood were family history for hypertension and type 2 diabetes. A previous study demonstrated that girls at the age of 13 who were classified having higher risk for MetS and its components, were more likely to have a family history of obesity, type 2 diabetes and gestational diabetes (Ventura *et al.* 2006). There are various potential mechanisms explaining the relations between MetS and positive family history. For example, there may be some underlying genetic variants that predispose some individuals to the development of MetS (Yang *et al.* 2007;Gomez-Abellan *et al.* 2008). Genes may also modify the impact of obesity and other conventional risk factors. Maintaining a high level of physical activity from youth to adulthood is associated with lower risk of abdominal obesity in women (Yang *et al.* 2006). In this study, the level of physical activity was not an independent determinant of adulthood MetS. In addition, there were no significant differences in the level of physical activity in serial measurements between obese adolescents who differed regarding metabolic syndrome status in adulthood.

Previous reports have shown that exposure to metabolic risk factors in adolescence may contribute to the development of structural and functional vascular markers of subclinical atherosclerosis (Raitakari *et al.* 2003;Juonala *et al.* 2005;Juonala *et al.* 2006b). Emerging data also link childhood exposure to metabolic risk factors to cardiovascular end-points decades later (Morrison *et al.* 2007;Juonala *et al.* 2011). The longitudinal Princeton LRC School Study demonstrated that children with a cluster of risk factors defined as pediatric MetS were significantly more likely to have CVD in adulthood than those without MetS (Morrison *et al.* 2007). These observations thus emphasize the importance of early identification of children and adolescents at increased risk of MetS.

6.6 Metabolic syndrome and carotid vascular changes (III)

MetS was associated with increased cIMT and decreased CAC in the population of asymptomatic young adults. All previously proposed diagnostic criteria for MetS equally identified subsets of individuals at risk for increased cIMT and decreased CAC, despite the overlap in their target populations. These findings indicate an increased burden of subclinical atherosclerosis and by inference, an increased risk of future cardiovascular events in young adults with MetS.

MetS has been previously linked to subclinical atherosclerosis in young adults in the Bogalusa Heart Study and in the Baltimore Longitudinal Study of Aging. In the Bogalusa Heart Study, cIMT was measured in 507 young adults aged 20 to 38 years showing that MetS, defined by either NCEP or WHO guidelines, was associated with increased cIMT (Tzou *et al.* 2005). In the same cohort, MetS and its components were associated with increased arterial stiffness in young adults (Urbina *et al.* 2004;Li *et al.* 2005). The Baltimore Longitudinal Study of Aging investigated the association between MetS and cIMT and stiffness in 471 participants, reporting that cIMT was 16 % and stiffness 32 % higher in participants with MetS compared to controls (Scuteri *et al.* 2004). The present thesis utilizing a population of over 2,000 participants confirms these findings. Recently, the Young Finns Study has demonstrated that MetS also associates with accelerated IMT progression in young adults (Koskinen *et al.* 2009).

The components of IDF and NCEP definitions are easily ascertainable, as they do not require the measurement of insulin or oral glucose tolerance test to allow diagnosis. In cohort used in this thesis, all the individual components of the updated NCEP and IDF definitions, except low HDL-cholesterol, correlated significantly with cIMT. All components by any MetS definition correlated significantly with CAC. However, hyperinsulinemia, which is generally considered as the primary underlying abnormality in MetS (Reaven 1988;Ferrannini *et al.* 1991) did not correlate with cIMT in this study population. Intuitively, the inclusion of a marker of insulin resistance to the diagnostic criteria of MetS should increase the predictive value for CVD and type 2 diabetes. Analysis of the NHANES data have suggested that the NCEP criteria may fail to detect a proportion of participants with insulin resistance (Ford and Giles 2003). Furthermore, the NCEP definition may be less sensitive than the WHO definition in predicting type 2 diabetes (Laaksonen *et al.* 2002a). Interestingly, findings from another study have shown that individuals with MetS defined by the NCEP were less insulin resistant but had higher future risk of cardiovascular disease than participants with MetS according to the WHO definition (Meigs *et al.* 2003). In the current cohort, however, all MetS definitions appeared to work equally well predicting cIMT.

Type 2 diabetes increases the risk of cardiovascular disease in women to a greater extent than in men (Juutilainen *et al.* 2004). One report has shown that the effects of MetS on cIMT were more pronounced in women than in men (Iglseder *et al.* 2005). However, no evidence was found in this study of an interaction by sex on the effects of MetS definitions on markers of subclinical atherosclerosis. In conclusion, MetS, which occurs very frequently in the general population, is burdened by a frequent incidence and progression of carotid atherosclerosis.

6.7 Interrelations between brachial flow-mediated dilatation and cIMT (III)

Conventional risk factors did not correlate very strongly with brachial FMD in the used cohort population (Juonala *et al.* 2004b). FMD is directly related with HDL-cholesterol

and BMI, and inversely with systolic blood pressure when these risk markers are considered as continuous variables (Juonala *et al.* 2004b). Previously, in the FATE study among 1,578 healthy middle-aged firefighters, there was an inverse correlation between FMD and blood pressure, but no correlations between FMD and other individual risk factors (Yan *et al.* 2005). In the present analysis, no evidence was found of an impaired FMD response among individuals with MetS. In fact, participants with MetS had higher average FMD responses due to the direct relation between FMD and body size, which was previously demonstrated in the same population (Juonala *et al.* 2004b). This was unexpected, because previous studies have linked obesity to impaired coronary and peripheral endothelial function (Al Suwaidi *et al.* 2001;Benjamin *et al.* 2004). Juonala et al. have earlier suggested that an increase in body size within the non-obese range in a population of healthy young adults may be associated with physiological changes that lead to enhanced FMD responses, and overcome the opposite influences of larger vessel size and increased oxidative stress associated with higher BMI's (Juonala *et al.* 2004b). Other possibility is that the relationship between body size and endothelial function is not linear (Higashi *et al.* 2003). In line with this, it was previously shown that the relationship between body size and endothelial function is curvilinear, and that the upward slope of this relationship can be observed in healthy adults (Juonala *et al.* 2004b).

Although participants with MetS did not have impaired endothelial function, the FMD response modified the relations between MetS and subclinical atherosclerosis. MetS was associated with higher cIMT in participants with impaired FMD, whereas participants with MetS and enhanced FMD had normal cIMT values, comparable to the population average. These findings indicate that systemic endothelial function may reflect the propensity of arteries to develop atherosclerotic changes in response to exposure to metabolic risk factors. Arterial endothelial damage or activation may be required before risk factors can induce atherosclerotic changes in the arterial wall. Conversely enhanced or preserved endothelial function may offer protection for arteries against the development of atherosclerosis.

6.8 The effects of apo A-I and B and inflammatory markers CRP and sPLA2 on cIMT (IV)

Changes in apoB and apoA-I concentrations are frequently seen in MetS and may importantly mediate the association between MetS and atherosclerosis. Several studies have suggested to include apoB, apoA-I and apoB/apoA-I ratio for risk assessment. Present study showed that young adults with MetS had increased serum apoB and decreased apoA-I values. Results showing elevated apoB and reduced apoA-I values in participants with MetS are in agreement with previous studies. Several studies have described that the elevated apoB/apoA-I ratio associates with MetS (Sierra-Johnson *et al.* 2006;Lind *et al.* 2006) and the insulin resistance (Sung and Hwang 2005;Sierra-Johnson *et al.* 2007). Lind et al. have investigated the prognostic independence of apoB/A-I ratio

and MetS for subsequent fatal and nonfatal myocardial infarction in a sample of 1,826 middle-aged men with over 20-year follow-up showing that both apoB/apoA-I ratio and MetS were independent predictors of the risk of myocardial infarction (Lind *et al.* 2006).

Previously, MetS has been associated with subclinical atherosclerosis indicated by increased cIMT suggesting a higher risk for future cardiovascular events (Tzou *et al.* 2005). In the present study, it was specifically examined to what extent the atherogenicity of MetS is explained by apolipoproteins A-I and B and inflammatory markers CRP and sPLA2. MetS associated with increased cIMT in cross-sectional and prospective analysis, and the association between MetS and incident high cIMT was considerably attenuated after controlling for apoB. These findings provide support for the concept that apolipoprotein A-I and B are significantly associated with MetS and they may provide additional information on atherogenicity over and above MetS. Particularly, in the participants with MetS, elevated apoB may intensify the development of subclinical atherosclerosis.

Previous study of 338 healthy 58-year-old men demonstrated that the likelihood of progressive change in cIMT was related to the apoB/apoA-I ratio (Wallenfeldt *et al.* 2004). In another study, the association between apoB/apoA-I ratio and cIMT was shown to be independent of conventional lipids, CRP and use of statins(Dahlén *et al.* 2009). Together, these observations indicate that these two apolipoproteins play an important role in the atherogenesis of MetS.

Chronic subclinical inflammation is shown to associate with MetS (Festa *et al.* 2000;Laaksonen *et al.* 2004). In addition, inflammation may be causally related to insulin resistance and development of atherosclerosis (Festa *et al.* 2000;Ridker *et al.* 2000). In the present study as well as in the previous studies (Ridker *et al.* 2003;Sattar *et al.* 2003), participants with MetS had elevated CRP levels. Among MetS components, obesity, high triglycerides, high insulin and hypertension were significantly associated with CRP levels. Novel to the present study was the observation of an association between sPLA2 enzyme activity and MetS. Previous studies have linked sPLA2 with insulin resistance and type 2 diabetes (Leinonen *et al.* 2003;Leinonen *et al.* 2004). In atherosclerotic lesions sPLA2 is synthesized by smooth muscle cells and macrophages and in close association with lesion lipid depots. sPLA2 may play a role in remodeling of HDL particles to become pro-atherogenic (Tietge *et al.* 2000), inducing release of pro-inflammatory lipid mediators and modifying apoB containing particles to a more atherogenic form thus enhancing lipoprotein retention and foam cell formation (Hakala *et al.* 2001). These potentially pro-atherogenic mechanisms could modify the risk associated to MetS. It is currently unclear whether inflammation is causally related to the development of atherosclerosis.

Most previous studies on this topic have examined the role of CRP. A previous study of healthy children reported that elevated serum CRP levels were associated with increased cIMT and decreased endothelial vasodilatory function (Järvisalo *et al.* 2002). However,

data from the Young Finns study has generally not supported the link between CRP and early atherosclerosis. Exposure to high CRP levels in childhood was not associated with increased cIMT in adulthood (Juonala *et al.* 2006c) and previous analysis using Mendelian Randomization approach failed to demonstrate a causal association between CRP and cIMT (Kivimäki *et al.* 2007). In line with these observations, a recent analysis did not observe a relation between genetically elevated CRP levels and ischemic vascular disease (Zacho *et al.* 2008). Nevertheless, other studies have shown that inflammation may play a pathophysiological role in MetS. Women with MetS had increased risk of peripheral artery disease that was largely mediated by the effects of CRP and another inflammatory marker soluble intracellular adhesion molecule-1 (Conen *et al.* 2009). In our population, however, neither CRP nor sPLA2 attenuated the association between MetS and cIMT. Therefore, our study provides new data suggesting that in young adults the inflammatory markers CRP and sPLA2 do not play a significant role in explaining the increased risk of atherosclerosis that is associated with MetS.

6.9 Strengths and limitations

The strength of the present thesis is its longitudinal population-based design with repeated data assessment. Moreover, physical, laboratory and ultrasound examination data were determined with well-established methods using a large number of participants. On the other hand, a potential limitation of the study is the non-participation in follow-ups that was discussed in more detail above (page 63). Another limitation is that waist circumference was not measured in 1980 and 1986, and for some analysis, it was estimated from the regression line between waist and BMI in 2001. Information on family history of coronary heart disease, type 2 diabetes and hypertension were collected in 2001, when participants were 24 to 39 years of age. Obviously, the rates for positive family histories would have been lower if collected in 1980. In addition, our study cohort was racially homogenous and therefore, results are generalizable only to white European participants.

7.0 Clinical implications

There are only a limited number of longitudinal studies that have followed healthy children from childhood to adulthood. Therefore, the observations made in the Cardiovascular Risk in Young Finns Study are unique and play an important role in contributing to the understanding of how early life risk factors translate into adult disease.

A major justification for a clinical definition of MetS is to identify participants with high risk of type 2 diabetes or CVD, leading to lifestyle or pharmacological intervention among those who may not otherwise be treated. However, there are no clear guidelines concerning the introduction of lifestyle modifications or drug treatment once an individual is found to have MetS. At present, the diagnosis of MetS, or the lack of it,

does not change the treatment of CVD risk factors in patients. It is still important for health professionals to keep MetS in mind to assess related risk factors when one risk factor is detected.

The present study showed that MetS occurs very frequently in young, apparently healthy, adults. The identification of these individuals with MetS may be useful from a clinical standpoint, as it can be anticipated that they would benefit from interventions aimed at reducing risk of future outcomes. In particular, a major effort is needed for the reduction of overweight in all age groups, children, adolescents and young adults.

In this study, adolescent determinants of adulthood MetS included obesity, family history of hypertension, family history of type 2 diabetes, high triglycerides, high insulin and high CRP. Identifying these risk factors in children and adolescents could be helpful in pediatric metabolic risk assessment. Especially, the assessment of childhood obesity, which continues to become more common, and can easily be measured, deserves to be the focus of intervention in clinical practice. Although not all obese children will develop MetS, the presence of obesity provides an opportunity to identify many children and adolescents at highest risk of developing MetS. Interventions targeted to this group may be most efficient toward prevention of associated future morbidity and mortality from type 2 diabetes and CVD.

Moreover, this study demonstrated that healthy young adults with MetS had greater cIMT and decreased carotid elasticity than those without the syndrome. Our results confirm previous findings that MetS, which occurs very frequently in the general population, is burdened by a frequent incidence and progression of carotid atherosclerosis. This study also showed that MetS was associated with increased cIMT in participants with impaired FMD suggesting that the status of systemic endothelial function may modify the relations between metabolic risk and atherosclerosis. In addition, the evidence reported in this study provides support for the concept that apolipoprotein abnormalities are significantly associated with MetS and they may provide additional information on the risk assessment for atherogenicity over and above MetS. Particularly, among individuals with MetS, elevated apoB may intensify the development of subclinical atherosclerosis.

7.1 Goals for the future research

The concept of MetS has been widely criticized. The main reason is the lack of consensus on how to define MetS. A major challenge is to establish how to best and easily define MetS, including which components and which component cut points should be part of the criteria and how they should be measured. It is still unknown which combination best predicts the risk of type 2 diabetes and CVD. The clinical utility has also been questioned. The currently used dichotomous approach may exclude too much clinically relevant information. Future studies should aim to develop novel risk scores for application both

in pediatric and adult populations that would take into account the magnitude of all risk factors, their interactions and also contributing factors.

Although the prevalence of MetS is very common and continuously increasing worldwide and it has been investigated widely, there are still many unknown factors underlying MetS. Further investigation is needed to discover detailed pathophysiological mechanisms that predispose vulnerable individuals to develop MetS. An increased understanding of these specific causes of MetS may also help to assess risk of developing CVD or type 2 diabetes. In addition, further genetic studies are needed to clarify the involvement of genetic variants associated with MetS. Future research should also focus on childhood determinants of metabolic risk and the relations between MetS and other clinical conditions such as fatty liver and polycystic ovary syndrome.

As the present study cohort is comprised of young adults without any clinical atherosclerotic disease, it has not been possible to study associations between risk factors and cardiovascular events. In the future, when participants start to develop cardiovascular events, such as acute myocardial infarction or stroke, it will be possible to further investigate these clinical end-points. It is also essential to gain more prospective data concerning the risk factors and changes in ultrasound markers especially in young people. Ultrasound measures are currently widely used for research purposes, but the clinical use is still limited.

8. CONCLUSIONS

1. MetS is common among young adults and increases substantially with age. A significant secular trend in MetS was observed for individuals aged 24 years between years 1986-2001 and that was driven mostly by an increase in the prevalence of obesity.

2. Childhood predictors of adult MetS included obesity, family history of hypertension, family history of type 2 diabetes, high triglycerides, high insulin and high CRP. Identifying these risk factors in children and adolescents could be helpful in pediatric metabolic risk assessment.

3. All diagnostic definitions of MetS identify subsets of young adults with greater cIMT and lower CAC indicative of increased burden of subclinical carotid atherosclerosis. Although brachial FMD response is not related to MetS, the status of systemic endothelial function seems to modify the relations between metabolic risk and atherosclerosis. Individuals with evidence of enhanced endothelial function may be protected against the development subclinical atherosclerosis in response to metabolic risk factors. In addition, the atherogenicity of MetS in this population assessed by incident high cIMT appears to be substantially mediated by elevated apoB but not the two inflammatory markers used CRP and sPLA2.

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