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METABOLIC SYNDROME

EARLY CARDIO-METABOLIC, VASCULAR AND HEPATIC CHANGES

The Cardiovascular Risk in Young Finns Study

by

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To my family

ABSTRACT

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Metabolic syndrome – early cardio-metabolic, vascular and hepatic changes. Departments of Medicine and Clinical Physiology, Turku University Hospital and the Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku. Annales Universitatis Turkuensis, Medica–Odontologica, Turku, Finland, 2011.

Background: Metabolic syndrome (MetS) is a combination of several cardio-metabolic risk factors including obesity, hyperglycemia, hypertension and dyslipidemia. MetS has been associated with increased levels of apolipoprotein B (apoB) and low-density lipoprotein oxidation (OxLDL) and with an increased risk of cardiovascular disease and non-alcoholic fatty liver disease.

Aims: To establish the relation of apoB and OxLDL with the MetS development and to determine the status of MetS as a risk factor for adverse liver changes and for subclinical atherosclerosis.

Subjects and Methods: The present thesis is part of the two large scale population-based, prospective, observational studies. Cardiovascular Risk in Young Finns study was launched in 1980 including 3,596 subjects aged 3-18 years. Thereafter follow-up studies have been conducted regularly. In the latest follow-ups that were performed in 2001 (N=2,283) and 2007 (N=2,204), non-invasive ultrasound studies were introduced to the study protocol to measure subclinical atherosclerosis i.e. carotid intima-media thickness (IMT), carotid artery distensibility (Cdist) and brachial flow-mediated dilatation (FMD). Alanine-aminotransferase (ALT) and gamma-glutamyltransferase (GGT) were measured in 2007 to assess liver function. The Bogalusa Heart Study is a long-term epidemiologic study of cardiovascular risk factors launched in 1972 in a biracial community of Bogalusa, Louisiana, USA. Total of 374 youths (aged 9-18 years at baseline in 1984-88) who underwent non-invasive ultrasound studies of the carotid artery as adults, were included in the analyses of the present thesis.

Results: The odds ratios (95% confidence intervals) for MetS incidence during a 6-year follow-up by quartiles of apoB were 2.0(1.0-3.8) for the second quartile, 3.1(1.7-5.7) for the third quartile and 4.2(2.3-7.6) for the fourth quartile. OxLDL was not independently associated with incident MetS. Youth (aged 9-18 years) with MetS or with high body mass index were at 2-3 times the risk of having MetS, high IMT, and type 2 diabetes 24-years later as adults. IMT increased 79±7µm (mean±SEM) in subjects with MetS and 42±2µm in subjects without the MetS ($P<0.0001$) during 6-years. Subjects who lost the MetS diagnosis during 6-year follow-up had reduced IMT progression compared to persistent MetS group (0.036±0.005vs.0.079±0.010 mm, $P=0.001$) and reduced Cdist change compared to incident MetS group (-0.12±0.05vs.-0.38±0.10 %/mmHg, $P=0.03$) over 6-year follow-up. MetS predicted elevated ALT (β±SEM=0.380±0.052, $P<0.0001$ in men and 0.160±0.052, $P=0.002$ in women) and GGT (β±SEM=0.240±0.058, $P<0.0001$ in men and 0.262±0.053, $P<0.0001$ in women) levels after 6-years.

Conclusions: These findings suggest that apoB may give additional information on early metabolic disturbances predisposing MetS. MetS may be used to identify individuals at increased risk of developing atherosclerosis and non-alcoholic liver disease. However, recovery from the MetS may have positive effects on liver and vascular properties.

Key Words: Metabolic syndrome, dyslipidemia, obesity, atherosclerosis, non-alcoholic fatty liver disease

TIIVISTELMÄ

Juha Koskinen
Metabolinen oireyhtymä - varhaiset muutokset kardiometabolisissa riskitekijöissä ja verisuonten sekä maksan toiminnassa. Departments of Medicine and Clinical Physiology, Turku University Hospital and the Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku. Annales Universitatis Turkuensis, Medica–Odontologica, Turku, Finland.

Tausta: Metabolinen oireyhtymä (MBO) on sydän- ja verisuonitautien riskitekijäkasauma, jonka keskeisiä piirteitä ovat keskivartalolihavuus, korkea verenpaine ja paastosokeri sekä rasva-aineenvaihdunnan häiriöt. MBO:n on todettu ennustavan suurentunutta valtimotaudin sekä ei-alkoholiperäisen rasvamaksan riskiä.

Tavoite: Tutkia lisääntyneen apolipoproteiini B pitoisuuden ja LDL hapettumisen yhteyttä MBO:n kehittymiseen, sekä MBO:n ominaisuutta ennustaa varhaisvaiheiden valtimo- ja maksamuutoksia.

Menetelmät: Tämä väitöskirjatutkimus toteutettiin osana laajaa, pitkittäistä Lasten Sepelvaltimotaudin Riskitekijät -projektia, jossa selvitetään sydän- ja verisuonitautien syntyyn vaikuttavia tekijöitä. Tutkimus alkoi vuonna 1980, jolloin 3 596 lasta ja nuorta (iältään 3-18-vuotiaita) osallistui. Viimeisimmät tutkimukset suoritettiin vuosina 2001 ja 2007, jolloin yli 2 000 tutkittavilta kartoitettiin varhaisvaiheiden valtimomuutoksia mittaamalla kaulavaltimoiden sisä- ja keskikerroksen paksuutta ja joustavuutta sekä olkavarsivaltimon sisäkalvon toimintakykyä. Alaniini-aminotransferaasi (ALAT) ja gamma-glutamyylitransferaasi (GT) arvot mitattiin vuonna 2007 maksan alkavan rasvoittumisen arvioimiseksi. Tutkimuksessa hyödynnettiin myös yhdysvaltalaisen vuodesta 1972 kerättyjä Bogalusa Heart -tutkimuksen aineistoja. Tässä väitöskirjatutkimuksessa mukana olivat ne 374 lasta ja nuorta, jotka olivat lähtötilanteessa 9-18-vuotiaita ja osallistuivat kaulavaltimon ultraäänitutkimuksiin 24-41-vuotiaina.

Tulokset: Suurentunut veren apolipoproteiini B pitoisuus (neljännessä kvartiilissa) lisäsi MBO:n kehittymisen riskiä 2-4 kertaiseksi. Hapettunut LDL kolesteroli ei ennustanut itsenäisesti MBO:n kehittymistä. Ne, joilla diagnosoitiin MBO lapsuudessa ja nuoruudessa olivat 2-3 kertaa suuremmassa riskissä potea aikuisena tyypin 2 diabetesta, MBO:ää sekä paksuuntunutta valtimoiden sisä- ja keskikerrosta (>90 %) verrattuna muihin. Suuren painoindeksin todettiin kuitenkin ennustavan yhtä hyvin aikuisiän sairastavuutta kuin MBO:n. Seurannan alussa MBO:n kriteerit täyttäneillä kaulavaltimoiden sisä- ja keskikerros paksuuntuivat merkitsevästi enemmän (79±7µm) kuin kriteerit täyttämättömillä (42±2µm) 6 vuoden aikana. MBO:sta toipuneiden kaulavaltimon sisä- ja keskikerroksen paksuuntuminen oli hitaampaa kuin oireyhtymää jatkuvasti poteneiden (0.036±0.005vs.0.079±0.010 mm, P=0.001). Kaulavaltimoiden joustavuus huonontui myös toipuneilla hitaammin kuin niillä, joille oireyhtymä ilmaantui seurannassa (-0.12±0.05vs.-0.38±0.10 %/10mmHg, P=0.03). Lisäksi MBO ennusti itsenäisesti suurentuneita ALAT (β±SEM=0.38±0.05, P<0.0001 miehillä ja 0.16±0.05, P=0.002 naisilla) sekä GT pitoisuuksia (β±SEM=0.24±0.06, P<0.0001 miehillä ja 0.26±0.05, P<0.0001 naisilla) 6-vuoden seurannassa.

Johtopäätökset: Tulosten perusteella apolipoproteiini B saattaa antaa lisätietoa alkavasta MBO:n kehityksestä. Diagnosoimalla MBO voidaan löytää potilaat, joilla on tulevaisuudessa suurentunut ateroskleroosin ja ei-alkoholiperäisen rasvamaksan vaara. Tosin MBO:ää potevien henkilöiden valtimoiden rakenne ja toiminta, sekä maksan toimintakyky voivat palautua MBO:n riskitekijöitä pienentämällä.

Avainsanat: Metabolinen oireyhtymä, rasva-aineenvaihduntahäiriö, lihavuus, valtimonkovettumatauti, ei-alkoholiperäinen rasvamaksa

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ABBREVIATIONS

apoB = Apolipoprotein B

apoA1 = Apolipoprotein A1

ALT = Alanine-aminotransferase

AUC = Area under receiver-operating characteristc curve

BMI = Body mass index

cMetS = Continuous metabolic syndrome risk score

Cdist = Carotid distensibility

CV = Coefficient of variation

CVD = Cardiovascular disease

CRP = C-reactive protein

EGIR = European Group for Insulin Resistance

FFA = Free fatty acid

FMD = Flow-mediated dilatation

GGT = Gamma glutamyl transferase

Harm = Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity

HDL = High density lipoprotein

HOMA = Homeostasis assessment index

IDF = International Diabetes Federation

IMT = Carotid intima-media thickness

LDL = Low-density lipoprotein

MetS = Metabolic syndrome

Mod = Modified

NAFLD = Non-alcoholic fatty liver disease

NCEP = National Cholesterol Education Program

NHANES = National Health and Nutrition Examintation Survey

NPV = Negative predictive value

NRI = Net reclassification improvement

OxLDL = Oxidized low-density lipoprotein

Peds = Pediatric

PPV = Positive predictive value

RR = Risk ratio

SD = Standard deviation

SEM = Standard error of the mean

T2DM = Type 2 diabetes mellitus

VLDL = Very low density lipoprotein

WHO = World Health Organization

LIST OF ORIGINAL PUBLICATIONS

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

I) **Koskinen J**, Magnussen CG, Würtz P, Soininen P, Kangas AJ, Viikari JSA, Kähönen M, Loo BM, Jula A, Ahotupa M, Ala-Korpela M, Juonala M, Raitakari OT. Apolipoprotein B, Oxidized Lipoprotein and Mean LDL Particle Size in Predicting Incident Metabolic Syndrome in Young Adults. The Cardiovascular Risk in Young Finns Study. *Europan Journal of Cardiovascular Prevention and Rehabilitation* 2011; published ahead of print

II) Magnussen CG, **Koskinen J**, Chen W, Thomson R, Schmidt MD, Srinivasan SR, Kivimäki M, Mattsson N, Kähönen M, Laitinen T, Taittonen L, Rönnemaa T, Viikari JS, Berenson GS, Juonala M, Raitakari OT. Pediatric Metabolic Syndrome Predicts Adulthood Metabolic Syndrome, Subclinical Atherosclerosis, and Type 2 Diabetes Mellitus but Is No Better Than Body Mass Index Alone. The Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study. *Circulation* 2010; 122:1604-11

III) **Koskinen J**, Kähönen M, Viikari JS, Taittonen L, Laitinen T, Rönnemaa T, Lehtimäki T, Hutri-Kähönen N, Pietikäinen M, Jokinen E, Helenius H, Mattsson N, Raitakari OT, Juonala M. Conventional Cardiovascular Risk Factors and Metabolic Syndrome in Predicting Carotid Intima-Media Thickness Progression in Young Adults: The Cardiovascular Risk in Young Finns Study. *Circulation* 2009;120:229-36

IV) **Koskinen J**, Magnussen CG, Taittonen L, Räsänen L, Mikkilä V, Laitinen T, Rönnemaa T, Kähönen M, Viikari JS, Raitakari OT, Juonala M. Arterial Structure and Function after Recovery from the Metabolic Syndrome: The Cardiovascular Risk in Young Finns Study. *Circulation* 2010;121:392-400

V) **Koskinen J**, Magnussen CG, Kähönen M, Loo BM, Marniemi J, Jula A, Saarikoski LA, Huupponen R, Viikari JS, Raitakari OT, Juonala M. Association of Liver Enzymes with Metabolic Syndrome and Carotid Atherosclerosis in Young Adults. The Cardiovascular Risk in Young Finns Study. *Annals of Medicine* 2011; published ahead of print

1. INTRODUCTION

The etiology of the MetS is multifaceted (Reaven, 1988). MetS is a constellation of several interrelated cardio-metabolic risk factors often described by obesity, hypertension, dyslipidemia, hyperglycemia and insulin resistance (Reaven, 1988; Balkau & Charles, 1999). These conditions coexist in an individual more often than might be expected by chance. Although controversy has surrounded both the pathophysiological basis and the clinical utility of the MetS (Kahn et al., 2005; Gale, 2005), it has been associated with an increased risk of developing type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Lakka et al., 2002; Gami et al., 2007). In addition, it has been hypothesized that the effect of MetS components are more than simply additive in the development of atherosclerosis. Evidence continues to accumulate as to the vast variety of clinical manifestations associated with the MetS in young adults (Wassink, Olijhoek, & Visseren, 2007; Eckel, Grundy, & Zimmet, 2005).

The concept of interrelated metabolic disturbances was first introduced 80 years ago as a constellation of hypertension, hyperglycemia and gout (Eckel et al., 2005). Ferrannini et al. stated in 1987 that essential hypertension correlates with impaired insulin-mediated glucose uptake (Ferrannini et al., 1987). One year later, Gerald Reaven proposed that insulin resistance and its compensatory hyperinsulinemia predisposed individuals to hypertension, dyslipidemia (high plasma levels of triglycerides and low levels of high-density lipoprotein particles) and diabetes thus being the underlying cause of CVD (Reaven, 1988). Since then the concept has progressively emerged into clinical practice and several definitions have been proposed to describe MetS as discussed in more detail later.

Due to high prevalence of CVD in Finland, the population-based, longitudinal study of Cardiovascular Risk in Young Finns was launched to assess the levels of cardiovascular risk factors and their determinants in children and adolescents of various ages in different parts of the country. The first cross-sectional (baseline) study was performed in 1980 in collaboration between five university hospitals (in Helsinki, Kuopio, Oulu, Tampere and Turku) and several other institutions in Finland. Total of 3,596 children and adolescents aged 3, 6, 9, 12, 15 and 18 years participated in the baseline study (Raitakari, 2008). The latest follow-up studies were conducted in 2001 and 2007 when ultrasound examinations were performed to assess early structural and functional atherosclerosis when the participants were young adults (aged 30 to 45 year at the 27-year follow-up in 2007).

In the present thesis the main objectives were to study 1) the early metabolic predictors of incident MetS, 2) the stability and effects of MetS from childhood to adulthood and 3) the associations of MetS and spontaneous recovery from the syndrome with early markers of adverse liver changes and vascular properties in young adults.

2. REVIEW OF LITERATURE

2.1 DEFINING THE METABOLIC SYNDROME

2.1.1 Definitions of the MetS

2.1.1.1 MetS as a pathophysiological model

Several attempts have been made to develop a set of components that constitutes MetS. Gerald Reaven proposed the first generally accepted concept of the MetS in his Banting lecture in 1988. Reaven hypothesized a pathophysiological construct where insulin resistance and its compensatory hyperinsulinemia predisposes clustering of hypertension, dyslipidemia and diabetes thus being the underlying cause of later T2DM and CVD in apparently healthy individuals (Reaven, 1988). Although, definition of obesity was not characterized, Reaven acknowledged the strong correlation between adipose tissue and insulin resistance. In addition, weight loss and physical activity were considered as the primary prevention and treatment of the MetS (Reaven, 1988). Next, the American Diabetes Association proposed that MetS is comprised of glucose intolerance, central obesity, dyslipidemia, hypertension, increased prothrombotic and antifibrinolytic factors. However, no specific cut-off points or definitions were determined.

2.1.1.2 MetS as a diagnostic category

In 1999, the World Health Organization published a definition of the MetS (MetS/WHO) for a research purposes with the aim to improve the recognition and comparability of the MetS. MetS/WHO was the first widely accepted definition of the MetS with specific thresholds including insulin resistance, obesity, dyslipidemia, hypertension and microalbuminuria (Alberti & Zimmet, 1998). Prior, research groups used their own modified set of components defining MetS. MetS/WHO emphasized the importance of insulin resistance as a central trait defining the syndrome. Insulin resistance was defined after 2-hour glucose tolerance test, which is an accurate method for defining insulin mediated glucose transport but unsuitable for clinical practice. The purpose of the definition of MetS by European Study of Insulin Resistance (MetS/EGIR) (Balkau et al., 1999) was to modify MetS/WHO to make it more ascertainable in clinical practice. MetS/EGIR used fasting insulin instead of 2-hour glucose tolerance test in defining insulin resistance and omitted microalbuminuria from the definition. In clinical practice, however, the use of MetS/EGIR may be limited, partly because the accurate measurement of insulin levels is difficult due to its interactions with liver and quick metabolism in the circulation. (Meigs, Haffner, Nathan, D'Agostino, & Wilson, 2001). Latest definitions of the MetS were the modified definition of the original National Cholesterol Education Program

by a joint expert group of the National Heart, Lung and Blood Institute and the American Heart Association (MetS/NCEP) (Lorenzo, Williams, Hunt, & Haffner, 2007), and International Diabetes Federation definition of the MetS (MetS/IDF) (Alberti, Zimmet, & Shaw, 2006). These definitions omitted the accurate measurement of insulin resistance and required only fasting assessment of plasma blood glucose. They also considered abdominal obesity as the central trait underlying the MetS. Therefore, MetS/NCEP and MetS/IDF that do not require measurement of insulin levels or oral glucose tolerance test may be more ascertainable in a clinical setting. However, the inclusion of a marker of insulin resistance/production may increase the power to predict increased CVD risk (Alberti et al., 1998; Balkau et al., 1999). Nevertheless, no unanimous consensus existed of the individual components that comprised the MetS or whether central obesity should be an obligatory trait in the MetS. The most recent definition proposed in a joint statement of the IDF Task Force on Epidemiology and Prevention, National Heart, Lung and Blood Institute (NHLBI), the American Heart Association, the World Heart Federation, the International Atherosclerosis Society, and the International Association for the Study of Obesity was to address this gap (Alberti et al., 2009). The new Joint Interim Societies definition of the MetS (MetS/Harm) is an attempt to unify criteria. It was agreed that there should not be an obligatory component. Three abnormal findings out of 5 would qualify a person for the MetS/Harm and for waist circumference national or regional cut points may be used. Definitions of MetS used in the present thesis are shown in *Table 1*. Although, the MetS as a diagnostic tool has received considerable attention over the last two decades, it has not provided conclusive evidence to support the clinical utility of the MetS as a diagnostic category.

Table 1

Guideline	Criteria for diagnosis	Central obesity	Hypertension	Dyslipidemia	Glucose/Insulin/ Other abnormality
MetS/WHO 1999	Presence of one of T2DM, impaired glucose tolerance or fasting glucose plus 2 other features	Waist/hip ratio > 0.90 (men); >0.85 (women) or body mass index >30 kg/m^2	≥140/90 mmHg	Triglycerides ≥ 1.7 mmol/l and HDL cholesterol ≤0.9 mmol/l (men); ≤1.0 mmol/l (women)	Microalbuminuria: urinary albumin excretion ≥ 20 µg/min or albumin:creatinine ≥ 30 mg/g
MetS/EGIR 1999	Insulin resistance plus 2 other features	≥94 cm (men), ≥80 cm (women)	≥140/90 mmHg**	Triglycerides ≥2.0 mmol/l and/or HDL cholesterol <1.0 mmol/l**	Insulin resistance: Fasting insulin in top 25 percentile and Plasma glucose >6.1 mmol/l
MetS/NCEP 2001	Any three features	>102 cm (men), >88 cm (women)	≥130/85 mmHg**	Triglycerides ≥1.7mmol/l and/or HDL cholesterol <1.0 mmol/l (men) <1.3 mmol/l (women)**	Plasma glucose ≥5.6 mmol/l**
MetS/IDF 2005	Central obesity plus 2 other features	≥94cm (men), ≥80cm (women)*	≥130/85 mmHg**	Triglycerides ≥1.7 mmol/l and/or HDL cholesterol <1.0 mmol/l (men) <1.3 mmol/l (women)**	Plasma glucose ≥5.6 mmol/l or previously diagnosed type 2 diabetes
MetS/Harm 2009	Any three features	>102 cm (men) >88 cm (women)*	≥130/85 mmHg**	Triglycerides ≥1.7 mmol/l and/or HDL cholesterol <1.0 mmol/l (men) <1.3 mmol/l (women)**	Plasma glucose ≥5.6 mmol/l or previously diagnosed type 2 diabetes**

***Population- and country-specific definitions (Alberti et al., 2009)**
** drug treatment is an alternate indicator

2.1.2 Issues concerning the concept of MetS

The concept of MetS has been criticized (Kahn et al., 2005; Gale, 2005). Constituent traits of the MetS are relatively common and they have been suggested to coexist in an individual independent of any common physiological antecedent. However, evidence from several epidemiological studies has shown that the components of MetS coexist in an individual more often than would be predicted by chance alone (Cornier et al., 2008). Several components of the MetS occur at a 2-fold higher degree than would be expected by chance. Further support for this aggregation comes from prospective studies using cluster analysis (Hanley et al., 2002).

Controversy has also surrounded the pathophysiologial basis of the MetS (Kahn et al., 2005; Gale, 2005). Kahn et al. stipulated the need for a single causation for the development of MetS (Kahn et al., 2005). The pathogenesis of the MetS however, is multifactorial in origin including underlying causes and exacerbating factors (Grundy, 2006). The development of MetS affected by a balance between cardiovascular risk factors, genetic factors, physical activity, dietary factors, age, sex and endocrine function (Eckel et al., 2005; Cornier et al., 2008). Although uncertainty about its underlying cause, the pathophysiology of the MetS is increasingly better understood and will be discussed later in detail. MetS is a condition that constitutes multiple risk factors that are metabolically interrelated and multifactorial in origin (Grundy, 2006).

Another matter of dispute is whether MetS is a useful marker of CVD risk over and above its individual components (Reaven, 2011). Studies have shown that fasting glucose concentration was as good as the diagnosis of the MetS in predicting T2DM and myocardial infarction (Wilson, D'Agostino, Parise, Sullivan, & Meigs, 2005; Sattar et al., 2008; Eddy, Schlessinger, & Heikes, 2008). Failure to predict T2DM and CVD have been suggested to inherent in being based on closely related abnormalities. Thus, as a diagnostic tool, MetS provides limited information on increased CVD risk (Reaven, 2011). However, several longitudinal studies have found that MetS was predictive of CVD after adjusting for its individual components (McNeill et al., 2005; Sattar et al., 2003; Schillaci et al., 2005; Sundstrom et al., 2006; Yusuf et al., 2005) and will be discussed later. It is also possible that part of the additional risk for CVD associated with the major risk factors of the syndrome is confounded by some unmeasured factors such as insulin resistance, oxidative stress, non-alcoholic fatty liver disease (NAFLD) and elevated apoB (Grundy, 2006). However, evidence whether the syndrome has any independent prognostic value over and above its components remains controversial (Kahn et al., 2005; Reaven, 2011; Sattar et al., 2008).

In addition, the dichotomization of risk factors included in the MetS has been criticized. Kahn et al proposed that risk assessment is a progressive function and may not be classified as present or absent (Kahn et al., 2005). Dichotomous variables in risk assessment may result in loss of crucial information concerning the magnitude of risk factors (Eddy et al., 2008). However, MetS definitions represent a simple way to identify individuals in clinical practice who are highly likely to have complex metabolic derangement thus having increased risk for CVD (Reaven, 1988). All people who meet either criterion deserve lifestyle intervention to reduce long-term risk for both CVD and T2DM and more detailed, short-term risk assessment for CVD such as Framingham Risk Score (Reaven, 2011).

Identifying the MetS in children and adolescents has recently been questioned because of the lack of established criteria in this population and evidence demonstrating marked short-term instability in the categorical diagnosis (Goodman, Daniels, Meigs, & Dolan, 2007; Steinberger et al., 2009; Gustafson et al., 2009). The complexity of pediatric growth patterns, effects of hormonal changes of puberty on insulin sensitivity and lipid profile make such criteria difficult to establish (Zimmet et al., 2007).

2.1.3 Prevalence

Due to the population ageing, global increase in obesity and sedentary lifestyles, the prevalence of the MetS is increasing in Finland and throughout the western world (Ilanne-Parikka et al., 2004; Grundy, 2008; Uusitupa, 2002; Cornier et al., 2008). The MetS affects approximately one-quarter of North Americans (Ford, Giles, & Dietz, 2002). Similar prevalence in MetS has been found in Scandinavian populations (Qiao et al., 2009). In the FINRISK cohort, that included 2,049 middle-aged individuals, MetS was present in 28.8 % of men and 22.2 % of women (Ilanne-Parikka et al., 2004). Mattsson et al. showed in the Young Finns study

population an increase in the prevalence of the MetS in ambulatory young adults already at the age of 24 driven mostly by the increase in obesity (Mattsson, Rönnemaa, Juonala, Viikari, & Raitakari, 2007). The overall prevalence in 24-year-old subjects was increased from 1.0 % in 1986 to 7.5 % in 2001. Similarly, the prevalence of MetS has increased in adults. According to NHANES study, the prevalence of the MetS in the USA increased in middle aged adults from 23.1 % (1988-1994) to 26.7 % (1999-2000) (Ford, Giles, & Mokdad, 2004).

MetS becomes more prevalent with increasing age paralleled by an increase in age-associated diseases and disabilities (Lechleitner, 2008). Previous studies have found that the MetS prevalence increased with age into approximately 60-75 years (Ford et al., 2004; Cameron, Magliano, Zimmet, Welborn, & Shaw, 2007). The plateau in prevalence estimates after the sixth and seventh decade is likely due to survival effect (Cornier et al., 2008).

Prevalence of the MetS is also dependent on the definition used, sex, race and ethnicity. Prevalence estimates between men and women have been somewhat different across cohorts. In white population, age-adjusted sex-related differences in MetS prevalence seems to be minor (Ford et al., 2002; Ilanne-Parikka et al., 2004). In addition, sex-related differences in MetS prevalence throughout the world are not consistent (Cornier et al., 2008). Differences may be due to cultural differences and different waist circumference thresholds used in different populations (MetS/IDF and MetS/Harm). In NHANES cohort, the prevalence of the MetS in women was caught up and then exceeded the prevalence in men after the age of 60, suggesting an interaction between age and sex on the prevalence of the MetS (Ford et al., 2004). Previous reports have found that prevalence using the MetS/IDF definition was often somewhat higher compared to other MetS definitions (Cornier et al., 2008). This discrepancy may be a consequence of different component thresholds used in different definitions.

Due to the lack of established criteria for pediatric MetS the prevalence estimates varies among children according to the age and the definition used in the study (Cornier et al., 2008). Among U.S. adolescents MetS was found highly prevalent in overweight subjects (32.1 %) (Duncan, Li, & Zhou, 2004). In addition, the overall prevalence among adolescents increased from 4.2 % (1988-1992) to 6.4 % (1999-2000) (Duncan et al., 2004). Further, the prevalence of overweight and obesity has also increased. The age-standardized prevalence of overweight (BMI ≥ the 85th percentile cut-off point for age- and sex-specific group) in children and adolescents (12-18 years old) during 22-years has increased from 4.0 % to 9.8 % in girls and from 7.2 % to 16.7 % among boys (Kautiainen, Rimpelä, Vikat, & Virtanen, 2002).

2.2 PATHOPHYSIOLOGY OF THE METABOLIC SYNDROME

The mechanisms underlying the MetS are not fully known (Eckel et al., 2005). The role of sedentary lifestyle habits in the development of MetS is well established (Reaven, 1988; Park et al., 2003). MetS is a complex syndrome affected by genetic and environmental factors, as well as their interactions. Although MetS is believed to have multifactorial causation, the most

accepted and unifying hypothesis to describe the pathophysiological basis of the MetS is insulin resistance and abdominal obesity (Reaven, 1988; Eckel et al., 2005; Wassink et al., 2007). In addition, the pathogenesis of hypertension as part of the MetS is only partly understood (Laaksonen et al., 2008).

Insulin resistance can be defined as an insufficient insulin action in liver, skeletal muscle and adipose tissue. Insulin resistance induces increased gluconeogenesis in the liver, decreased glucose disposal in the muscle, endothelial dysfunction in the arteries and increased release of free fatty acids (FFAs) from the adipose tissue. Impaired insulin action leads to a compensatory increased production of insulin thus leading to hyperinsulinemia to maintain euglycemia. If this compensatory mechanism fails, hyperglycemia will occur (Reaven, 2005).

Elevated levels of circulating FFAs contribute to the development of insulin resistance by inhibiting insulin signaling (Wassink et al., 2007). Plasma FFAs are derived mainly from adipose tissue by the action of lipases (Eckel et al., 2005). Insulin inhibits lipolysis in adipose tissue and glucose production in the liver (Jensen, Caruso, Heiling, & Miles, 1989). Thus, when insulin resistance develops, the inhibitory effect of insulin on lipolysis is suppressed. The increased amount of lipolysis in adipose tissue produces more FFAs, which could further inhibit the antilipolytic effect of insulin, creating additional lipolysis. In addition, increased FFAs may result in ectopic lipid formation in the liver leading to the non-alcoholic fatty liver disease that will be discussed later in detail. Ectopic lipid accumulation in liver, muscle and pancreas further increases insulin resistance in these sites (Eckel et al., 2005).

Adipose tissue is not merely a passive storage depot, but functions as a highly active metabolic organ producing FFAs and vast variety of bioactive molecules. In the case of obesity, particularly abdominal obesity, the release of FFAs is increased. In addition, there is an increased production of several inflammatory cytokines and reduced production of anti-inflammatory adipokines (Eckel et al., 2005; Ross, 1999; Wassink et al., 2007; Cornier et al., 2008). This imbalanced production of inflammatory cytokines favors not only the inflammatory state associated with obesity but also induces insulin resistance by impairing insulin signalling transduction (Wassink et al., 2007).

In the case of insulin resistance, the increased flux of FFAs to the liver increases the hepatic production of apoB containing triglyceride rich VLDL particles. ApoB serves as a structural protein for cholesterol and triglyceride containing lipoproteins that are carried from the liver to the site of use, whereas apoprotein A1-containing particles mediate the reverse transport from the peripheral tissue to the liver (Bamba & Rader, 2007; Ginsberg, Zhang, & Hernandez-Ono, 2006). Several studies have recently identified hepatic VLDL overproduction as a critical underlying factor in the development of metabolic dyslipidemia (Bamba et al., 2007; Walldius & Jungner, 2006; Adiels, Olofsson, Taskinen, & Boren, 2008). The presence of hypertriglyceridemia induces changes in lipoprotein composition and reduction of HDL cholesterol. The composition of LDL is modified producing small dense LDL (SdLDL) (Barter

et al., 2003). Potential atherogenic mechanisms of SdLDL particles low affinity to the LDL receptor (Chen et al., 1994) and long retention time in the circulation (Packard et al., 2000). In addition, SdLDL contains relatively smaller amounts of antioxidants compared to LDL cholesterol and therefore is more prone to oxidation (Esterbauer, Gebicki, Puhl, & Jurgens, 1992) forming oxidized LDL (OxLDL) particles.

Recent studies have suggested that OxLDL may have an important role in the pathogenesis of obesity and insulin resistance (Holvoet, Lee, Steffes, Gross, & Jacobs, Jr., 2008). Further, all MetS components have been found to increase oxidative stress (Halle et al., 1999; Park et al., 2009) which may induce the production of OxLDL. Previously Maddux et al. (Maddux et al., 2001) exposed cultured rat muscle cells to oxidative stress and found that the insulin stimulation of glucose transport was abolished, suggesting a link between insulin resistance and oxidative stress. Another biological study by Masella et al. (Masella et al., 2006) reported that oxidative stress affected the balance between cultured adipose cell proliferation and differentiation. They proposed causative role for oxidative stress in obesity and its clinical complications. Recently, Holvoet et al. found an association between OxLDL and incident MetS independent of potentially confounding factors during a 5-year follow-up suggesting a causal effect of OxLDL on MetS (Holvoet et al., 2008).

Hypertension have been suggested to relate to insulin resistance in several mechanisms (Wassink et al., 2007; Eckel et al., 2005; Reaven, 1988). First, in the presence of insulin resistance the vasodilatory effect of insulin (Westerbacka, Seppälä-Lindroos, & Yki-Järvinen, 2001) in the endothelium may be suppressed resulting vasoconstriction (Montagnani & Quon, 2000). Compensatory hyperinsulinemia increases the activity of the sympathetic nervous system, where the effect on insulin action is preserved (Egan, 2003). Renal sodium reabsorption in the kidney is increased directly by adipose tissue (Sharma, Janke, Gorzelniak, Engeli, & Luft, 2002) and via increased sympathetic nervous activation. In addition, FFAs produced by adipose tissue may directly mediate vasoconstriction (Tripathy et al., 2003). Adipocytes also produce a variety of vasoactive peptides, which may impair the vasodilatory effect of insulin. Indeed, the relation between insulin resistance and hypertension is more evident in the case of obesity, suggesting that the effect may be mediated by adipose tissue (Vaccaro et al., 1996). In addition, it has been suggested that LDL and triglycerides may damage the arterial epithelium, impair nitric oxide release and cause endothelial dysfunction. Therefore, dyslipidemia characterized by elevated levels of apoB containing lipoproteins could cause hypertension by mechanisms only partly related to obesity and insulin resistance (Laaksonen et al., 2008; Vogel, 1999).

Results from multiple genome-wide studies have shown genetic basis for the individual components of the MetS (obesity, hypertension, dyslipidemia, hyperglycemia) (Sutton et al., 2005; Waterworth et al., 2000; Pollex & Hegele, 2006). Such associations might facilitate or enable the development of the MetS. In addition, some candidate genes have been suggested to affect more than one MetS component (Rosmond, 2002; Ohashi et al., 2004). Although genetic contribution on the development of MetS exists (Vaag, 2008; Peeters et al., 2008; Pietiläinen et

al., 2006), the proportion of variance explained has been low (Sale, Woods, & Freedman, 2006; Pollex et al., 2006; Joy, Lahiry, Pollex, & Hegele, 2008). No genetic test is available that may be used in the diagnosis of the MetS. The lack of association is likely due to the complex interplay between gene and environment necessary for expression of this phenotype (Joy et al., 2008). Genetics of MetS involves a large number of genes having weak effects but they may interact with each other and work synergistically with environmental factors (diet, physical activity, alcohol intake, smoking) in the pathogenesis of the MetS (Andreassi & Botto, 2003).

Birth weight is an indicator of prenatal growth, which is affected by numerous variables. It is suggested that the fetus makes physiological adaptations in response to changes in its environment to prepare itself for postnatal life (de Boo & Harding, 2006). It has been hypothesized that overnutrition of the fetus may lead to permanent changes in appetite control, insulin metabolism, and to an increased birth weight. On the other hand, Barker et al. (Barker et al., 1993) have proposed that intrauterine malnutrition, marked by low birth weight may induce reduced insulin sensitivity especially if they experience a rapid catch-up growth during the first years of life. This favors the occurrence of hypertension, insulin resistance, hypercholesterolemia i.e. MetS in adult life. Thus, the risk for MetS may be present already at birth.

2.3 LIFESTYLE HABITS AND METABOLIC SYNDROME

Even if there is full agreement that lifestyle changes that induce weight loss, are the first-line to treat and prevent the MetS, the ideal diet and form of exercise for the treatment of the MetS remains uncertain (Blair, Cheng, & Holder, 2001; Magkos, Yannakoulia, Chan, & Mantzoros, 2009).

2.3.1 Physical activity and fitness

Increased physical activity promotes weight loss, improves insulin sensitivity by enhancing glucose transport and insulin action in muscles, increases HDL cholesterol levels, lowers triglyceride levels and prevents hypertension (Cornier et al., 2008; Lakka et al., 2003; Stewart, 2002; Hu et al., 2004). Physical activity is associated with fitness but these terms describe different concepts. Physical activity is a behaviour while fitness represents the ability to perform physical activity (Caspersen, Powell, & Christenson, 1985). Both physical activity and fitness are shown to protect against development of MetS through their effects on individual components (Cornier et al., 2008). However, due to their close interrelationship and methodological problems, it is not known whether physical activity or fitness is more important for health (Blair et al., 2001). Physical activity is multidimensional, and a complex behavior to measure (Westerterp, 2009). Techniques include behavioral observation, questionnaires in the form of diaries, recall questionnaires and interviews, and physiological markers such as heart rate, calorimetry, and motion sensors. The doubly labeled water method has become the gold

standard for assessing physical activity. Cardio-respiratory fitness on the other hand can be assessed even more quantitatively by measuring maximal oxygen uptake (VO_{2max}).

Previous studies have found an association between physical activity and low levels of inflammatory cytokines and markers of oxidative stress (Roberts, Won, Pruthi, Lin, & Barnard, 2006; Roberts et al., 2006). Laaksonen et al. showed in their 4-year follow-up study that men engaging in >3 h/week of moderate or vigorous physical activity were half as likely to have the MetS compared to sedentary men (Laaksonen et al., 2002). In addition, Lakka et al. (Lakka et al., 2003) demonstrated that men with a poor cardiorespiratory fitness (VO_{2max}) < 29.1 ml x kg^{-1} x min^{-1}) were almost seven times more likely to have the MetS than those with a $VO_{2max} \geq 35.5$ ml x kg^{-1} x min^{-1}. Pahkala et al. showed that leisure time activity associated beneficially with BMI, HDL cholesterol, systolic blood pressure in adolescents. Further, it is still unclear whether exercise independent of weight loss decreases the risk of developing the MetS (Magkos et al., 2009). Whether or not physical activity is accompanied by weight loss, it is an important mediator in beneficial changes on the individual components of the MetS (Magkos et al., 2009).

2.3.2 Dietary habits

The association of dietary habits with the risk of metabolic syndrome is well established. Diet is usually associated with weight loss, which is beneficial for treating all of the components of the MetS (Pasanisi, Contaldo, de Simone, & Mancini, 2001; Raitakari et al., 2004; Dattilo & Kris-Etherton, 1992; Dengel et al., 2006). It has been shown that even a modest loss of initial body weight by dietary habits can reduce the prevalence of MetS (Pasanisi et al., 2001). Data suggests that hypocaloric diets that are either high in protein or monounsaturated fat facilitate weight loss (McManus, Antinoro, & Sacks, 2001; Foster et al., 2003). Although dietary intake has been linked to individual components of MetS, the role of diet in the origin of MetS is not well understood and the data are scarce regarding the effect of macronutrient changes in patients with the MetS (Lutsey, Steffen, & Stevens, 2008; Magkos et al., 2009). Appel et al. showed improvements in lipid profile and blood pressure by modifying macronutrient composition of the diet based on stable body weight suggesting an independent effect of diet on health (Appel et al., 2005). Previously it has been reported that greater intakes of fruit and vegetables are associated with a lower prevalence of MetS (Esmaillzadeh et al., 2006). Dietary pattern analyses have shown that a diet which includes cereals, fish, vegetables, and fruits was independently associated with reduced levels of clinical and biological markers linked to the MetS, whereas a greater prevalence of MetS has been found among individuals with "Western" dietary patterns characterized by high intakes of refined grains, processed meat, fried foods and red meat (Esmaillzadeh et al., 2006; Esmaillzadeh et al., 2007; Lutsey et al., 2008). Calorie restriction and a low-fat diet or a high low saturated fatty acid diet has been found to have a beneficial effect on the MetS (Watkins et al., 2003). Esposito et al. showed that Mediterranean dietary patterns (characterized by increased consumption of whole grains, fruits, vegetables, nuts, and olive oil) improved the components of the MetS and thus suggested to provide a benefit on CVD risk. Mean body weight however, was decreased more in subjects in the

intervention group (-4.0±1.1 kg) than in those in the control group (-1.2±0.6 kg). Thus the beneficial changes may have occurred due to weight loss (Esposito et al., 2004).

In infants, the effect of breastfeeding on the mean BMI later in life appears to be quite small (Owen et al., 2005). Studies on the impact of single nutrients in childhood on the risk of CVD have given inconclusive results (Juonala et al., 2004a). In children and adolescents, the data from the Finnish Prospective Randomized Trial of Atherosclerosis Prevention in Childhood - study has shown that restriction of saturated fat from infancy until 15 years of age decreases childhood and adolescent blood pressure (Niinikoski et al., 2009). Dietary and lifestyle counselling at childhood seems to reduce the clustering of CVD risk factors in adolescents (Hakanen et al., 2010). Mikkiä et al. showed that diet characterised by high consumption of rye, potatoes, butter, sausages, milk and coffee in childhood and adolescence (aged 3-18) were independently associated with total and LDL cholesterol and apoB among both sexes, and also with systolic blood pressure and insulin levels among women 21-years later. Contrary, a high consumption of vegetables, legumes and nuts, tea, rye, cheese and other dairy products and alcoholic beverages was inversely, but less strongly associated with CVD risk factors (Mikkilä et al., 2007).

2.4 CLINICAL MANIFESTATIONS OF THE METABOLIC SYNDROME

2.4.1 The risk of cardiovascular disease

Subjects with the MetS are at increased risk of developing T2DM and clinical CVD (Lakka et al., 2002; Gami et al., 2007; Mottillo et al., 2010).The complications of metabolic syndrome such as coronary heart disease, myocardial infarction and stoke are the leading cause of all deaths in industrialized countries. Pyörälä et al. showed that MetS predicted CVD and stroke after 22-year follow-up in nearly 1000 Finnish men (Pyörälä, Miettinen, Halonen, Laakso, & Pyörälä, 2000). This finding was further supported by several studies showing an increased risk for incident CVD events in the MetS (Wilson et al., 2005). Lakka et al showed in a prospective study of Finnish men that CVD-death and all-cause mortality were increased in subjects with the MetS even in the absence of baseline CVD or diabetes (Lakka et al., 2002). This association remained significant after adjustments for risk factors not included in the MetS definition (LDL cholesterol, smoking, family history of coronary heart disease, fibrinogen levels, white blood cell levels, alcohol consumption and socioeconomic status). In line, results from the factor analysis gave equivalent results (Lakka et al., 2002). These statistical methods allow us to determine unifying correlation patterns underlying variables that are intercorrelated (Bollen, Van de, Hagberg, & Chute, 2009). Moreover, meta-analysis with over 170,000 subjects showed that individuals with the MetS have nearly 2-fold risk of CVD-death, and 1.6-fold risk of all-cause mortality compared to individuals without MetS (Gami et al., 2007). Recently, Mottillo et al. conducted another large scale meta-analysis identifying 87 studies, which included over 950,000 patients (Mottillo et al., 2010). Their results were similar with previous meta-analysis

with 2-fold risk of CVD-death and 1.5-fold risk of all-cause mortality. In addition, they reported that patients with the MetS, but without diabetes, maintained a high cardiovascular risk (RR, 95 % CI: 1.62, 1.31-2.01 for myocardial infarction and 1.86, 1.10-3.17 for stroke). These findings are consistent with cross-sectional studies assessing subclinical atherosclerosis (Mattsson et al., 2008). In addition, all MetS components have been associated with atherosclerosis and the risk for CVD rises as the number of these risk factors increases (Yusuf et al., 2005). However, previous studies have also established that atherosclerosis is a dynamic and even reversible process – favorable changes in markers of subclinical atherosclerosis have been shown to occur in response to modifications in cardiovascular risk factors (Salonen et al., 1995; Nissen et al., 2006; Girerd et al., 1998; Nathan et al., 2003; Meyer, Kundt, Lenschow, Schuff-Werner, & Kienast, 2006). The reversibility of arterial structure and function associated with MetS however is unknown.

Previous meta-analyses showed that the MetS was associated with higher CVD risk in women relative to men (Gami et al., 2007; Galassi, Reynolds, & He, 2006; Mottillo et al., 2010). The mechanisms explaining a potentially higher CVD risk in women with the MetS are only partly understood. Possible mechanisms include differences in fat distribution, cholesterol profiles and post-menopausal hormone changes (Donato, Fuchs, Oppermann, Bastos, & Spritzer, 2006; Blake, Otvos, Rifai, & Ridker, 2002). In a meta-analysis, it was shown that an increase in triglycerides of 0.21 mmol/l was associated with a 76% increased CVD risk in women compared with a 32% increased risk in men suggesting that triglycerides are more highly associated with CVD in women compared to men (Hokanson & Austin, 1996). However, no data exists whether the MetS is associated with higher mortality in women relative to men.

Previously (Raitakari et al., 2003) it has been shown that elevated risk factor levels already in childhood predict increased adulthood IMT. Although clinical manifestations of atherosclerotic diseases do not occur until middle age, the development of atherosclerosis begins early in life (Juonala et al., 2008). In line, studies in postmortem subjects have described a high prevalence of atherosclerosis already in young adulthood and increased thickening in coronary arteries in children (Newman, III et al., 1986; McGill, Jr. & McMahan, 1998). Due to limited data that track individuals from childhood to adulthood, data are lacking whether pediatric MetS predicts adult atherosclerosis (Steinberger et al., 2009).

2.4.2 The risk of type 2 diabetes

Type 2 diabetes is characterized by insulin resistance and reduced insulin production. Insulin, produced by pancreatic β-cells, has the ability to stimulate glucose uptake particularly in adipose tissue, muscle and in the liver. Individuals with MetS are considered as insulin resistant. Therefore more insulin is required to induce insulin dependent glucose uptake. The development of type 2 diabetes results from a failure of insulin producing cells to maintain the degree of compensatory hyperinsulinemia necessary to prevent loss of glucose tolerance in insulin-resistant tissues thus leading to increased plasma levels of glucose. Hyperglycemia is a

risk factor of diabetic complications including retinopathy, nephropathy, neuropathy and angiopathy, all of which have been associated with lower life expectancy and substantial loss in quality of life. Patients with type 2 diabetes have increased risk of CVD complications such as coronary heart disease, peripheral vascular disease and cerebrovascular disease (Gerstein, 1997; Stratton et al., 2000; Juutilainen, Lehto, Rönnemaa, Pyörälä, & Laakso, 2005). MetS is a precursor of type 2 diabetes (Reaven, 1988). Type 2 diabetes is increasingly prevalent. Almost all individuals with type 2 diabetes has MetS (Liese, Mayer-Davis, & Haffner, 1998). Ford et al. reported the relative risk of 3.5-5.2 (depending on ethnicity) for incident type 2 diabetes in subjects with the MetS compared to those without it (Ford, Li, & Sattar, 2008).

2.4.3 The risk of non-alcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD), characterized by liver damage is one of the most common causes of liver disease in adults (Angulo, 2002). NAFLD refers to fat accumulation in liver exceeding 5 % of liver weight (Kotronen, Westerbacka, Bergholm, Pietiläinen, & Yki-Järvinen, 2007). The diagnosis requires the exclusion of alcohol-induced fatty liver disease and the exclusion of viral, toxic, autoimmune or other rare causes of liver disease (Becker et al., 1996). Fatty liver in NAFLD may proceed to steatohepatitis and further to cirrhosis and liver failure (Day, 2005). Liver cirrhosis due to NAFLD has been implicated as the leading cause of liver transplantations by the year 2020 in the United States (Charlton, 2008). NAFLD is associated with hepatic insulin resistance, characterized by an impaired ability of insulin to suppress hepatic glucose production. This results in mild hyperglycemia and stimulation of insulin secretion, leading to hyperinsulinemia (Angulo, 2002; Clark, 2006). Liver fat is associated with the components of the MetS independently of BMI (Westerbacka et al., 2004). As discussed previously, insulin normally inhibits the production of VLDL, especially apoB containing VLDL particles from the liver. Overproduction of VLDL and the defect in insulin suppression of VLDL production correlates with the amount of fat in the liver (Adiels et al., 2006; Adiels et al., 2007). The epidemic of MetS and obesity has been accompanied by an increase in the prevalence of NAFLD (Kotronen & Yki-Järvinen, 2008). Kotronen et al. reported that in subjects (aged 20-65 years) with MetS, liver fat content was four-fold higher (8.2 % vs. 2.0 %) compared to subjects without MetS (Kotronen et al., 2007). The close association of MetS and NAFLD suggests that they may share a common physiologic antecedent (Kotronen et al., 2008). As MetS becomes even more common, the impact of NAFLD will also increase. Elevated alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) are markers of liver damage that have been associated with fat accumulation in liver (Kotronen et al., 2008). In epidemiological studies, elevated liver enzymes predict CVD (Kotronen et al., 2008) Recently, accumulating data has shown association of NAFLD with IMT progression (Sookoian & Pirola, 2008) and the risk of CVD (Targher, Day, & Bonora, 2010). The strong association between NAFLD and MetS has stimulated interest to investigate its role in the development of CVD (Targher et al., 2010). Recent evidence has suggested that NALFD may not only be a marker of CVD but also involved in its pathogenesis. Potential

mechanisms may include systemic release of proatherogenic mediators from the damaged liver or through the contribution of NAFLD to insulin resistance and atherogenic dyslipidemia, which are important risk factors for CVD (Tarantino, Savastano, & Colao, 2010; Targher et al., 2010).

2.4.4. Youth MetS and the risk of adult outcomes

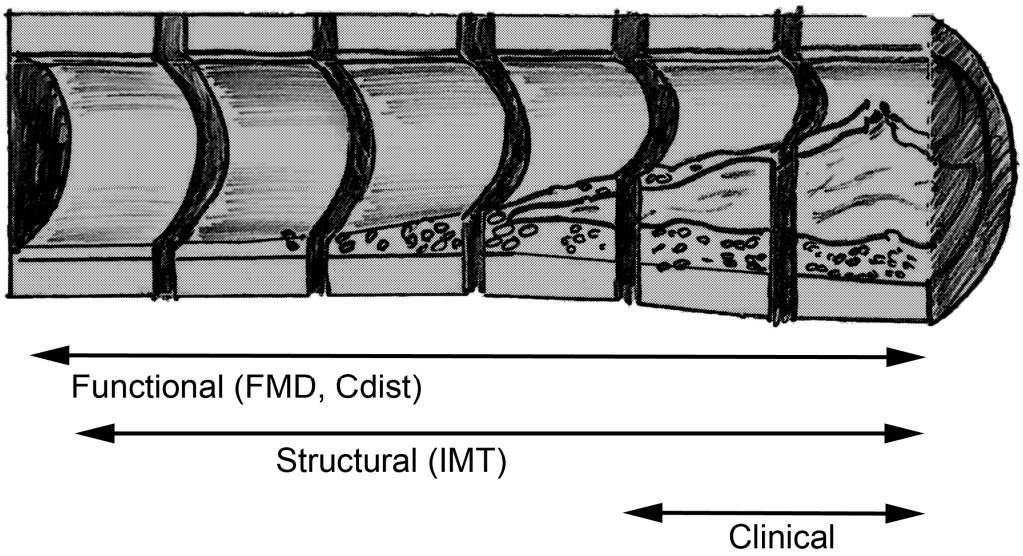
Data are lacking whether or not pediatric MetS identifies those at increased risk of subsequent disease later in life (Steinberger et al., 2009). While some studies suggest that pediatric MetS predicts adult MetS, few studies have also found an association between MetS in youth and risk of future CVD and T2DM in adulthood (Morrison, Friedman, & Gray-McGuire, 2007; Morrison, Friedman, Wang, & Glueck, 2008). The existing data, however, are limited by very small case numbers, and did not fully consider the contribution of each MetS component to risk prediction (Brambilla et al., 2007).

2.4.5. Other manifestations

MetS is associated with several other manifestations. Well-known manifestations include obstructive sleep apnea and polycystic ovary syndrome. Obesity and MetS occur frequently in patients with obstructive sleep apnoea. Individuals with obstructive sleep apnea have a higher incidence of cardiovascular morbidity and mortality (Hamilton, Solin, & Naughton, 2004). This association may be partly explained by the presence of MetS (Vgontzas, Bixler, & Chrousos, 2005; Coughlin, Mawdsley, Mugarza, Calverley, & Wilding, 2004). Polycystic ovary syndrome is characherized by anovulation, androgen excess and insulin resistance (Glueck, Papanna, Wang, Goldenberg, & Sieve-Smith, 2003). Etiology of the polycystic ovary syndrome is still somewhat unclear (Legro, 2007). Insulin resistance with compensatory hyperinsulinemia are suggested to have pathogenic role in the development of polycystic ovary syndrome leading to increased androgen production (Apridonidze, Essah, Iuorno, & Nestler, 2005). MetS is common in women with polycystic ovary syndrome, suggesting that these individuals may have increased risk for CVD. Gluek et al. reported that in NHANES population 46.4 % of women with polycystic ovary syndrome had the MetS (Glueck et al., 2003).

2.5. ULTRASONIC EXAMINATION OF ARTERIES

Although clinical manifestations of atherosclerosis do not occur until middle age, the development of vascular changes begins early in life (Raitakari et al., 2003). Carotid IMT, carotid artery distensibility (Cdist) and brachial flow-mediated dilatation (FMD) assessed non-invasively by ultrasound are preclinical markers of vascular health (*Figure 1*). These studies are reliable and producible methods of studying early atherosclerosis.

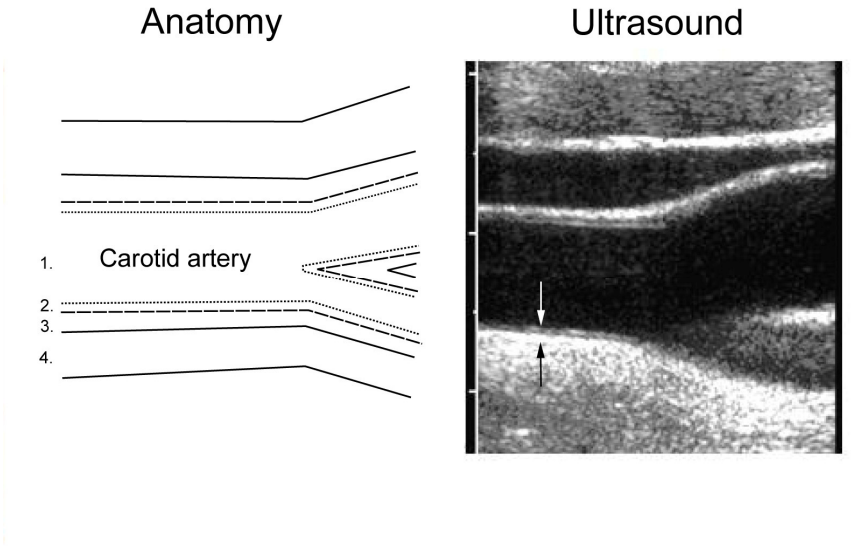


Blocks indicate decades. The initial lesions of atherosclerosis contains atherogenic lipoproteins that elicit an increase in macrophages in the arterial wall causing adaptive intimal thickening that may be detectable by decreased function of the artery. Later fatty streaks can be found and directly detected by thickening of the intima media layer of the artery. Finally, atheromas and fibroatheromas are potentially symptom producing lesions.

Figure 1. Development of atherosclerosis.

2.5.1. Arterial wall-thickness

Measurement of IMT was first introduced in 1986 by Pignoli et al. showing strong association between histological measurements of aortic arterial wall IMT and ultrasonically measured intima-media thickness (Pignoli, Tremoli, Poli, Oreste, & Paoletti, 1986). IMT measurement involves a simple distance measurement between the leading edges of the lumen–intima and media–adventitia ultrasound interfaces. With this imaging method, the typical double-line pattern of the carotid arterial wall can be identified (*Figure 2*). The carotid arteries have been shown to be ideal for ultrasonic measurement due to their size and attainable anatomy. In addition, a strong association has been established between carotid IMT and lesions found in the coronary arteries. Therefore IMT is considered as a surrogate marker of coronary atherosclerosis (de Groot et al., 2004). Typically, IMT progresses from 0.4 mm at birth to 0.8 mm by the age of 80 years, if no risk factors are present. With an increased number of cardiovascular risk factors, IMT grows more rapidly over the lifetime (de Groot et al., 2004).



1. Artery lumen, 2. Intima layer, 3. Media layer, 4. Adventitia.
Area between the arrows indicates intima-media layer in the ultrasound image.

Figure 2. Intima-media thickness

Increased IMT associates with increased risk of CVD (de Groot et al., 2004), coronary event (O'Leary, 1999) and cardiovascular mortality (Salonen & Salonen, 1991; Chambless, 1997; Lorenz, Markus, Bots, Rosvall, & Sitzer, 2007; Bots & Grobbee, 2002). IMT is also associated with the severity of CVD (Hodis et al., 1998). Hodis et al. reported that 0.03 mm increase per year in IMT associated with over 2-fold increase in coronary event risk. In ARIC study that involved 15,800 adults, it was reported that an increase in IMT of 0.20 mm was associated with an increase in relative risk for myocardial infarction and stroke of 33% and 28% (Lorenz et al., 2007; Chambless, 1997; Chambless et al., 2002) during the 9-year follow-up. Further, a meta-analysis by Lorenz et al. using 8 relevant studies adjusted for age and sex reported that relative risk of myocardial infarction was increased by 15 % per 0.10 mm IMT difference (Lorenz et al., 2007). In cross-sectional settings, conventional risk factors in adulthood have been related with IMT. Previous studies have also proposed that elevated risk factor levels in childhood predict increased adulthood IMT (Raitakari et al., 2003; Li et al., 2003; Davis, Dawson, Riley, & Lauer, 2001). Cross-sectional studies have also demonstrated that young adults with the MetS have increased IMT (Mattsson et al., 2008). Large follow-up studies have shown solid evidence that IMT progression can be used to indicate the degree of existing generalized atherosclerosis and future CVD risk (O'Leary et al., 1999). Statin intervention studies using IMT as a surrogate marker have reported that a reduction in IMT thickening of 0.012 mm per year was translated into a significant reduction (odds ratio of 0.48) of diagnosed CVD (Espeland et al., 2005). Therefore, these small changes in IMT appear to be clinically significant.

2.5.2. Arterial distensibility

Decreased elasticity have been considered to represent the early pathophysiological changes in arteries relevant to the development of atherosclerosis (Oliver & Webb, 2003). These parameters characterize the elastic behavior of arteries. The elasticity of an artery can be estimated by measuring its distensibility (Cdist). Adverse changes in carotid artery elasticity are apparent in carotid arteries already at relatively young age (Reneman, Meinders, & Hoeks, 2005). Cdist measures the ability of the arteries to expand as the response to pulse pressure caused by cardiac contraction and relaxation thus reflecting the mechanical load of the artery wall *(Figure 3*) (Juonala et al., 2005). The elasticity of the large arteries, such as carotid artery, is the result of the high elastin to collagen ratio in their walls. The decrease in arterial elasticity that occurs with age is largely the result of progressive elastic fiber degeneration and increase in collagen content (Avolio, Jones, & Tafazzoli-Shadpour, 1998). However, several conventional risk factors have also been associated with decreased arterial elasticity in cross-sectional studies (Aggoun et al., 2000; Mattsson et al., 2008). Obesity and insulin resistance already in young childhood is associated with decreased arterial elasticity (Tounian et al., 2001; Juonala et al., 2005). Furthermore, decreased arterial elasticity has been implicated as an independent predictor of cardiovascular events (Leone et al., 2008; Blacher et al., 1998; Haluska, Jeffries, Carlier, & Marwick, 2010). In addition, the reduction in arterial elasticity associates with left ventricular hypertrophy (Boutouyrie et al., 1995). These data suggest that reduced arterial elasticity is not only age-related, but also reflects increased atherosclerotic burden.

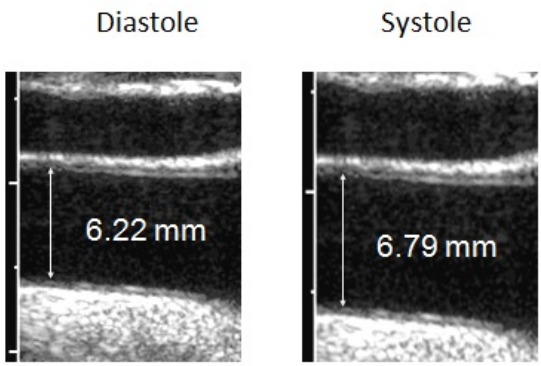


Figure 3. Carotid distensibility

2.5.3. Endothelial function

FMD was introduced in 1992 as a functional marker of endothelial health (Celermajer et al., 1992). The vascular endothelium is the thin layer of cells that lines the interior surface of blood vessels and works as an endocrine organ with several functions. A normal endothelium mainly regulates vascular tone and growth in addition to maintaining balance in thrombosis, fibrinolysis, angiogenesis and inflammation (Raitakari & Celermajer, 2000). Endothelial dysfunction is an early event of atherosclerosis preceding structural atherosclerotic changes in

the vascular wall and has been shown to occur mainly in the response to endothelial release of nitric oxide (Mullen et al., 2001). To assess FMD, the vasodilatory effect mediated by the nitric oxide release, is measured (*Figure 4*). FMD response correlates significantly with invasive methods testing coronary and endothelial function (Takase, Matsushima, Uehata, Ishihara, & Kurita, 2008; Anderson et al., 1995). Several studies have demonstrated the independent prognostic value of FMD predicting CVD (Gokce et al., 2002; Chan et al., 2003). Conventional cardiovascular risk factors in children and adults are associated with FMD (Celermajer et al., 1992; Anderson et al., 1995). However, the integrity of FMD is complex (Bonetti, Lerman, & Lerman, 2003). FMD response is greatly affected by balance between CVD risk factors, vasculoprotective elements, genetic predisposition and some unknown variables. Previous studies (Juonala et al., 2004b)(Higashi et al., 2003) have shown that the association between body size and FMD is curvilinear in the population of young adults. In addition, FMD has been found to modify the association between CVD risk and atherosclerosis (Juonala et al., 2004b). In other words, enhanced FMD may protect against the development of subclinical atherosclerosis in response to metabolic risk factors.

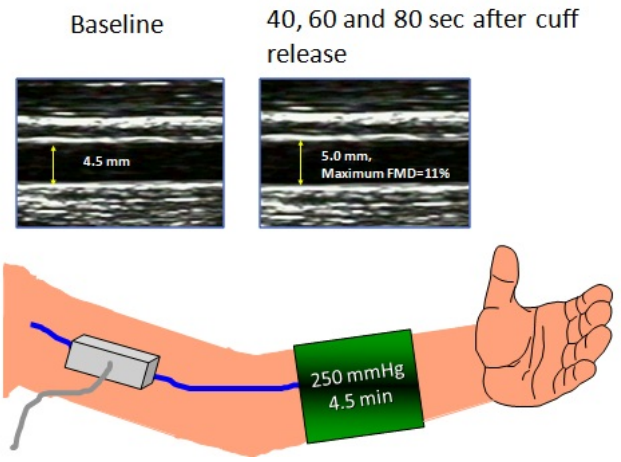


Figure 4. Flow mediated dilatation

3. AIMS

The Cardiovascular Risk in Young Finns Study is an on-going epidemiological study to assess risk factors underlying CVD. The first cross-sectional survey was conducted in 1980 when 3,596 randomly selected children and adolescents (age range 3-18 years) participated. Thereafter, several follow-up studies have been performed. The present study is based on findings from the 21-year follow-up performed in 2001 and from the 27-year follow-up performed in 2007. In addition, total of 374 youths from another on-going epidemiological study of Bogalusa Heart Study (aged 9-18 years at baseline in 1984-88) were included in the analyses of the present thesis. The specific aims of the present thesis are as follows:

1. To study the relations of cardiovascular risk factors with the 6-year incidence of MetS and its components (I).

2. To investigate the status of pediatric MetS as a risk factor for adult MetS, subclinical atherosclerosis and type 2 diabetes mellitus, and compare and contrast this prediction with its individual components (II).

3. To examine whether MetS and spontaneous recovery from MetS is associated with early markers of liver damage and vascular properties in young adults (III, IV, V).

4. SUBJECTS AND METHODS

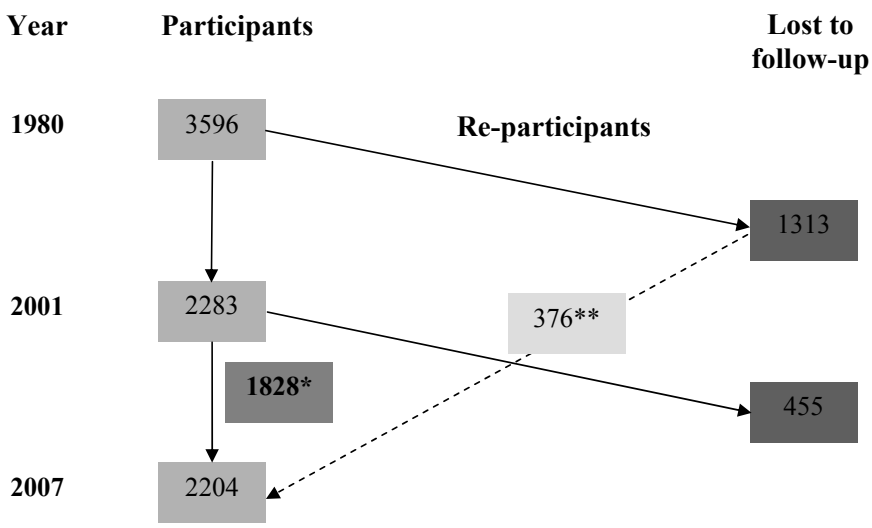
4.1. DESCRIPTION OF THE STUDY COHORTS

The Cardiovascular Risk in Young Finns Study was designed as a collaborative effort between five university hospitals in Finland (Turku, Tampere, Helsinki, Kuopio and Oulu) (Raitakari, 2008). The aim was to assess risk factors underlying cardiovascular disease in children and young adults of various ages in different parts of the country. Two pilot studies were performed in 1978 (N=264, age 8 years) and in 1979 (N=634, aged 3, 12 and 17 years). The first cross-sectional (baseline) study was conducted in 1980 and included 3,596 children and adolescents (83.2% of those invited) aged 3, 6, 9, 12, 15 and 18 years. These subjects were randomly chosen from the national register from study areas to produce a representative sample of Finnish children. Thereafter, follow-up studies have been conducted at 3-year intervals. In 2001, a total of 2,283 subjects (63.5 %) from the original cohort participated in the follow-up study. The participation rates in the follow-up studies among Young Finns participants are shown in *Table 2*. In addition, the study group has been dynamic during the study years as many of those lost-to follow-up have participated in later follow-ups (*Figure 5*).

Table 2. Cohorts and design of the Young Finns Study

Year	N	Age															
1980	3596	3	6	9	12	15	18										
1986	2799			9	12	15	18	21	24								
2001	2283								24	27	30	33	36	39			
2007	2204										30	33	36	39	42	45	

In the 21-year follow-up, non-invasive ultrasound studies were introduced to the study protocol to assess markers of subclinical atherosclerosis. The latest follow-up was performed in 2007, when the study subjects had reached the age of 30 to 45 years. In 2007 a total of 2,204 subjects were examined. A total of 1,828 subjects participated both in 2001 and 2007. Study flow diagram is shown in *Figure 5*.



**Subjects that participated both in 2001 and 2007*
*** Subjects who were lost-to follow-up in 2001, but re-participated in 2007*

Figure 5. Study flow diagram

The Bogalusa Heart Study is a long-term epidemiologic study of cardiovascular risk factors launched in 1972 in a biracial population (65 % white and 35 % black). The study is conducted in a community of Bogalusa, Louisiana, USA. The focus is to understand the early natural history of coronary artery disease and essential hypertension. Seven cross-sectional surveys of children aged 4 to 17 years, each including more than 3,500 children, were conducted between 1973 and 1988. In addition, five cross-sectional surveys of young adults aged 18 to 32 years who had been previously examined as children and remained accessible were conducted between 1979 and 1991. In the 2001 to 2002 survey, 1,143 participants (mean age 36.4 ± 4.4 years; 70% white; 43% men) underwent non-invasive ultrasound studies of the carotid artery. In the 2003 to 2007 survey, IMT was measured in 958 participants (39.0 ± 4.3 years old). The participation rate was approximately 80 % for children to approximately 60 % for the young adult cohort.

The present thesis (Studies I-V) is part of The Cardiovascular Risk in Young Finns Study. Study II is a collaboration study between the Cardiovascular Risk in Young Finns Study and the Bogalusa Heart Study including subjects from both cohorts.

4.2. STUDY DESIGN

Study I examined the association of cardiovascular risk factors and more specifically apoB and OxLDL with incident MetS and its components during 6-year follow-up in young adulthood. The study included those participants who had full metabolic risk factor data from both 2001 and 2007 follow-ups and free of MetS in 2001. A total of 1,429 subjects were included in this analysis.

Study II examined the utility of four categorical definitions of youth MetS and their components in predicting adult high carotid IMT and T2DM among 1,781 participants aged 9-18 years at baseline (1984-88) who were then examined 14-27 years later (2001-2007) when aged 24-41 years. In this study subjects were included from the Bogalusa Heart- and the Cardiovascular Risk in Young Finns studies. For the Bogalusa Heart Study, youths aged 9-18 years who participated in either the 1984-85 or 1987-88 surveys and attended either the 2001-02 or 2003-07 adult surveys (then aged 25-41 years) were included in the analyses (N=374). To harmonize the study designs, those subjects from the Cardiovascular Risk in Young Finns Study were included who participated in the 1986 survey when aged 9, 12, 15, or 18 years and in either the 2001 or 2007 adult follow-ups (then aged 24-39 years, N=1,407). For individuals that participated in multiple baseline or follow-up surveys, those measures that provided the longest time-period between baseline and follow-up, were used.

In Study III, the relations of conventional risk factors and MetS to the 6-year progression of IMT, Cdist and FMD were studied. The study included 1,809 subjects (aged 24-39 in 2001), who had IMT measured in 2001 and 2007.

Study IV examined whether spontaneous recovery from MetS over 6-year follow-up (2001-2007) has a favorable effect on vascular properties and the associations between lifestyle factors and MetS recovery. In this study IMT, Cdist, and FMD were assessed by ultrasound in 1,673 subjects to assess vascular properties.

Study V examined whether MetS (in 2001) predicts early laboratory markers of liver damage in young adults after 6 years. In addition, it was evaluated whether spontaneous recovery during follow-up (2001-2007) from MetS has a favorable effect on the activity of these markers and whether these markers contribute to the atherogenicity of MetS assessed by IMT. In this analysis total of 1,553 subjects were included.

4.3. BIOCHEMICAL ANALYSES

In Young Finns study, all venous blood samples were drawn from the right antecubital vein after fasting for 12 hours.

At the 1986 survey, serum cholesterol and triglycerides were measured using fully enzymatic Boehringer CHOD-PAP kits with an OLLI 3000 analyzer. Glucose concentration was determined using β-D-glucose: nicotinamide adenine dinucleotide oxidoreductase method in 1986. Due to changes in determination methods and kits during study years, lipid levels for 1986 were corrected accordingly. Total cholesterol (2001-2007) = 1.091 * total cholesterol (1983-1986) – 0.271 mmol/l. HDL cholesterol (2001-2007) = 1.068*HDL cholesterol (1983-1986) – 0.0277 mmol/l. Triglycerides (2001-2007) = 1.00756 * triglycerides(1983-1986) + 0.0582 mmol/l. Porkka et al previously presented details of the methods and correction factor equations of lipid levels (Porkka et al., 1997; Viikari et al., 1991).

At follow up-studies in 2001 and 2007, serum cholesterol and triglyceride concentrations were determined enzymatically (Olympus System Reagent; Germany) in a clinical chemistry analyzer (AU400, Olympus). LDL cholesterol was calculated using the Friedewald's formula for subjects with <4 mmol/l triglycerides (Friedewald, Levy, & Fredrickson, 1972). Serum HDL cholesterol was measured by the dextran sulphate 500,000 method (Kostner, 1976). Coefficient of variation (CV) was 2.2 % for total cholesterol, 2.3% for HDL cholesterol and 3.8% for serum triglycerides. Serum apoliporoteins (apoA1 and apoB) were analyzed immunoturbidometrically (Orion Diagnostica, Espoo, Finland). $OxLDL_{prot}$ was determined with an enzyme-linked immunosorbent assay with antibodies directed against the oxidized apoB molecule (Oxidized LDL ELISA kit, Mercodia, Sweden). The inter-assay coefficient of variation was 12 %. Analysis of $OxLDL_{lip}$ was based on determination of the baseline level of conjugated dienes in LDL lipids. The assay procedure consisted of isolation of the lipoprotein fraction, extraction of lipoprotein lipids and spectrophotometric analysis of conjugated dienes in the lipoprotein lipids at 234 nm. Validation studies for the assay have ruled out interference by nonspecific substances, and shown that diene conjugation is a measure of oxidative LDL modification found in all LDL lipid classes. CV was 4.5 % (Ahotupa & Vasankari, 1999).

Plasma glucose concentrations were analyzed enzymatically with a clinical chemistry analyzer (Olympus, AU400; CV 2.0%) and serum insulin concentration was measured by microparticle enzyme immunoassay kit (CV 2.1%) (Abbott Laboratories, Diagnostic division, Dainabot). Insulin resistance was estimated according to the homeostasis model assessment (HOMA-index) as the product of fasting glucose and insulin divided by 22.5 (Matthews et al., 1985). Serum C-reactive protein (CRP) was analyzed by an automated analyzer (Olympus AU400) using a latex turbidimetric immunoassay kit (CRP-UL-assay, Wako Chemicals, Neuss, Germany). The detection limit reported by the manufacturer for the assay was 0.06 mg/l. The interassay CV was 3.3%.

In the Bogalusa Heart Study, at the 1984-85 baseline survey, HDL cholesterol and triglycerides were measured using chemical procedures with a Technicon Auto Analyzer II (Technicon Instrument Corp, Tarrytown, NY), according to the laboratory manual of the Lipid Research Clinics program. Since this time, HDL cholesterol and triglycerides were determined by enzymatic procedures (Chen, Srinivasan, Li, Xu, & Berenson, 2005) using the Abbott VP instrument (Abbott: Laboratories, North Chicago, IL). Plasma insulin was measured using a commercial radioimmunoassay kit (Padebas Pharmacia, Piscataway, NJ), and plasma glucose was measured enzymatically using the Beckman Instant Glucose Analyzer (Beckman Instruments, Palo Alto, CA) (Berenson et al., 1998).

4.4. PHYSICAL EXAMINATION

Weight, height and waist circumferences (measured in duplicate at the level of the 12th rib or level with the navel in thin subjects) were measured with an accuracy of 0.1 kg and 0.5 cm. Body mass index was calculated using the formula: weight[kg]/(height[m])^2. Blood pressure was

measured by using a random zero sphygmomanometer in supine position from the right arm after fasting for 12 hours. Average of three measurements was used in the analyses (Raiko et al., 2010).

4.5. QUESTIONNAIRES

Smoking habits, alcohol intake, physical activity, attention paid to health habits and family history of coronary disease were ascertained with the use of questionnaires (Juonala et al., 2004a). Family history was considered positive if either study subjects' father or mother had been diagnosed with coronary heart disease at or before the age of 55 years. Subjects were asked to report their alcohol consumption of cans or bottles (1/3 l) of beer, glasses (12 cl) of wine, and shots (4 cl) of strong alcohol per week. The values of different beverages consumed during the week allowed the total alcohol intake per day to be determined. Physical activity is represented as metabolic equivalent index by assessing the intensity, frequency and duration of physical activity, including leisure-time physical activity and commuting. Intensity was evaluated by asking how one usually performs physical execercise: 1) usually not becoming out of breath or sweating, 2) becoming out of breath and sweating slightly 3) becoming out of breath and sweating considerably. Frequency was evaluated by asking how often one performs physical exercise during spare time to become out of breath or sweat: 1) not at all, 2) once a month, 3) once a week, 4) 2-3 times a week, 5) 4-6 times a week, 6) daily. Duration was evaluated by asking the average duration of a single instance of physical exercise: 1) under 20 minutes, 2) 20-40 minutes, 3) 40-60 minutes, 4) >60 minutes. Commuting was evaluated by asking the length of the commute and whether it was traveled by foot, bicycle or motorized vehicle. One metabolic equivalent index represents the consumption of 1 kcal per weight kilogram per hour at rest. As part of the questionnaire, participants were asked to indicate the degree to which they paid attention to healthy lifestyle habits: 1) considerable, 2) quite a lot, 3) quite little 4) not at all. Subjects were asked to report if they had any physician diagnosed medical conditions (such as type 1 diabetes, hypertension, dyslipidemia etc.). Information on food consumption was assessed with food frequency questionnaires (FFQ). In 2001, a short non-quantitative FFQ with 20 items (representing the main food groups) was used to estimate the number of weekly portions of selected food groups. In the latest follow-up in 2007 the participants filled in a more comprehensive semi-quantitative 128-item FFQ that provided an estimate of food consumption in grams per day (Paalanen et al., 2006; Mikkilä, Räsänen, Raitakari, Pietinen, & Viikari, 2005).

4.6. DEFINING METABOLIC SYNDROME AND TYPE 2 DIABETES

In the present thesis, 3 definitions for MetS were used for adults: the European Group of Insulin Resistance classification (MetS/EGIR) (Balkau et al., 1999), International Diabetes Federation classification (MetS/IDF) (Alberti, Zimmet, Shaw, & IDF Epidemiology Task Force Consensus Group, 2005) and the recent definition proposed in a joint statement of the IDF Task Force on Epidemiology and Prevention, National Heart, Lung and Blood Institute (NHLBI), the AHA, the World Heart Federation, the International Atherosclerosis Society, and the International Association

for the Study of Obesity (MetS/Harm) (Alberti et al., 2009). The results for MetS/Harm are shown in Studies I-V and additionally for MetS/EGIR in Study III and for MetS/IDF in Study IV. Definitions of the MetS criteria used in the present thesis are shown in *Table 1*.

In Studies IV and V, subjects were classified further into four groups according to their MetS status at the two time-points: recovery group (MetS at baseline 2001 but not at follow-up 2007), incident group (MetS at follow-up but not at baseline), persistent group (MetS both at baseline and at follow-up) and control group (no MetS at baseline or at follow-up by any MetS definition).

Due to different exclusion criteria and MetS definitions used between the studies in the present thesis, the numbers of subjects in the MetS status groups are not consistent. In Study I, total of 205 subjects had incident MetS according to MetS/Harm. In study IV according to MetS/IDF, subjects were stratified as follows: recovery group N=71, incident group N=194, persistent group N=166, control group N=1,242. According to MetS/Harm prevalences were somewhat similar: recovery group N=83, incident group N=198, persistent group N=178, control group N=1,214. In study V, prevalences were: recovery group N=76, incident group N=183, persistent group N=166 and control group N=1,128 according to MetS/Harm definition.

In Study II, 5 approaches were used to diagnose pediatric MetS. Due to lack of universal definition of pediatric MetS, this thesis introduces a similar approach used in previous reports that characterize pediatric MetS using multiple alternate definitions (Goodman et al., 2007). BMI was used as the measure of adiposity since waist circumference was not available for either cohort in childhood. For the first two definitions, age-, sex-, race- (Bogalusa Heart Study), cohort-, and study-year-specific z-scores of BMI, systolic and diastolic blood pressures, HDL cholesterol, triglycerides, and glucose was generated. For the modified National Cholesterol Education Program (modNCEP) definition, a participant was categorized as having MetS if the individual had any three of the following five components: BMI $\geq 75^{th}$ percentile, systolic or diastolic blood pressure $\geq 75^{th}$ percentile, HDL cholesterol $\leq 25^{th}$ percentile, triglycerides $\geq 75^{th}$ percentile, or glucose $\geq 75^{th}$ percentile. For the modified International Diabetes Federation (modIDF) definition, the same cut-points as those for the modNCEP definition were used but the combination of the components differed. The modIDF required elevated BMI plus any two of the remaining four components to be classified as having MetS. The third and fourth definitions utilized age- and sex-standardized pediatric cut-points available in the literature to denote each component risk factor. Overweight or obesity was defined according to the Cole classification; (Cook, Chambers, & Coleman, 2009) prehypertension or hypertension was defined according to the fourth report on high blood pressure in children and adolescents from the National High Blood Pressure Education Program; low HDL cholesterol and high triglycerides were defined using cut-points recently proposed from growth-curve data that were linked to adult definitions (Cook, Auinger, & Huang, 2009); and hyperglycemia was defined as fasting plasma glucose ≥ 5.60 mmol/l, as growth-curve data linking youth glucose levels to adult hyperglycemia have shown levels to remain consistent in the pediatric setting (Jolliffe & Janssen, 2007). Pediatric NCEP (pedNCEP) definition required any three of these

five criteria whereas the pediatric IDF (pedIDF) required overweight or obesity plus any two of the remaining four components. To complement the dichotomous definitions, a continuous MetS risk score (cMetS) was created using the methods described by Wijndaele et al (Wijndaele et al., 2006). Similar to previous studies using this method (Chen, Srinivasan, Elkasabany, & Berenson, 1999; Wijndaele et al., 2006) two principal components were identified as shown in *Table 3*. The principal components were then summed, with weights determined by the relative proportion of variance explained, in order to compute cMetS where a higher score is indicative of a less favorable MetS profile (Wijndaele et al., 2006).

Participants were classified as having T2DM if they had a fasting plasma glucose ≥7.0 mmol/l; or reported receiving oral hypoglycemic agents and/or insulin injections and did not have type 1 diabetes; or reported a history of physician-diagnosed T2DM, consistent with the WHO definition (Wijndaele et al., 2006).

Table 3, Rotated factor loadings from principal components factor analysis to derive the continuous MetS score in childhood in the Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study

	Factor 1	Factor 2
Bogalusa (1984-1985)		
BMI	0.539	0.271
Systolic BP	0.137	0.827
Diastolic BP	-0.029	0.817
HDL cholesterol	-0.794	0.059
Triglycerides	0.837	-0.045
Glucose	0.005	0.224
Variance explained, %	27.3	24.7
Total variance explained, %	52.0	
Bogalusa (1987-1988)		
BMI	0.672	0.289
Systolic BP	0.097	0.802
Diastolic BP	-0.099	0.769
HDL cholesterol	-0.706	0.132
Triglycerides	0.697	0.090
Glucose	0.130	0.195
Variance explained, %	24.5	23.0
Total variance explained, %	47.5	
Young Finns (1986)		
BMI	0.552	0.404
Systolic BP	0.819	0.031
Diastolic BP	0.722	-0.083
HDL cholesterol	0.067	-0.814
Triglycerides	0.211	0.746
Glucose	0.216	0.101
Variance explained, %	26.5	23.3
Total variance explained, %	49.8	

4.7. ULTRASOUND STUDIES

An ultrasound imaging device with a high-resolution system (Sequoia 512, Acuson, CA, USA) was used. Ultrasound studies were performed by trained sonographers following a standardized protocol. Measurements were made off-line from stored digital images. All ultrasound scans were analyzed by one reader (same reader in 2001 and 2007) blinded to subjects' details (Raitakari et al., 2003; Juonala et al., 2005). To assess intra-individual reproducibility of ultrasound measurements 57 subjects were re-examined 3-months after the initial visit in 2001 (2.5 % random sample). The 3-month between visit coefficients of variations were 6.4 % for IMT, 14.3 % for Cdist and 26.0 % for FMD measures in the Cardiovascular Risk in Young Finns Study.

Mean IMT was derived using a minimum of four IMT measurements from the posterior (far) wall of the left common carotid artery approximately 10 mm proximal to the carotid bifurcation. In Study II, high IMT in adulthood was defined as a maximum IMT $\geq 90^{th}$ percentile for age-, sex-, race- (Bogalusa Heart Study), study-year-, and cohort-specific values (Magnussen et al., 2009).

To assess Cdist, the best quality cardiac cycle was selected from a continuous 5-second image file. The common carotid diameter was measured at least twice during end-diastole. Ultrasound and concomitant brachial blood pressure measurements were used to calculate Cdist = $([D_s - D_d]/D_d)/(P_s - P_d)$, where D_d is the diastolic diameter, D_s the systolic diameter, P_s systolic blood pressure, and P_d diastolic blood pressure.

To assess brachial FMD, the left brachial artery diameter was measured at rest and during reactive hyperemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 4.5 minutes followed by release. The average of 3 measurements at rest, 40, 60 and 80 seconds after cuff release were used to derive maximum FMD. The maximal vessel diameter in scans after reactive hyperemia was expressed as the percentage relative to resting scan (100%).

In the US cohort (Bogalusa Heart Study), B-mode ultrasound examinations were performed according to established protocols (Urbina et al., 2002; The ARIC Study Group, 1991b; The ARIC Study Group, 1991a). Maximum IMT measurements of 3 right and 3 left far walls for common carotid, carotid bifurcation, and internal carotid segments were recorded. Seventy-five participants underwent repeat ultrasound examinations 10-12 days after their initial visit to determine intra-individual reproducibility. The average absolute difference and standard deviation between measurements for all IMT segments was 0.05±0.03 mm.

4.8. STATISTICAL METHODS

Values for plasma triglycerides, insulin, HOMA-index, CRP, metabolic equality index and dietary habits were $\log_e$ transformed to correct for skewness. The normality assumptions were

assessed by examining histograms and normal probability plots. Statistical analyses were performed with SAS 9.1. with statistical significance inferred as a two-tailed P value ≤0.05.

ATTRITION

The representativeness of the present study subjects was tested by comparing (1980) characteristics with non-participants using regression modeling adjusted for age. The baseline (2001) characteristics of the study subjects were compared using the t-test for continuous variables and chi-square test for categorical variables. To examine whether selection bias was present between 2001-2007, a dichotomous (yes/no) variable was created for participation in 2001 and 2007 (yes) or 2001 only (no) and used this as the outcome variable in logistic regression model that included age, sex and baseline MetS. Characteristics of study subjects in 2001 and 2007 and changes in risk factors and lifestyle factors between 2001 and 2007 were summarized for each study group (control, recovery, incident, persistent) and linear regression models adjusted for age and sex were used to test for significant trends.

STUDY I

Pearson's correlation coefficient adjusted for age and sex was calculated between lipid parameters. Next, subjects were stratified into quartiles of apoB and oxLDL. Multivariable logistic regression was used to evaluate the association of the quartiles of apoB and oxLDL with incident MetS. Adjusted models were fitted by the inclusion of covariates that were considered important: Model 2 adjusted for age, sex, baseline BMI, HOMA-index, CRP, smoking and in model 3 and 4 further adjusted for $OxLDL_{prot}$ (Table 9, Model 3), $OxLDL_{lip}$ (Table 9, Model 4) and finally adjusted for continuous risk factors included in MetS (Table 9, Model 5). To study the relations of continuous apoB and OxLDL with incidence of MetS components, standardized values (z-score) at baseline for risk components were calculated. Second, multivariable logistic regression was constructed to assess the odds ratios of incident MetS components. BMI was omitted from the model assessing the incidence of abdominal obesity due to a high multicollinearity between outcome- and dependent variable.

STUDY II

Relative risks and 95% confidence intervals estimated using log binomial regression or Poisson regression with robust standard errors were used to examine associations between MetS phenotypes (number of MetS components in youth; youth MetS status; cMetS score) and outcomes of: adult MetS; adult high IMT; and adult T2DM. Analyses were performed for both cohort-stratified and cohort-pooled data. All estimates were adjusted for length of follow-up. Adjustments were made for length of follow-up to account for any within-cohort differences observed between length of follow-up and risk of adult outcomes (Magnussen et al., 2009). Race was also included as a covariate for Bogalusa Heart Study (BHS) analyses. For pooled estimates, a two-level variable for cohort was included. Interactions between cohort and the predictor variables were assessed by including product terms as additional covariates. The association between each MetS component and the adult outcomes were examined using two

models. Model 1 was adjusted for length of follow-up and cohort; model 2 additionally included all MetS components.

The ability of each MetS definition in youth to predict MetS, high IMT, and T2DM in adulthood was assessed using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under receiver-operating characteristic curves (AUC). In addition, comparisons between three models were performed: (A) high youth BMI (referent model); (B) modNCEP (or pedNCEP) MetS definition; and (C) modIDF (or pedIDF) MetS definition to predict adult outcomes of MetS, high IMT, and T2DM. Differences in AUC between models were estimated using the DeLong algorithm (DeLong, DeLong, & Clarke-Pearson, 1988). Net reclassification improvement (NRI) was also calculated to determine the extent to which MetS definitions reassigned participants to a risk status that better reflected their final outcome (case or control) (Pencina, D'Agostino, Sr., D'Agostino, Jr., & Vasan, 2008).

STUDY III

Pearson's correlation coefficients were calculated to assess associations between risk factors and ultrasound variables. Analysis of covariance was used to compare change in ultrasound variables between subjects with and without MetS diagnosis. Linear and polynomial (second degree) regression models were used to examine the associations between the number of MetS components and change in IMT, Cdist and FMD.

In line with prior reports (Johnson et al., 2007; Hassinen et al., 2006; Yanez, III, Kronmal, Shemanski, Psaty, & Cardiovascular, 2002), the present thesis demonstrates an inverse correlation between baseline ultrasound variables and their 6-year change. Therefore, all analyses were adjusted for baseline variable and also repeated after using correction techniques for possible measurement error. If regression analysis is made using explanatory variables which include measurement error, the parameter estimates are biased (Yanez, III, Kronmal, & Shemanski, 1998). The situation where the change in outcome variable is the dependent variable and baseline measurement of the outcome variable is included as an explanatory variable creates such a problem. A technique described by Yanez et al. was used to perform corrections to the usual least square estimates of regression analysis (Yanez, III et al., 1998). Thus, the models including the change in ultrasound variable as the outcome variable and the baseline variable as a covariate were evaluated also using correction techniques for possible measurement error in IMT. The results remained similar to those when baseline measure was included as the covariate into the multivariable models.

STUDY IV

Pair-wise comparisons between study groups were performed using logistic regression model adjusted for age and sex. To study the associations between change in metabolic risk factors and change in lifestyle variables, age- and sex-specific z-score values was calculated (standardized values) at baseline and follow-up for metabolic risk components (waist circumference, systolic blood pressure, triglycerides, HDL cholesterol and glucose) and lifestyle variables (physical

activity, attention paid to health habits, alcohol-, vegetable-, fruit-, meat-, and fish consumption). Second, a change variable by subtracting the baseline value from the follow-up value was generated. Next, a series of multiple regression models was examined. The models included age- and sex-specific z-score values for change in physical activity, attention paid to health habits, alcohol-, vegetable-, fruit-, meat- and fish consumption as dependent variables and individual change in metabolic risk component as the outcome variable.

STUDY V

Analysis of covariance was used to assess liver enzyme levels between subjects with and without MetS as well as to calculate mean ALT and GGT activities across groups according to the number of MetS components (0, 1, 2, 3 and >4 components). Pearson's correlation coefficients adjusted for age were calculated to assess bivariate associations between liver enzymes and risk factors. To study the independent relations between MetS and liver enzymes standardized values (z-score) at baseline and follow-up for risk components were calculated. Second, a multivariable regression was constructed adjusting for components not included in the MetS definition (age, BMI, LDL cholesterol, CRP, alcohol intake and adiponectin). Analysis of covariance (age and sex as covariates) was used to compare mean liver enzyme levels of recovery to incident, persistent and control groups. Linear regression was used to examine for significant ALT*MetS and GGT*MetS interactions on IMT. Age, sex, alcohol intake and MetS adjusted analysis of covariance was used to examine the mean IMT levels in subjects with and without NAFLD. Multivariable regression was used to examine associations between IMT and liver enzymes at follow-up.

5. RESULTS

5.1. CLINICAL CHARACTERISTICS

5.1.1. Attrition

A total of 3,596 subjects participated in 1980. After that, several follow-up studies of the Young Finns cohort have been conducted. Study flow diagram is shown in *Figure 5*. Childhood characteristics in 1980 were compared between participants of the present study and non-participants with age-adjusted analysis. Non-participants were younger in both sexes than participants. BMI was higher in female non-participants than in participants. No statistically significant differences were observed for other risk factors. The representativeness of the present study cohort is shown in *Table 4*. Amongst those lost-to follow-up in 2001 or 2007, there were more males than females (55% vs. 45%, P<0.0001). No difference was observed in MetS prevalence (in 2001) between subjects who were lost-to follow-up between 2001 and 2007 (17.3 %) compared to participants (14.5 %, P=0.14).

Table 4. Comparison of childhood characteristics (in 1980) between study participants (those who participated in 2001 and 2007) and non-participants.

	MEN		WOMEN	
	Participants	Non-Participants§	Participants	Non-Participants§
Number of subjects	794	970	1015	817
Age in 1980	10.9	9.9*	10.9	10.1*
Body mass index (kg/m^2)	17.5	17.6	17.5	17.8†
Systolic blood pressure (mmHg)	113	113	112	112
Diastolic blood pressure (mmHg)	73	74	73	74
Total cholesterol (mmol/l)	5.21	5.23	5.38	5.34
LDL cholesterol (mmol/l)	3.37	3.38	3.50	3.47
HDL cholesterol (mmol/l)	1.56	1.56	1.57	1.55
Triglycerides (mmol/l) ‡	0.57	0.58	0.63	0.64
Insulin (IU/l) ‡	5.8	5.5	6.9	7.5
Smoking prevalence (%)‖	13.7	17.1	9.9	12.5

§ Non-participants (N=1,787), including subjects lost to follow-up or missing IMT data in 2001 (1,326) or in 2007 (N=461). * P<0.0005, differences in age between participants and non-participants were examined with t-test. † P=0.005, in age-adjusted regression model. ‡ Results are geometric mean values, ‖ Among subjects aged 12-18 years

5.1.2. Characteristics

The baseline (2001) and follow-up data (2007) of study subjects according to MetS groups are shown in *Table 5*. Significant linear trends from control group to persistent group were observed for waist circumference, BMI, weight, blood pressure, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, insulin, CRP and metabolic equality index at follow-up.

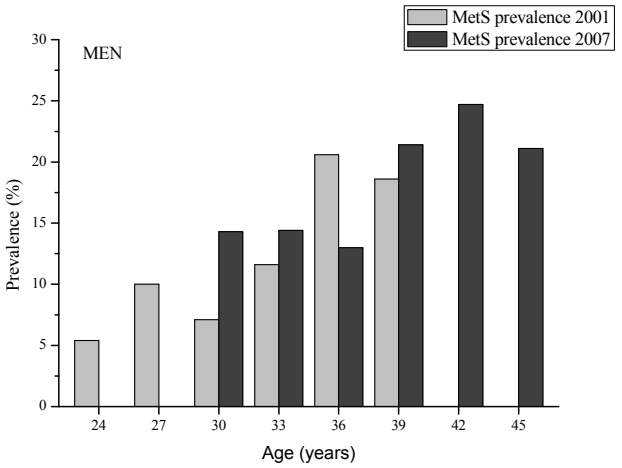
Table 5. Characteristics of study subjects

			Control	Recovery	Incident	Persistent	P for trend
Number of subjects			1242	71	194	166	0.40
Age in 2001 (years)			32.7±4.9	32.8±4.7	33.4±4.6	31.5±5.0	<0.0001
Sex, male (%)			47	55	63	41	<0.0001
Waist circumference (cm)		2001	80±10	95±10	89±10	102±10	<0.0001
		2007	84±11	95±12	99±10	106±11	<0.0001
BMI (kg/m^2)		2001	23.6±3.4	29.2±4.3	26.5±3.6	31.2±4.4	<0.0001
		2007	24.5±3.7	28.5±4.4	28.9±3.8	32.2±4.9	<0.0001
Weight (kg)		2001	70.1±12.9	86.1±16.1	79.8±12.9	94.8±16.3	<0.0001
		2007	72.7±13.7	83.5±14.8	87.2±14.9	97.8±18.3	<0.0001
Systolic blood pressure (mmHg)		2001	114±12	121±13	121±13	128±14	<0.0001
		2007	118±13	120±11	128±14	131±16	<0.0001
Diastolic blood pressure (mmHg)		2001	69±9	76±10	75±10	80±12	<0.0001
		2007	74±10	77±10	82±10	85±12	<0.0001
Total cholesterol (mmol/l)		2001	5.00±0.89	5.44±0.96	5.39±1.01	5.64±1.05	<0.0001
		2007	4.94±0.84	5.10±0.89	5.36±0.94	5.43±0.97	<0.0001
LDL cholesterol (mmol/l)		2001	3.18±0.80	3.49±0.86	3.52±0.89	3.64±0.95	<0.0001
		2007	3.03±0.75	3.21±0.79	3.33±0.86	3.39±0.85	<0.0001
HDL cholesterol (mmol/l)		2001	1.34±0.30	1.10±0.23	1.23±0.29	1.00±0.24	<0.0001
		2007	1.41±0.32	1.27±0.27	1.15±0.27	1.07±0.28	<0.0001
Triglycerides* (mmol/l)		2001	1.00 (0.80-1.30)	1.76 (1.60-2.10)	1.28 (1.00-1.70)	2.09 (1.70-2.70)	<0.0001
		2007	1.02 (0.75-1.36)	1.24 (0.95-1.46)	1.77 (1.36-2.38)	2.05 (1.56-2.78)	<0.0001
apoB (g/l)		2001	0.98±0.22	1.24±0.24	1.13±0.24	1.35±0.26	<0.0001
		2007	0.95±0.22	1.08±0.24	1.18±0.15	1.28±0.25	<0.0001
apoA1 (g/l)		2001	1.51±0.14	1.39±0.23	1.46±0.25	1.37±0.21	<0.0001
		2007	1.63±0.24	1.51±0.27	1.52±0.24	1.47±0.24	<0.0001
Glucose (mmol/l)		2001	4.93±0.40	5.14±0.44	5.14±0.37	5.42±0.91	<0.0001
		2007	5.16±0.45	5.23±0.41	5.66±0.69	5.85±1.13	<0.0001
Insulin* (IU/l)		2001	5.6 (4.0-8.0)	9.5 (7.0-13.0)	7.3 (5.0-10.0)	12.6 (9.0-18.0)	<0.0001
		2007	5.7 (3.8-8.5)	7.8 (5.2-12.1)	11.36 (8.1-16.4)	14.7 (9.9-20.1)	<0.0001
CRP* (mmol/l)		2001	0.65 (0.27-1.34)	1.91 (0.86-3.76)	0.89 (0.36-1.88)	1.75 (0.91-3.05)	<0.0001
		2007	0.74 (0.34-1.46)	1.18 (0.54-2.38)	1.36 (0.67-2.51)	1.84 (0.95-3.52)	<0.0001
Metabolic equality index (kcal/kg/h)		2001	11.0 (5.0-32.5)	10.0 (2.0-28.4)	7.7 (1.9-29.5)	5.6 (0.7-18.9)	<0.0001
		2007	11.3 (5.0-32.6)	10.8 (3.0-31.3)	7.20 (1.1-19.6)	6.0 (0.7-19.5)	<0.0001
Vegetable consumption*	(frq/wk)	2001	5.8 (3.0-9.5).	5.3 (3-9.5)	5.4 (3.0-6.3)	5.0 (3.0-6.3)	0.02
	(g/day)	2007	224 (157-330)	205 (136-352)	212 (151-325)	213 (157-331)	0.63
Fruit consumption*	(frq/wk)	2001	5.9 (3.0-9.5)	5.6 (3.0-9.5)	5.3 (3.0-9.5)	5.0 (3.0-6.3)	0.03
	(g/day)	2007	182 (84-284)	174 (77-275)	154 (60-259)	158 (67-238)	0.26
Meat consumption*	(frq/wk)	2001	3.3 (1.3-6.3)	3.5 (3.0-6.3)	3.6 (3.0-6.3)	3.5 (3.0-6.3)	0.20
	(g/day)	2007	136 (92-187)	154 (104-214)	145 (105-188)	180 (111-234)	0.0005
Fish consumption*	(frq/wk)	2001	1.1 (0.3-1.3)	1.0 (0.3-1.3)	1.0 (0.3-1.3)	1.0 (0.3-1.3)	0.11
	(g/day)	2007	33 (21-48)	34 (23-53)	35 (20-52)	36 (18-54)	0.99

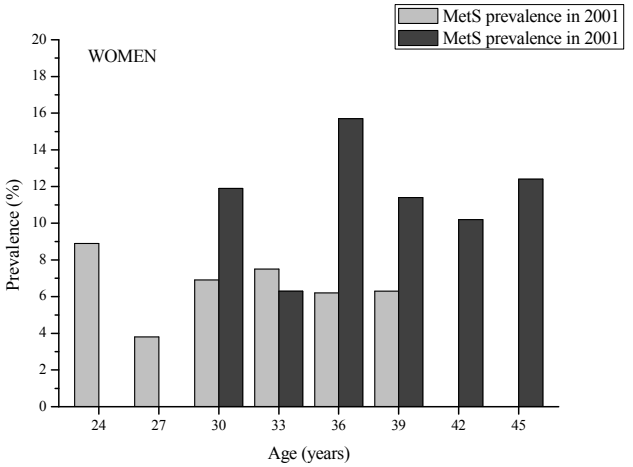
Values are mean±SD or geometric mean (25-75 percentiles)
P-values are from linear regression models adjusted for age and sex.
* Geometric mean values
Abbreviations: MetS, metabolic syndrome; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein

Figure 6, shows the prevalence of MetS stratified according to the age groups of 24 to 45-year-olds in study years 2001 and 2007. In men (A), the prevalence of MetS increased linearly by age both in 2001 (P<0.0001) and 2007 (P=0.01). In women (B), no such age trend was observed (in 2001, P=0.88; in 2007 P=0.66). Waist circumference increased significantly during 2001 and 2007 by age in both study years and in both sexes (P always <0.001). Similar trends were observed for systolic blood pressure and glucose (P always <0.0007). The age trends in serum triglycerides, HDL cholesterol, and insulin levels were non-significant in analysis when sexes were pooled.

A)



B)



MetS prevalence in (A) men and (B) women in 2001-2007

Figure 6A) Prevalence of metabolic syndrome

5.2. CARDIOVASCULAR RISK FACTORS IN PREDICTING INCIDENT METABOLIC SYNDROME

5.2.1. Risk factors conveying early metabolic derangements

Correlation coefficients between risk factors in 2001 and the 6-year MetS incidence are shown in *Table 6*. The strongest correlates of future MetS were adiposity measures, blood pressure, triglycerides and apoB. The correlation coefficients between lipid parameters are shown in *Table 7*.

Table 6. Pearson correlation coefficients for associations between risk factors and 6-year MetS incidence.

	MetS/Harm	
Variable 2001	r	P-value
Waist circumference	0.30	<0.0001
BMI	0.27	<0.0001
Systolic blood pressure	0.20	<0.0001
Diastolic blood pressure	0.23	<0.0001
Total Cholesterol	0.13	<0.0001
LDL cholesterol	0.13	<0.0001
HDL Cholesterol	-0.13	<0.0001
Triglycerides	0.20	<0.0001
apoB	0.21	<0.0001
apoA1	-0.07	0.01
$OxLDL_{prot}$	0.16	<0.0001
$OxLDL_{lip}$	0.14	<0.0001
Glucose	0.18	<0.0001
Insulin	0.18	<0.0001
CRP	0.10	<0.0001

Table 7. Age- and sex-adjusted Pearson correlation coefficients between lipid parameters

	apoB	$OxLDL_{prot}$	$OxLDL_{lip}$	LDL cholesterol	Total Cholesterol	non-HDL	Triglycerides	HDL cholesterol	apoA1
apoB	-	0.63	0.85	0.87	0.94	0.94	0.63	-0.03*	0.14
$OxLDL_{prot}$	0.73	-	0.42	0.69	0.68	0.73	0.38	0.006*	0.07
$OxLDL_{lip}$	0.63	0.42	-	0.41	0.51	0.55	0.67	-0.05	0.14
LDL cholesterol	0.85	0.69	0.41	-	0.92	0.96	0.22	0.06	0.09
Total Cholesterol	0.87	0.68	0.51	0.93	-	0.95	0.41	0.34	0.40
non-HDL	0.94	0.73	0.55	0.96	0.95	-	0.47	0.04*	0.13
Triglycerides	0.63	0.38	0.67	0.22	0.41	0.47	-	-0.08	0.18
HDL cholesterol	-0.03*	0.006*	-0.05	0.06	0.34	0.04*	-0.08	-	0.89
apoA1	0.14	0.07	0.14	0.09	0.40	0.13	0.18	0.89	-

*P-value >0.05

Age- and sex-specific standardized MetS components (waist circumference, blood pressure, triglycerides, HDL cholesterol and glucose) as well apoB, OxLDL, total cholesterol, LDL cholesterol were regressed against incident MetS. In these models variables were included individually into the model as continuous covariate. The results are presented in *Table 8*. Odds ratio for apoB was lower than those of waist circumference and triglycerides, but was higher than those of apoB/apoA1-ratio, OxLDL and non-HDL in predicting incident MetS.

Table 8. Standardized, age- and sex-specific (1±SD) odds ratios (OR) and their 95 % confidence intervals (95% CI) between continuous risk factor and incident MetS during the 6-year follow-up.

	Incident MetS	
Variable	**OR (95% CI)**	**P-value**
Waist circumference	2.05 (1.77-2.37)	**<0.0001**
Triglycerides	1.79 (1.51-2.11)	**<0.0001**
apoB	1.74 (1.49-2.04)	**<0.0001**
Systolic BP	1.62 (1.39-1.88)	**<0.0001**
apoB/apoA1 ratio	1.62 (1.39-1.87)	**<0.0001**
Glucose	1.55 (1.03-1.85)	**<0.0001**
Non-HDL	1.50 (1.29-1.74)	**<0.0001**
OxLDL	1.49 (1.27-1.74)	**<0.0001**
HDL cholesterol	0.71 (0.61-0.84)	**0.0003**
apoA1	0.87 (0.74-1.01)	0.07

Results are standardized (1±SD) odds ratios (OR) and their 95 % confidence intervals (95% CI).
Risk factors were included individually into the models.

5.2.2. ApoB in predicting MetS incidence

Next, the role of apoB in predicting MetS incidence was assessed in more detail. As shown in *Table 9*, the incidence of MetS was associated with higher quartiles of apoB in model adjusted for age, sex, baseline BMI, HOMA-index, CRP, smoking and OxLDL (Table 9, Models 1, 2, 3 and 4). Similar results were obtained when BMI was replaced by waist circumference. In addition, the results for apoB were similar when LDL cholesterol was included into the models. When the model was adjusted for the components of MetS as continuous variables (waist circumference, systolic blood pressure, triglycerides, HDL cholesterol, glucose) the association remained significant for apoB as shown in *Table 9, Model 5*. The association between $OxLDL_{prot}$ and incident MetS as well as between $OxLDL_{lip}$ and incident MetS was attenuated when apoB was included into the model as a covariate (P always >0.40).

Table 9. ApoB predicting incidence of MetS

N=numbers of subjects remain consistent across models.

		Model 1		Model 2		Model 3		Model 4		Model 5	
apoB	N*	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI
Quartile1	352	1.0	(Ref)	1.0	(Ref)	1.0	(Ref)	1.0	(Ref)	1.0	(Ref)
Quartile2	350	2.0	(1.0-3.8)	1.7	(0.9-3.6)	1.6	(0.8-3.2)	1.7	(0.8-3.8)	1.5	(0.7-2.9)
Quartile3	350	3.1	(1.7-5.7)	2.4	(1.3-4.6)	2.8	(1.3-4.6)	2.8	(1.4-5.9)	2.4	(1.2-4.8)
Quartile4	377	4.2	(2.3-7.6)	2.8	(1.5-5.1)	2.0	(0.9-4.2)	3.0	(1.4-6.6)	2.5	(1.3-4.9)
P for trend		**≤0.0001**		**0.001**		**0.01**		**0.005**		**0.03**	

Model 1: Adjusted for age and sex
Model 2: Model 1 + baseline BMI, HOMA-index, CRP, smoking
Model 3: Model 2 + $OxLDL_{prot}$
Model 4: $OxLDL_{prot}$ replaced with $OxLDL_{lip}$
Model 5: Adjusted for waist circumference, systolic blood pressure, triglycerides, HDL cholesterol, glucose

The results of continuous apoB in predicting incidence of each dichotomous MetS component are shown in *Table 10*. The model included apoB, $OxLDL_{prot}$, age, sex, BMI, LDL cholesterol, HOMA-index, CRP and smoking as explanatory variables. Essentially similar results were observed for apoB when $OxLDL_{prot}$ was replaced with $OxLDL_{lip}$. ApoB was significantly associated with 6-year incidence of central obesity, hypertension, triglyceridemia and low-HDL

Table 10. ApoB levels predicting incidence of MetS components.

		apoB	
MetS component	**Number of cases**	**OR (95% CI)**	**P-value**
Abdominal obesity	159	1.5 (1.0-2.2)	**0.03**
Hypertension	145	1.5 (1.0-2.2)	**0.04**
Triglyceridemia	146	4.4 (2.6-7.4)	**<0.0001**
Low-HDL	79	3.2 (1.8-5.6)	**0.0001**
Hyperglycemia	202	1.2 (0.9-1.7)	0.31

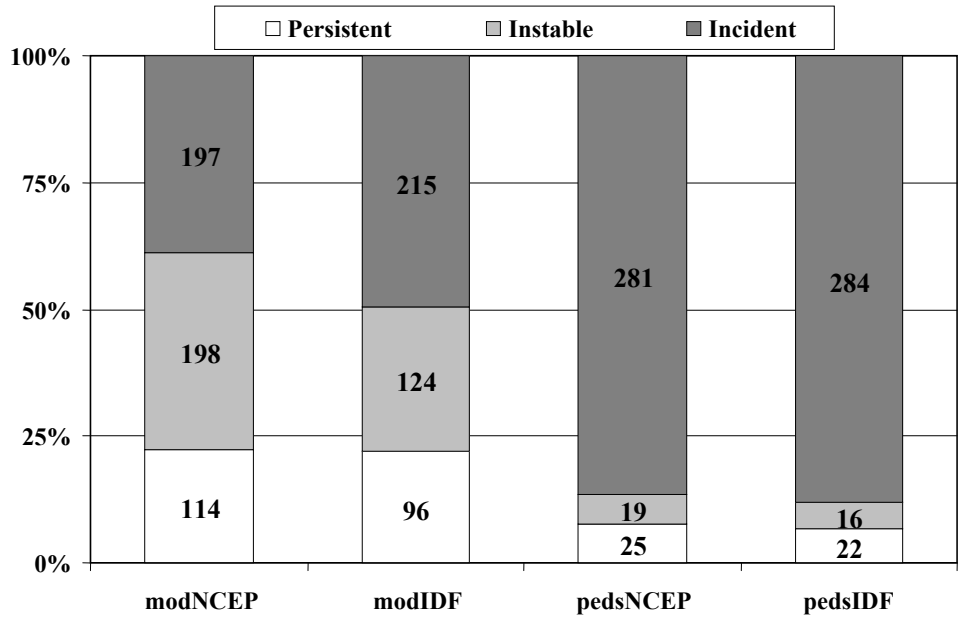
Results are odds ratios (OR) and their 95 % confidence intervals (95% CI) for 1-SD change in apoB. The model included $OxLDL_{prot}$, apoB, age, sex, BMI, LDL cholesterol, HOMA-index, CRP, smoking as explanatory variables. (BMI was omitted from the model when assessing incident obesity).

5.3. PEDIATRIC METABOLIC SYNDROME AS A RISK FACTOR FOR ADULT OUTCOMES

5.3.1. Stability of MetS between youth and adulthood

Stability of MetS definitions between youth and adulthood are presented according to three groups: (1) persistent MetS (MetS positive youth who were also MetS positive as adults); (2) instable (those MetS positive at baseline but MetS negative at follow-up); and (3) incident MetS (MetS negative youth who were MetS positive as adults). The number of participants in each of

these three groups is expressed as a proportion of the total MetS cases identified and are presented graphically in *Figure 7*. Those with persistent MetS accounted for approximately 20% based on the modified definitions and ~7% using the pediatric definitions. Irrespective of the youth definition employed, the major proportion of participants with MetS had acquired it since youth.



Proportions of participants with persistent, baseline only, and incident MetS according to two definitions of youth MetS. y-axis indicates the proportion of total MetS cases identified (youth and adult). Number of cases for each group is shown in the center of each bar.

Figure 7. Stability of the metabolic syndrome from childhood to adulthood

5.3.2. Pediatric MetS in predicting adult outcomes

Cohort pooled analyses (Young Finns and Bogalusa) showed youth with MetS to have between 2.7 and 3.4 times greater risk of adult MetS compared with those without baseline MetS (*Table 11 A*). The risk of adult MetS tended to increase as the number of youth MetS components increased (*Figure 8A)*. Pediatric MetS definitions were associated with ~2-fold increase in risk for developing high IMT in adulthood (*Table 11 B*). The risk of high IMT increased as the number of youth MetS components increased shown in *Figure 8B*. Pooled analyses showed youth with MetS had 2-3 times the risk of developing T2DM in adulthood compared to those without youth MetS (*Table 11 C*). There was a trend toward increased risk of T2DM as MetS components increased (*Figure 8C*). Results are presented on the next two pages.

*Table 11. Relative risk (RR) and 95% confidence intervals (95% CI) of A)MetS/Harm, B) high IMT, and C) T2DM in adulthood according to MetS risk variables in childhood**

A) MetS/Harm

	Bogalusa		Young Finns		Pooled	
	RR	95% CI	RR	95%CI	RR	95%CI
ModNCEP	2.3	(1.5-3.4)	2.8	(2.3-3.5)	2.7	(2.2-3.3)
ModIDF	3.0	(2.0-4.4)	3.2	(2.5-4.0)	3.1	(2.6-3.8)
pedsMetS	2.2	(1.1-4.6)	3.8	(2.8-5.2)	3.1	(2.5-4.4)
pedsIDF	2.2	(1.1-4.6)	3.9	(2.9-5.4)	3.4	(2.5-4.5)
cMetS	1.5	(1.3-1.6)	1.5	(1.4-1.6)	1.5	(1.4-1.6)

B) High IMT

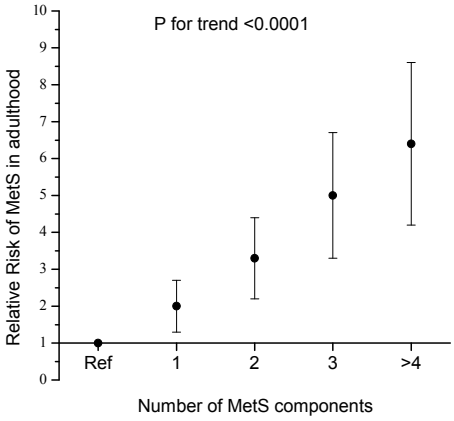
	Bogalusa		Young Finns		Pooled	
	RR	95% CI	RR	95%CI	RR	95%CI
ModNCEP	2.6	(1.3-5.0)	1.8	(1.3-2.5)	1.9	(1.4-2.6)
ModIDF	3.7	(1.9-7.3)	1.9	(1.3-2.7)	2.2	(1.6-3.0)
pedsMetS	2.3	(0.7-8.2)	2.1	(1.1-4.1)	2.1	(1.2-1.2)
pedsIDF	2.3	(0.7-8.2)	1.8	(0.8-4.0)	1.9	(1.0-3.8)
cMetS	1.6	(1.3-2.0)	1.2	(1.1-1.3)	1.3	(1.1-1.4)

C) T2DM

	Bogalusa		Young Finns		Pooled	
	RR	95% CI	RR	95%CI	RR	95%CI
ModNCEP	1.5	(0.7-3.5)	4.1	(1.3-13.5)	2.1	(1.1-4.2)
ModIDF	2.6	(1.2-6.0)	6.0	(1.8-19.9)	3.4	(1.7-6.7)
pedsMetS	2.9	(0.8-11.1)	4.2	(0.5-34.6)	3.6	(1.1-11.7)
pedsIDF	2.9	(0.8-11.5)	5.2	(0.7-40.2)	3.8	(1.2-12.6)
cMetS	1.1	(0.8-1.6)	1.4	(1.0-2.1)	1.3	(1.0-1.6)

*All models adjusted for length of follow-up; pooled estimates additionally adjusted for cohort. Reference category for dichotomous predictor variables (modNCEP, modIDF, pedsNCEP, pedsIDF) is no MetS. †Relative risks and 95% CIs expressed for a 1SD increase in cMetS.

A)



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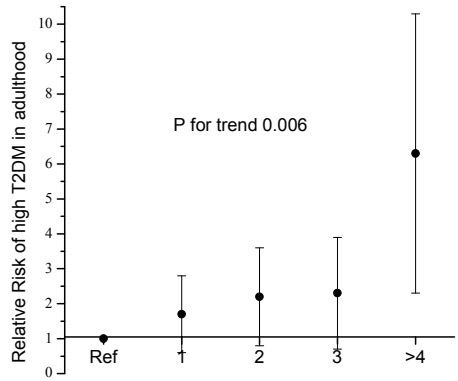

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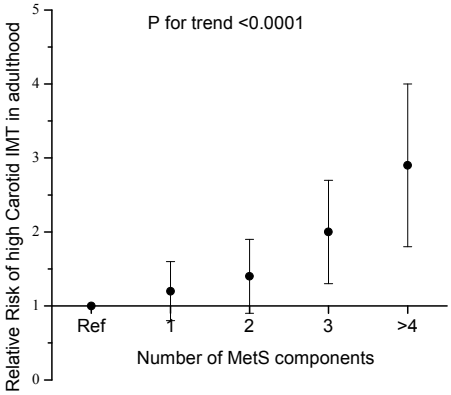


MetS components: BMI ≥75th percentile, systolic or diastolic blood pressure ≥75th percentile, HDL cholesterol ≤25th percentile, triglycerides ≥75th percentile, or glucose ≥75th percentile

Figure 8, Relative risk and 95 % confidence intervals of A) MetS, B) high IMT, and C) T2DM in adulthood according to number of MetS risk variables in childhood.

5.3.3. Pediatric MetS components in predicting adult outcomes

Relative risks from pooled data for predicting MetS, high IMT, and T2DM in adulthood according to each component of youth MetS and insulin are shown in *Table 12*. Cohort-stratified data were essentially similar. High BMI was the only consistent component associated with increased risk of adult outcomes in multivariable models.

Table 12. Unadjusted and adjusted† relative risks (RR) and 95% confidence intervals (95%CI) of adult MetS, high IMT, and T2DM according to each component of the youth MetS definitions as well as insulin*

	MetS				High IMT				T2DM			
	Model 1*		Model 2†		Model 1*		Model 2†		Model 1*		Model 2†	
modNCEP/IDF	RR	(95%CI)	RR	(95%CI)	RR	(95%CI)	RR	(95%CI)	RR	(95%CI)	RR	(95%CI)
BMI ≥75th percentile	3.0	(2.5-3.7)	2.4	(1.9-3.0)	2.2	(1.7-2.9)	2.1	(1.5-2.8)	3.4	(1.8-6.4)	2.9	(1.6-5.5)
BP ≥75th percentile	1.5	(1.2-1.8)	1.2	(1.0-1.5)	1.4	(1.0-1.8)	1.3	(1.0-1.6)	1.0	(0.5-2.0)	0.9	(0.5-1.8)
HDL C ≤25th percentile	1.9	(1.6-2.4)	1.5	(1.2-1.8)	1.3	(1.0-1.8)	1.1	(0.8-1.6)	1.8	(0.9-3.4)	1.5	(0.7-3.1)
TG ≥75th percentile	2.0	(1.6-2.5)	1.3	(1.0-1.6)	1.3	(1.0-1.7)	1.0	(0.7-1.4)	1.3	(0.6-2.6)	0.9	(0.4-1.8)
Glucose ≥75th percentile	1.5	(1.2-1.9)	1.2	(1.0-1.5)	1.1	(0.8-1.6)	1.0	(0.7-1.4)	1.8	(0.9-3.4)	1.5	(0.8-2.8)
Insulin ≥75th percentile	2.0	(1.7-2.5)	1.3	(1.0-1.6)	1.4	(1.1-1.9)	1.1	(0.8-1.5)	1.9	(1.0-3.7)	1.1	(0.6-2.1)

**Adjusted for length of follow-up and cohort*
† Adjusted for length of follow-up, cohort and all other MetS components

5.3.4. Comparison between high BMI and MetS definitions

Data that compare high BMI with MetS definitions in youth in predicting adult outcomes are displayed in *Table 13*. Prediction of adult outcomes by BMI in youth was either equal to or superior than the prediction provided by any of the youth MetS definitions. Substantial gains in sensitivity at relatively modest trade-offs in specificity were observed using high BMI or overweight or obesity in youth, which translated to improved discrimination (AUC). Evidenced by negative NRI, accuracy of classification reduced significantly (all $P<0.03$) by using either of the youth MetS definitions in place of high BMI.

Table 13. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the curve (AUC), and net reclassification index (NRI) values for youth MetS definitions in predicting adult MetS, high IMT, and T2DM

Adult outcome	Child MetS definition	N	Sensitivity, %	Specificity, %	PPV, %	NPV, %	AUC	(95%CI)	P-value	NRI, %	P-value
MetS											
	BMI ≥75th percentile	1755	50.8	80.0	35.4	88.3	0.654	(0.624-0.684)	-	-	-
	modNCEP	1755	36.7	86.3	36.5	86.3	0.615	(0.586-0.643)	0.008	-7.9	<0.001
	modIDF	1755	30.9	91.4	43.6	86.0	0.611	(0.585-0.638)	<0.001	-8.9	<0.001
High IMT											
	BMI ≥75th percentile	1743	43.1	76.6	17.6	92.1	0.599	(0.561-0.636)	-	-	-
	modNCEP	1743	29.3	83.6	17.2	91.1	0.565	(0.530-0.599)	0.07	-7.2	0.03
	modIDF	1743	23.8	88.8	19.7	91.0	0.563	(0.531-0.595)	0.02	-7.2	0.02
T2DM											
	BMI ≥75th percentile	1767	52.8	75.1	4.2	98.7	0.639	(0.556-0.723)	-	-	-
	modNCEP	1767	33.3	82.6	3.8	98.3	0.580	(0.501-0.658)	0.08	-20.8	0.004
	modIDF	1767	33.3	87.9	5.4	98.5	0.606	(0.5.28-0.685)	0.33	-14.5	0.02
MetS											
	Overweight or obese*	1708	33.7	88.8	39.6	86.0	0.612	(0.585-0.640)	-	-	-
	pedsNCEP	1708	8.2	98.6	56.8	83.1	0.534	(0.518-0.550)	<0.001	-15.4	<0.001
	pedsIDF	1708	7.2	98.9	57.9	83.0	0.530	(0.515-0.545)	<0.001	-15.9	<0.001
High IMT											
	Overweight or obese*	1696	28.1	86.8	19.9	91.1	0.574	(0.540-0.608)	-	-	-
	pedsNCEP	1696	5.1	97.9	22.0	89.8	0.515	(0.498-0.531)	<0.001	-19.4	0.005
	pedsIDF	1696	3.9	98.2	20.0	89.7	0.510	(0.496-0.525)	<0.001	-19.3	0.005
T2DM											
	Overweight or obese*	1720	48.6	85.5	6.5	98.8	0.670	(0.586-0.755)	-	-	-
	pedsNCEP	1720	8.6	97.6	6.8	98.1	0.531	(0.484-0.578)	0.001	-9.1	0.23
	pedsIDF	1720	8.6	97.9	7.9	98.1	0.533	(0.485-0.580)	0.001	-9.1	0.23

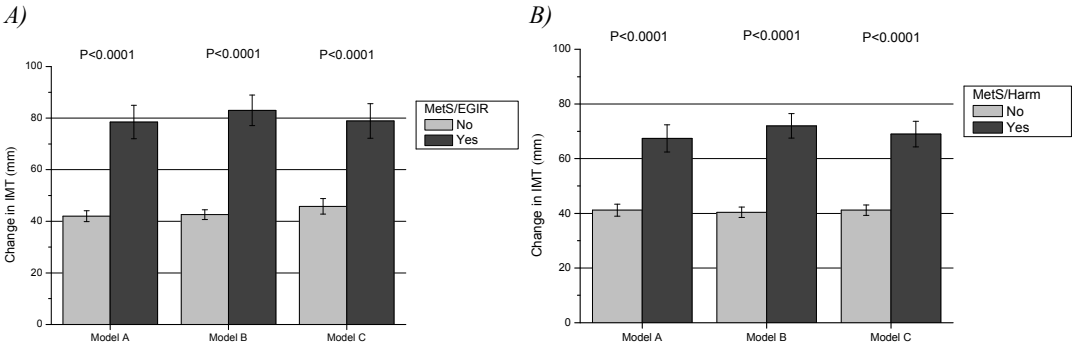
*Overweight or obese according to Cole definition (Cook et al., 2009).

5.4. METABOLIC SYNDROME IN PREDICTING VASCULAR CHANGES

5.4.1. MetS and IMT change

To examine the association between MetS and IMT, comparison between subjects with and without the MetS in 2001 in IMT progression was assessed. The rate of IMT progression was increased approx. 3 μm per year in MetS/EGIR group compared to subjects without MetS. *Figure 9A, Model A* shows unadjusted mean values for the 6-year IMT progression according to the MetS status, and *Model B* mean values in MetS groups adjusted for age, sex, and baseline IMT. In *Model C,* mean values are additionally adjusted for risk variables not included in the MetS definition (smoking,

CRP, LDL cholesterol and family history of coronary disease). When adjusting for measurement error bias the results were essentially similar. Results for MetS/Harm were similar (*Figure 9B).*

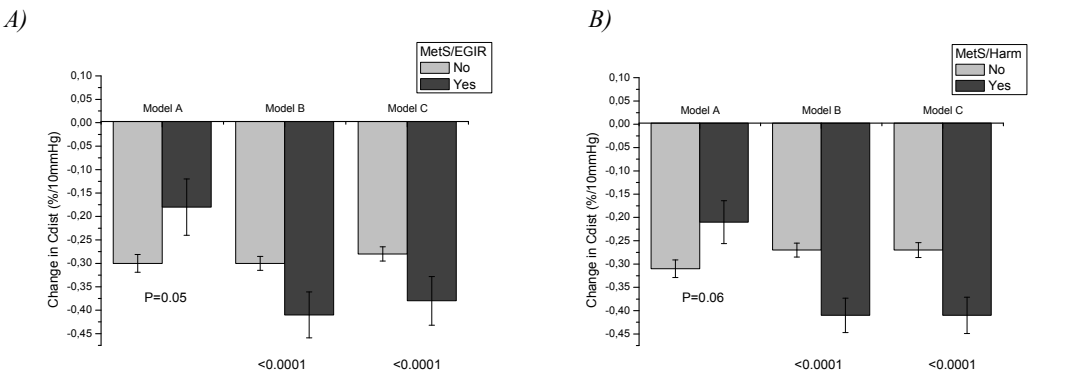


Model A) Mean±SEM values of IMT progression in subjects with and without MetS.
Model B) Comparison of IMT progression in subjects with and without MetS after adjusting for age, sex, and baseline IMT.
Model C) IMT progression values between subjects with and without MetS after further adjustment for smoking, LDL cholesterol, CRP and family history of coronary disease.

Figure 9. IMT progression in subjects with and without A)MetS/EGIR, B)MetS/Harm

5.4.2. MetS and Cdist change

Figures 10A and B displays the association between MetS and change in Cdist during the 6-year follow-up. No significant difference was observed between the MetS groups in the unadjusted model (*Model A*). When adjusting for baseline Cdist, subjects with MetS in 2001 had significantly higher regression rate (approx. 0.02 %/10mmHg per year) in Cdist compared to subjects without MetS. The results were essentially similar when adjusting for age and sex (*Model B*) and further for smoking, CRP, LDL cholesterol and family history of coronary heart disease (*Model C*).

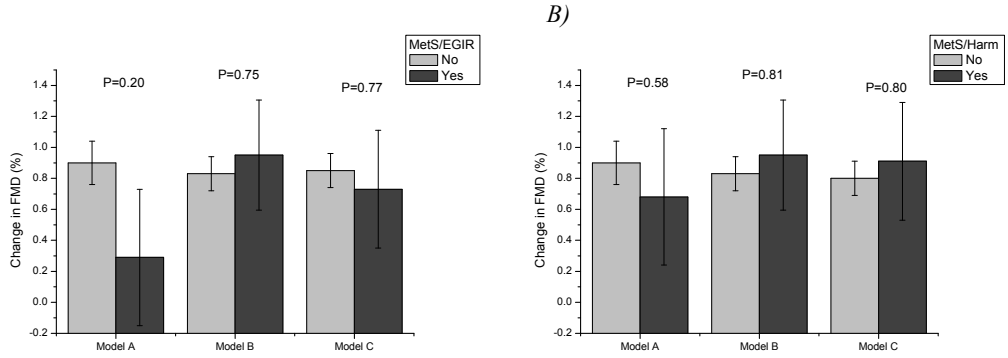


Model A) Mean±SEM values of Cdist regression in subjects with and without MetS.
Model B) Comparison of Cdist regression in subjects with and without MetS after adjusting for age, sex, and baseline Cdist.
Model C) Cdist regression values between subjects with and without MetS after further adjustment for smoking, LDL cholesterol, CRP and family history of coronary disease.

Figure 10. Cdist regression in subjects with and without A)MetS/EGIR, B)MetS/Harm

5.4.3. MetS and FMD change

No significant difference in FMD change during the 6-year follow-up between the subjects with and without MetS was observed (*Figures 11A and B).*



Model A) Mean±SEM values of FMD change in subjects with and without MetS.
Model B) Comparison of FMD changes in subjects with and without MetS after adjusting for age, sex, and baseline Cdist.
Model C) FMD change values between subjects with and without MetS after further adjustment for smoking, LDL cholesterol, CRP and family history of coronary disease.

Figure 11. FMD change in subjects with and without A)MetS/EGIR, B)MetS/Harm

5.4.4. Does MetS provide information over and above individual risk factors?

To analyze whether MetS provides information beyond individual risk factors, a multivariable model was created that included the 2-level MetS variable and MetS components as continuous covariates (waist circumference, systolic blood pressure, triglycerides, HDL cholesterol, glucose and insulin (MetS/EGIR) assessing 6-year change in IMT, Cdist and FMD (*Table 14*). MetS/EGIR was significantly associated with IMT progression in the multivariable model, but the model's R^2-value did not improve (24.0 % vs. 24.0 % for MetS/EGIR) after its inclusion (*Table 14A*). Results were similar when age and sex were included into the model. To assess components of the MetS in predicting IMT progression, multivariable regression analysis was used including individual risk components (waist circumference, blood pressure, triglycerides, HDL cholesterol, glucose and insulin) as continuous variables in the same model. In a model adjusted for age, sex and baseline IMT, the multivariable correlates of IMT progression included waist circumference (β=0.011±0.002, P<0.0001) and insulin (β=0.010±0.004, P=0.01). No association between MetS and change in Cdist or between MetS and change in FMD was observed when MetS components were included in the models (*Tables 14 B and 14 C*).

Table 14, Multivariable association between MetS and its components 2001 with A) IMT progression B) Cdist regression and C) FMD regression 2001-2007.

A)

	MetS/EGIR		MetS/Harm	
	Beta±SEM	P-value	Beta±SEM	P-value
MetS	0.017±0.008	0.03	0.012±0.006	0.06
Waist circumference	0.015±0.002	<0.0001	0.015±0.002	<0.0001
Systolic BP	0.004±0.002	0.08	0.003±0.002	0.12
Triglycerides	0.0003±0.002	0.88	-0.0002±0.002	0.92
HDL cholesterol	-0.003±0.001	0.18	-0.003±0.002	0.24
Glucose	0.0003±0.002	0.85	0.0003±0.002	0.85
Insulin	0.0003±0.002	0.88	-	-
Model	R^2=24.0 %		R^2=23.9 %	

B)

	MetS/EGIR		MetS/Harm	
	Beta±SEM	P-value	Beta±SEM	P-value
MetS	0.065±0.064	0.30	-0.010±0.052	0.86
Waist circumference	-0.113±0.020	<0.0001	-0.113±0.22	<0.0001
Systolic BP	-0.034±0.017	0.05	-0.033±0.017	0.06
Triglycerides	0.002±0.018	0.89	0.003±0.018	0.85
HDL cholesterol	-0.010±0.017	0.54	-0.012±0.017	0.47
Glucose	-0.016±0.015	0.31	-0.016±0.015	0.30
Insulin	-0.018±0.018	0.32	-	-
Model	R^2=35.7 %		R^2=35.7 %	

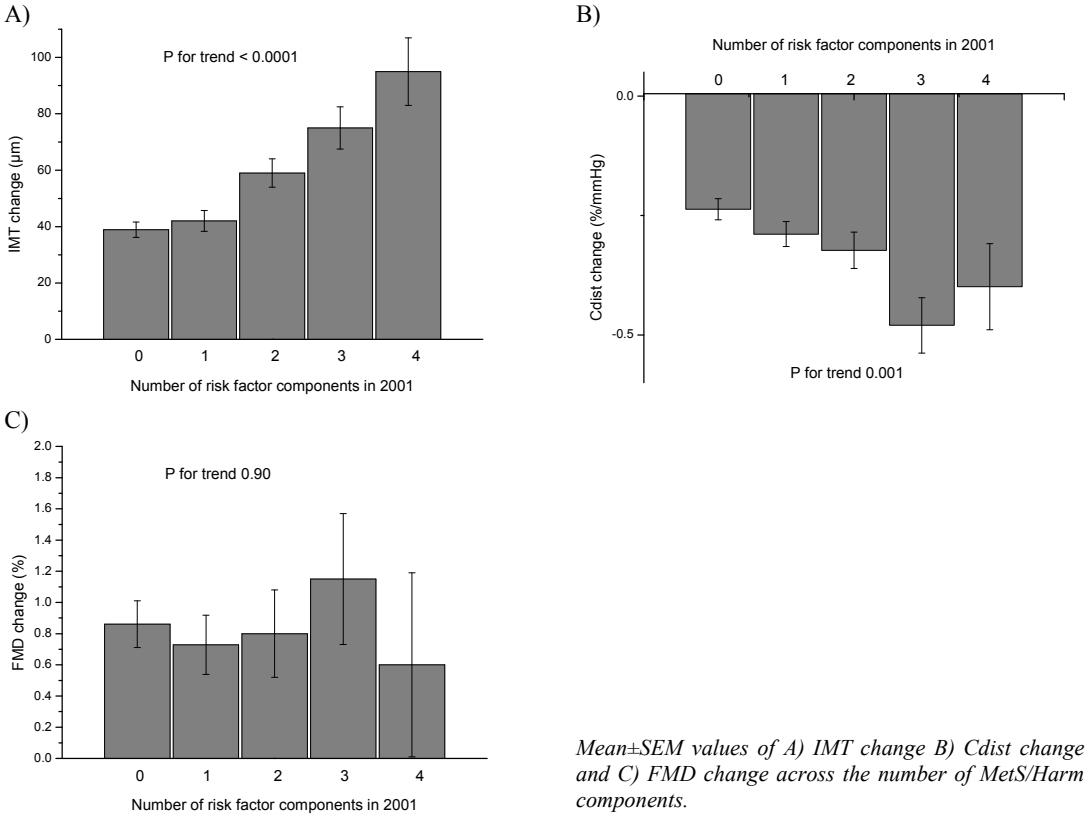
C)

	MetS/EGIR		MetS/Harm	
	Beta±SEM	P-value	Beta±SEM	P-value
MetS	-0.425±0.459	0.35	-0.451±0.391	0.25
Waist circumference	0.312±0.157	0.05	0.283±0.148	0.06
Systolic BP	-0.092±0.121	0.44	-0.122±0.123	0.32
Triglycerides	-0.110±0.128	0.39	-0.158±0.130	0.22
HDL cholesterol	-0.053±0.128	0.67	-0.012±0.130	0.92
Glucose	0.056±0.112	0.61	0.045±0.112	0.68
Insulin	0.142±0.138	0.30	-	-
Model	R^2=39.2 %		R^2=39.1 %	

Values are regression coefficients (expressed in millimeters) for a 1 standard deviation change in continuous variables. Adjusted for baseline ultrasound variable.

When the dichotomous MetS definition was replaced by the number of MetS components present, the MetS component risk score was not significantly associated with the 6-year change in IMT, Cdist or FMD in the multivariable models.

Finally, it was assessed whether the association between the number of MetS components and change in ultrasound variables showed evidence of non-linearity. To examine this, change in IMT/Cdist/FMD was plotted against the number of MetS components (*Figure 12*). There was an increasing trend in IMT progression and decreasing trend in Cdist with increasing number of MetS components. Second order polynomial regression showed no quadratic association between the number of MetS components and IMT progression (metabolic score*metabolic score P=0.19). Similarly, no quadratic association was observed for Cdist regression (P=0.06) and FMD regression (P=0.62).



Mean±SEM values of A) IMT change B) Cdist change and C) FMD change across the number of MetS/Harm components.

Figure 12, Change in ultrasound variables according to the number of risk factors

5.5. ARTERIAL FUNCTION AND STRUCTURE AFTER RECOVERY FROM THE METABOLIC SYNDROME

5.5.1. 6-year risk factor and lifestyle habit changes

Table 15 shows the age-and sex adjusted changes in risk factors and lifestyle habits between 2001 and 2007. The most favorable changes were observed in the recovery group during the 6-year follow-up period. Next, age- and sex specific standardized changes in multiple lifestyle variables (physical activity, attention paid to health habits, alcohol-, vegetable-, fruit-, meat-, and fish consumption) were regressed against change in metabolic components as shown in *Table 16*. In these models all lifestyle variables were included simultaneously into the models as covariates. Decrease in physical activity and decrease in attention paid to health habits were independently associated with increase in waist circumference. Decrease in attention paid to health habits was independently associated with increase in systolic blood pressure and increase in physical activity associated with increase in HDL cholesterol.

Table 15, Changes in risk factors and lifestyle habits between 2001-2007 according to MetS groups

Change between 2001-2007	Recovery	Control	P-value*	Incident	P-value†	Persistent	P-value‡
N	71	1242		194		166	
ΔWaist (cm)	0.8±8.4	4.2±6.0	**<0.0001**	9.3±7.0	**<0.0001**	4.4±6.5	**0.006**
ΔBMI (kg/m^2)	-0.7±3.3	0.9±1.9	**<0.0001**	2.5±2.3	**<0.0001**	1.0±2.4	**0.0002**
ΔWeight (kg)	-2.6±10.1	2.6±5.5	**<0.0001**	7.5±7.2	**<0.0001**	3.1±7.2	**<0.0001**
ΔSystolic BP (mmHg)	-1.5±12.6	4.0±9.9	**<0.0001**	7.6±11.0	**<0.0001**	3.5±13.4	**0.02**
ΔDiastolic BP(mmHg)	0.6±9.6	5.2±8.6	**<0.0001**	7.6±9.5	**<0.0001**	4.3±10.1	**0.001**
ΔTotal cholesterol (mmol/l)	-0.34±0.74	-0.07±0.67	**0.001**	-0.03±0.88	**0.03**	-0.21±0.91	0.20
ΔLDL (mmol/l)	-0.27±0.69	-0.15±0.58	0.12	-0.17±0.75	0.44	-0.26±0.88	0.70
ΔHDL (mmol/l)	0.17±0.22	0.07±0.23	**0.0003**	-0.08±0.20	**<0.0001**	0.07±0.18	**0.0005**
ΔTriglycerides (mmol/l)	-0.34±0.36	0.02±0.38	**<0.0001**	0.32±0.47	**<0.0001**	-0.01±0.43	**<0.0001**
ΔGlucose (mmol/l)	0.09±0.39	0.23±0.43	**0.005**	0.51±0.67	**<0.0001**	0.42±0.83	**0.0005**
ΔInsulin (IU/l)	-0.19±0.62	-0.005±0.61	**0.009**	0.44±0.51	**<0.0001**	0.17±0.52	**0.0001**
ΔCRP (mmol/l)	-0.47±1.16	0.13±1.10	**<0.0001**	0.43±1.06	**<0.0001**	0.05±0.97	**0.02**
ΔMetabolic equality index (kcal/kg/h)	0.20±2.40	0.04±2.33	0.96	-0.40±2.40	0.16	-0.03±2.0	0.35
ΔAlcohol consumption (drinks/day)	0.008±0.47	0.013±0.53	**0.03**	0.010±0.47	0.10	0.024±0.45	0.12
ΔSmokers (%)	-8	-5	0.57	-2	0.97	-5	0.26
ΔAttention paid to health habits (points)	-0.34±0.11	-0.10±0.02	**0.03**	0.04±0.07	**0.004**	-0.24±0.07	0.34

Values are mean ± SD
P-values are from logistic regression models adjusted for age and sex.
* P-value between recovery and control group, † P-value between recovery and incident group, ‡ P-value between recovery and persistent group

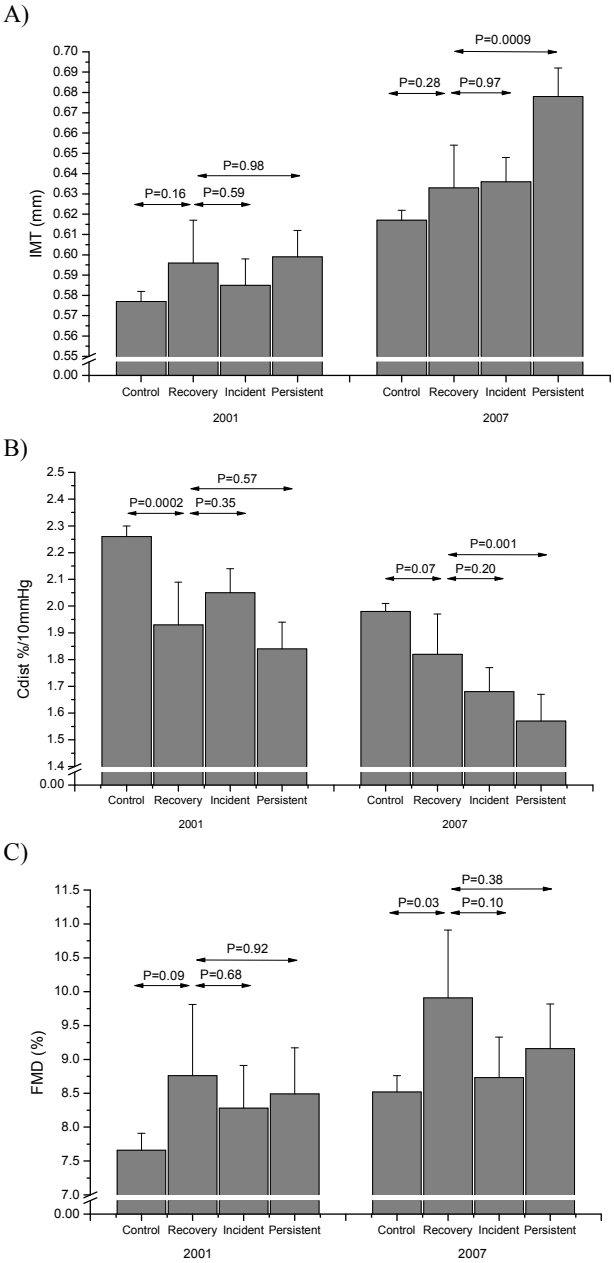
Table 16, Age- and sex specific standardized association between changes in multiple lifestyle variables and change in individual metabolic components.

	Model 1		Model 2		Model 3		Model 4		Model 5	
	ΔWaist circumference		ΔSystolic BP		ΔTrigly-cerides		ΔHDL cholesterol		ΔGlucose	
	Beta±SE	P-value	Beta±SE	P-value	Beta±SE	P-value	Beta±SE	P-value	Beta±SE	P-value
ΔPhysical activity	**-0.05±0.02**	**0.005**	0.06±0.03	0.06	0.002±0.03	0.94	**0.06±0.03**	**0.04**	-0.001±0.02	0.93
ΔAttention paid to health habits	**-0.08±0.02**	**0.0001**	**-0.07±0.03**	**0.02**	-0.01±0.03	0.70	0.007±0.03	0.80	-0.01±0.02	0.42
ΔAlcohol consumption	0.006±0.04	0.87	0.03±0.06	0.56	0.17±0.07	0.08	0.09±0.06	0.11	0.05±0.04	0.20
ΔVegetable consumption	-0.009±0.02	0.60	-0.03±0.03	0.28	0.01±0.02	0.73	-0.08±0.02	0.73	0.02±0.02	0.25
ΔFruit consumption	0.008±0.02	0.63	0.008±0.02	0.74	0.01±0.03	0.65	-0.004±0.02	0.85	-0.005±0.01	0.76
ΔMeat consumption	0.02±0.02	0.23	-0.02±0.02	0.30	-0.02±0.03	0.53	-0.002±0.02	0.38	-0.005±0.01	0.71
ΔFish consumption	0.003±0.02	0.85	0.01±0.02	0.48	-0.05±0.03	0.08	-0.01±0.02	0.68	-0.004±0.01	0.79

The models included age- and sex-specific z-score values for change in physical activity, attention paid to health habits, alcohol-, vegetable-, fruit-, meat- and fish consumption as dependent variables and individual change in metabolic risk component as the outcome variable (Model 1 = ΔWaist circumference, Model 2 =ΔSystolic blood pressure (BP), Model 3 = ΔTriglycerides, Model 4 = ΔHDL cholesterol, Model 5 = ΔGlucose)

5.5.2. Associations between MetS status and vascular properties in 2001 and 2007

Figure 13 shows the age- and sex-adjusted mean values of IMT, Cdist and FMD between study groups in 2001 and 2007. At baseline, in 2001, subjects in the recovery group had lower Cdist compared to control group. At follow-up, in 2007, subjects in the recovery group had smaller IMT (*Figure 13 A*) and higher Cdist (*Figure 13 B*) compared to subjects with persistent MetS. The difference in Cdist at baseline between recovery and control group was diminished at follow-up in 2007. In addition, those in the recovery group had higher FMD compared to subjects in the control group at follow-up in 2007 (*Figure 13 C*). When MetS was defined according to the MetS/Harm definition, additional difference in Cdist measured in 2007 between recovery and incident groups was observed (1.84±0.07 vs. 1.65±0.04 %/mmHg, P=0.05). Otherwise the results were similar to the results displayed in *Figure 13*.



Age- and sex adjusted mean±SEM values of (A) IMT (B) Cdist and (C) FMD in 2001 and 2007. P-values corrected for multiple comparisons (Tukey's). Abbreviations: IMT, carotid intima-media thickness; Cdist, carotid artery distensibility; FMD, brachial flow mediated dilatation

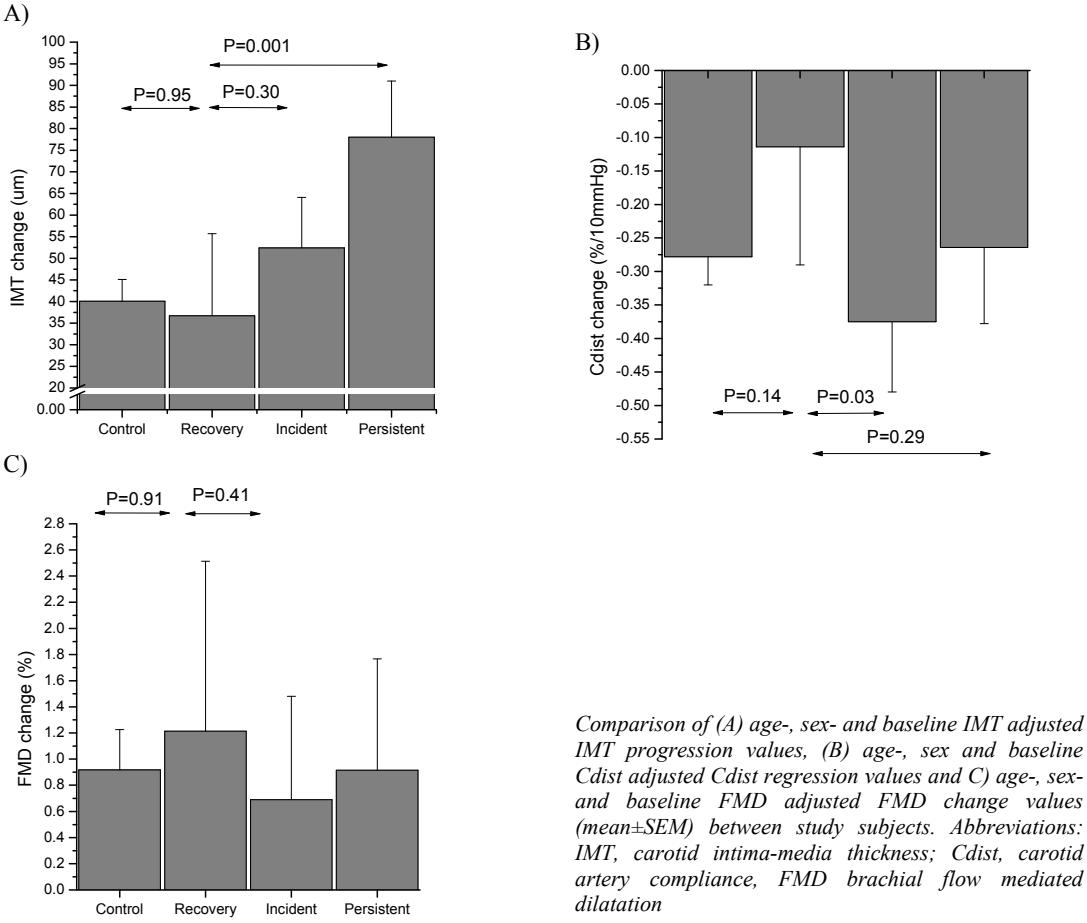
Figure 13, Associations between MetS status and vascular properties in 2001 and 2007.

Next, it was assessed whether weight change or MetS component changes have accounted for the findings. These analyses were performed as follows: 1) one at a time, changes in weight, waist circumference, systolic blood pressure, triglycerides, HDL cholesterol and glucose were included into the models to assess the effect of individual risk components; 2) included all variables simultaneously as covariates. Results for IMT and FMD in 2007 remained similar after including covariates individually or simultaneously into the model. The difference in Cdist between the

recovery group and persistent group at follow-up in 2007 was attenuated when 6-year weight change was included as a covariate (P=0.11 for MetS/IDF and P=0.06 for MetS/Harm).

5.5.3. Associations between MetS status and 6-year vascular changes

Figure 14 shows the age and sex adjusted mean values for the 6-year change in IMT, Cdist and FMD according to MetS status. Subjects in the recovery group had decreased rate of IMT progression compared to persistent group (*Figure 14 A*) as well as decreased rate of Cdist regression compared to incident group (*Figure 14 B*). No differences were observed for change in FMD between study groups (*Figure 14 C*). The results were similar when MetS was defined by the MetS/Harm definition.



Comparison of (A) age-, sex- and baseline IMT adjusted IMT progression values, (B) age-, sex and baseline Cdist adjusted Cdist regression values and C) age-, sex- and baseline FMD adjusted FMD change values (mean±SEM) between study subjects. Abbreviations: IMT, carotid intima-media thickness; Cdist, carotid artery compliance, FMD brachial flow mediated dilatation

Figure 14, Change in A) IMT B) Cdist and C) FMD stratified by MetS status

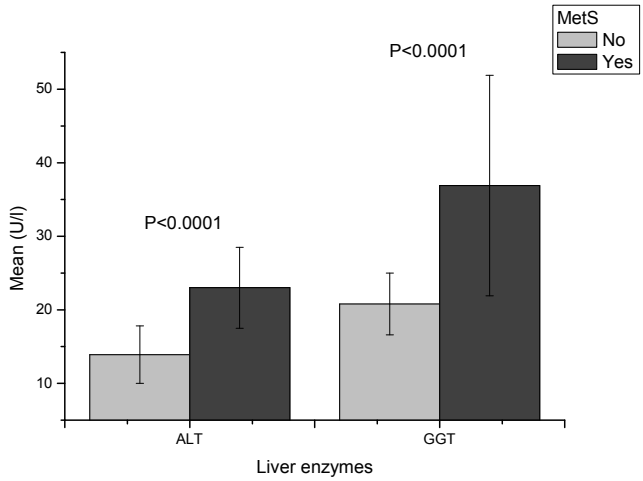
The results were similar for IMT and FMD progression when adjusting separately or simultaneously for 6-year change in weight, waist circumference, systolic blood pressure, triglycerides, HDL cholesterol and glucose. Similar results were also obtained after adjusting

for baseline IMT or baseline FMD, respectively. The difference in Cdist change between recovery group and persistent group was attenuated (P=0.90) when weight change was included into the model as a covariate. Of the individual MetS component changes, inclusion of the 6-year change in waist circumference and triglycerides also diluted the difference between recovery group and persistent group (P for Cdist regression 0.54 and 0.20 respectively). The results remained similar after inclusion of baseline Cdist into the model.

5.6. ASSOCIATION OF LIVER ENZYMES WITH METABOLIC SYNDROME AND THEIR INTERACTION ON ARTERIAL STRUCTURE

5.6.1. Cross-sectional analysis

Age- and sex-adjusted ALT and GGT levels in 2007 were higher in subjects with MetS compared to those without MetS (*Figure 15)*.



Cross-sectional age- and sex-adjusted comparison of liver enzyme levels between subjects with MetS and without it in 2007

Figure 15, ALT and GGT levels in 2007 in subjects with and without MetS

To assess whether the association of MetS on liver enzyme levels were mediated by alcohol intake or obesity, the levels of ALT and GGT were compared according to MetS status in subgroups of similar alcohol intake or BMI in 2007. The age- and sex-adjusted results are displayed in *Table 17*. Subjects with MetS had higher liver enzyme activities compared to those without MetS at all levels of alcohol intake and BMI status.

To further assess whether MetS was cross-sectionally associated with increased liver enzymes, regression models that included standardized age, LDL cholesterol, CRP, alcohol intake and adiponectin were constructed (*Table 18*). MetS was cross-sectionally (in 2007) associated in

both men and women with ALT and GGT. The results were similar when BMI or insulin was included into the models. Finally, MetS and age, LDL cholesterol, CRP, alcohol intake and adiponectin were regressed against insulin. MetS was associated with insulin in both men and women (β=0.37±0.03, P<0.0001 and β=0.42±0.12, P=0.0007 respectively).

Table 17, Cross-sectional age- and sex adjusted comparison of liver enzyme levels between subjects with MetS (+) and without MetS (-) in populations with similar alcohol intake and BMI in 2007.

		ALT			GGT		
		MetS(-)	MetS(+)	P-value	MetS(-)	MetS(+)	P-value
Drinks per day <1	N=1099	13.0(10.0-19.0)	18.9(13.0-26.0)	<0.0001	18.8(13.0-25.0)	27.9(19.0-48.0)	<0.0001
Drinks per day 1 to <2	N=346	14.9(12.0-22.0)	24.3(23.0-52.0)	<0.0001	22.6(15.0-30.0)	38.9(30.0-66.0)	<0.0001
Drinks per day >2	N=87	14.3(14.0-34.0)	23.6(23.0-52.0)	0.004	27.1(17.0-38.0)	38.9(22.0-76.0)	0.06
BMI <25 kg/m^2	N=732	11.8(9.0-16.0)	15.2(13.0-23.0)	0.04	16.8(12.0-23.0)	27.8(14.0-50.0)	<0.0001
BMI 25 to <30 kg/m^2	N=540	15.2(10.0-26.5)	18.3(13.0-26.5)	0.002	23.2(14.0-35.0)	29.6(21.0-66.0)	0.002
BMI >30 kg/m^2	N=267	18.7(12.5-25.5)	23.6(16.0-39.0)	0.003	29.1(18.0-40.0)	34.4(23.0-58.0)	0.03

Table 18, Multivariable cross-sectional associations between risk variables and liver enzymes in 2007

	ALAT		GT	
MEN	Beta±SEM	P-value	Beta±SEM	P-value
MetS	0.364±0.050	**<0.0001**	0.354±0.051	**<0.0001**
Age	-0.012±0.021	0.57	0.050±0.021	**0.02**
LDL cholesterol	0.051±0.020	**0.01**	0.112±0.020	**<0.0001**
CRP	0.083±0.024	**0.0006**	0.159±0.024	**<0.0001**
Alcohol intake	0.022±0.023	0.33	0.053±0.023	**0.02**
Adiponectin	-0.068±0.024	**0.005**	-0.071±0.024	**0.004**
WOMEN				
MetS	0.266±0.046	**<0.0001**	0.202±0.046	**<0.0001**
Age	0.001±0.016	0.94	0.053±0.016	**0.001**
LDL cholesterol	0.041±0.018	**0.02**	0.065±0.018	**0.0003**
CRP	0.060±0.016	**0.0002**	0.102±0.016	**<0.0001**
Alcohol intake	0.026±0.015	0.10	0.103±0.015	**<0.0001**
Adiponectin	-0.026±0.020	0.19	-0.046±0.020	**0.02**

Values are standardized regression coefficients (expressed in U/l) for 1-SD change in explanatory variables.

5.6.2. Prospective analysis

Age adjusted correlation coefficients for liver enzymes in 2007 with baseline 2001 MetS, risk factors and alcohol intake are shown in *Table 19*. The strongest correlates of future liver enzymes were adiposity measures and MetS in both sexes.

Results for multivariable regression analyses in men and women evaluating the independent baseline determinants in predicting increased liver enzymes activities 6-years later are shown in *Table 20*. Baseline MetS was associated in both men and women with ALT and GGT at follow-up in 2007. The results were similar when further adjusted for concurrent alcohol intake (2007). When BMI was included into the models as a dependent variable the association in women between MetS and ALT (P=0.42) and between MetS and GGT (P=0.30) 6 years later was diminished. When BMI was replaced with baseline insulin the association between MetS and GGT in women was diminished (P=0.27). To further assess whether MetS is associated with increased risk of liver damage, models that regressed standardized age, LDL cholesterol, CRP,

alcohol intake and adiponectin in 2001 against insulin measured in 2007 were constructed. MetS in 2001 was associated with insulin in both men and women (β=0.54±0.07, P<0.0001 and β=0.59±0.25, P=0.02 respectively).

Table 19, Age adjusted Pearson correlation coefficients for bivariate associations between risk variables measured in 2001 and liver enzymes 6-years later.

	MEN		*WOMEN*	
Variable 2001	ALT	GGT	ALT	GGT
MetS	0.26	0.21	0.13	0.20
Waist	0.37	0.36	0.28	0.33
BMI	0.34	0.32	0.29	0.33
Systolic blood pressure	0.17	0.16	0.11	0.11
Diastolic blood pressure	0.15	0.19	0.08§	0.13
Total cholesterol	0.15	0.22	0.08§	0.12
LDL cholesterol	0.11	0.17	0.09§	0.09§
HDL cholesterol	-0.19	-0.17	-0.06†	-0.04*
Triglycerides	0.28	0.33	0.09	0.18
Glucose	0.11	0.07†	0.05†	0.07†
Insulin	0.30	0.25	0.19	0.24
CRP	0.20	0.26	0.12	0.19
Alcohol intake	-0.01*	0.06*	0.05*	0.13

All P-values <0.001 unless informed otherwise
*P>0.12, †<0.05, §<0.01

Table 20, Multivariable associations between risk variables in 2001 and liver enzymes in 2007

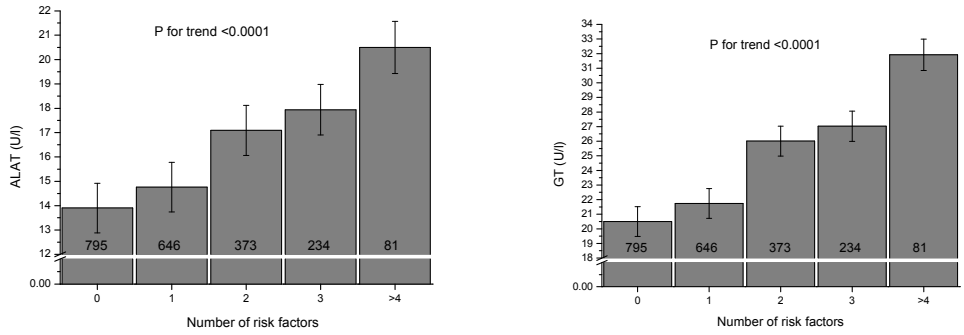
	ALT (2007)		GGT (2007)	
MEN	Beta±SE	P-value	Beta±SE	P-value
MetS	0.320±0.062	**<0.0001**	0.222±0.067	**<0.0001**
Age	-0.003±0.022	0.89	0.055±0.023	**0.02**
LDL cholesterol	0.051±0.020	**0.01**	0.088±0.022	**0.0009**
CRP	0.077±0.023	**0.01**	0.128±0.025	**<0.0001**
Alcohol intake	0.005±0.017	0.78	0.041±0.018	**0.03**
Adiponectin	-0.080±0.026	**0.002**	-0.110±0.028	**<0.0001**
WOMEN				
MetS	0.134±0.059	**0.02**	0.236±0.060	**<0.0001**
Age	0.012±0.017	0.45	0.063±0.017	**<0.0001**
LDL cholesterol	0.042±0.018	**0.02**	0.041±0.018	**0.02**
CRP	0.040±0.016	**0.02**	0.066±0.016	**<0.0001**
Alcohol intake	0.049±0.021	**0.02**	0.110±0.022	**<0.0001**
Adiponectin	-0.061±0.018	**0.0007**	-0.073±0.018	**<0.0001**

Values are standardized regression coefficients (expressed in U/l) for 1-SD change in explanatory variables.

Next it was assessed whether ALT and GGT levels in 2007 were associated with increasing number of MetS components diagnosed in 2001 (central obesity, hypertension, triglyceridemia, low HDL cholesterol, hyperglycemia). There was an increasing trend in ALT and GGT activity

with increasing number of baseline MetS components (age, sex, BMI, LDL cholesterol, CRP, alcohol intake and adiponectin adjusted) (*Figure 16 A and B*).

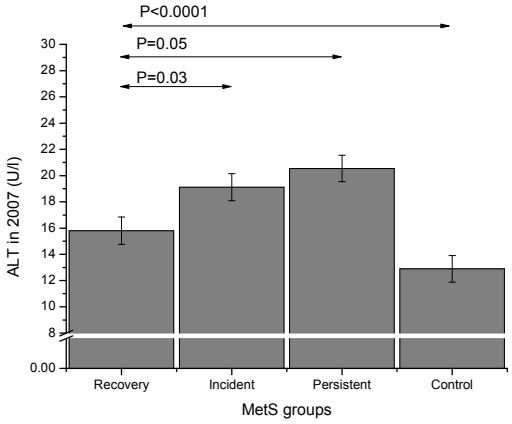
A) *B)*



Mean±SEM values for (A) ALAT and (B) GT activities across the number of MetS components. Adjusted for age, sex, BMI, LDL cholesterol, CRP, alcohol intake and adiponectin.

Figure 16, A) ALT and B) GGT activity with increasing number of baseline MetS components

To assess liver enzyme activities after recovery from the MetS, comparison of age- and sex-adjusted levels of liver enzymes between subjects with baseline (in 2001) only MetS and those with incident (only at follow-up in 2007) or with persistent (both at baseline and follow-up) MetS was performed. In 2007, subjects in the recovery group had significantly lower ALT activities compared to incident and persistent group (*Figure 17*). No difference between recovery and incident groups (P=0.17) or between recovery and persistent groups (P=0.17) in GGT levels was observed. Subjects in the control group had significantly lower GGT activities compared to recovery group.



Age- and sex adjusted mean and 95 % confidence intervals for ALT activity in recovery group vs. incident, persistent and control group.

Figure 17, ALT activities stratified by MetS status

5.6.3. The relations of liver enzymes with IMT in subjects with and without MetS

Multivariable regression model was used to examine cross-sectional association (in 2007) between liver enzymes and IMT. ALT and GGT were associated with IMT independent of age, sex and alcohol intake (*Table 21, Model 1*). When MetS was introduced into the models the association was diluted to non-significant as shown in *Table 21, Model 2*. Next, IMT was compared between subjects with and without biochemically proven non-alcoholic fatty liver (for men ALT >40 U/l, for women ALT >30 U/l). The model was adjusted for age, sex, alcohol intake and MetS. No difference was observed in IMT between subjects with and without biochemically proven non-alcoholic fatty liver (0.623±0.002 vs. 0.636±0.009, P=0.35). Results were similar when MetS was replaced by BMI (data not shown). Further, to examine the associations between liver enzymes and IMT according to the presence of MetS, cross-sectional interaction between liver enzymes and MetS on IMT was assessed. No statistically significant interaction for MetS*ALT (P=0.81) or MetS*GGT (P=0.92) on IMT was observed. Finally, IMT was regressed against liver enzyme activity stratified by MetS status. Models were adjusted for age, sex, alcohol intake and BMI. No linear association between ALT and IMT (β=0.004±0.012, P=0.71 in subjects with MetS and β=0.0001±0.04, P=0.99 in subjects without MetS) or between GGT and IMT (β=0.006±0.010, P=0.57 in subjects with MetS and β=-0.005±0.004, P=0.29 in subjects without MetS) was observed. Similar results were observed when subjects were stratified into ALT and GGT quartiles.

Table 21, Multivariable cross-sectional association between liver enzymes and IMT in 2007

	Model 1*		Model 2**	
	β±SE	P-value	β±SE	P-value
ALT	0.015±0.004	**0.0005**	0.008±0.004	0.06
GGT	0.013±0.004	**0.002**	0.006±0.004	0.16

* Adjusted for age, sex and alcohol use
** Further adjustment for MetS

6. DISCUSSION

6.1. SUBJECTS AND ATTRITION

The subjects of the present thesis are participants of the Cardiovascular Risk in Young Finns Study launched in 1980. The study subjects were randomly selected in different parts of the country, equally from both genders and from rural and urban areas to represent Finnish children and adolescents as closely as possible. A total of 3,596 (83.2 % of those invited), participated in the initial study in 1980 and were concluded to be representative of the total random sample (Åkerblom et al., 1985). Losts-to follow-up is inevitable in longitudinal studies. The participation rates in the follow-up studies among Young Finns participants have been satisfactory and shown in *Table 2*. In addition, the study group has been dynamic during the study years as many of those lost-to follow-up have participated in later follow-ups (*Figure 5*). Those lost-to follow-up were more likely to be males than females. Non-participants were younger in both sexes than participants. BMI was somewhat higher in female non-participants than in participants. However, the childhood risk factors in 1980 were similar between participants and non-participants. Therefore, the present study cohort seems to be representative of the original study population. In addition, no difference in MetS prevalence was observed at follow-up in 2001 among subjects who were lost-to follow-up between 2001 and 2007 compared to participants at both time-points. For the present thesis, 374 participants from the Bogalusa Heart study aged 9-18 years at baseline (11% of those eligible, 42% male, 34% Black) were included in Study II. The Bogalusa Heart Study is biracial (65 percent white and 35 percent black) community-based longitudinal epidemiologic study of cardiovascular risk factors from birth through the age of 38 years (Berenson et al., 1998). As shown previously (Magnussen et al., 2008; Magnussen et al., 2009), those lost to follow-up were more likely to be black, younger and male in the Bogalusa cohort (all $P<0.05$), but no significant differences were present between participants and non-participants in total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels in age- and sex-adjusted analyses suggesting that a major bias is unlikely and that study cohort seems representable. Due to the low number of blacks, the generalizability of the results in Study II is limited to white population and caution is advised of conclusions to biracial populations until larger scale data are available. The population of the present follow-up study has been randomly selected and the sample size is sufficient for statistical analyses and the follow-up bias does not seem to affect the representativeness of the original study cohort. Therefore the results in the present thesis may be generalized in white, ambulatory populations.

6.2. METHODS

6.2.1. Carotid intima-media thickness

Increased IMT associates with increased risk of CVD (de Groot et al., 2004), coronary event (O'Leary, 1999) and cardiovascular mortality (Salonen et al., 1991; Chambless, 1997; Lorenz et al., 2007; Bots et al., 2002). IMT is also associated with the severity of CVD (Hodis et al., 1998). The implications of IMT progression in population based samples, however, are not entirely clear, and more studies are needed to confirm the role of IMT progression as an indicator of clinical atherosclerotic disease (Costanzo et al., 2010). O'leary et al. reported that IMT was associated with the incidence of myocardial infarction and stroke in adults 65 years of age or older without pre-existing CVD (O'Leary et al., 1999). Further, Hodis et al. showed in their large follow-up study that IMT progression can be used to indicate the degree of existing generalized atherosclerosis and future CVD (Hodis et al., 1998). IMT was measured in the far wall of the common carotid artery. Measurements of IMT in the common carotid artery are more reliable and less difficult to obtain than IMT measurements in the carotid bifurcation or in the internal carotid artery, but also less sensitive to local atherosclerotic changes (Kanters, Algra, van Leeuwen, & Banga, 1997). Therefore, it is possible that the IMT data from only one site may underestimate the relationships between MetS and IMT progression compared to using data from all three segments (O'Leary et al., 1999). There is also controversy regarding the effect of measurement error bias in analyses evaluating IMT progression with baseline IMT used as a covariate (Yanez, III et al., 2002; Chambless et al., 2002). In the present thesis, however, the results from analyses correcting for measurement error bias were similar to those obtained from models adjusted for baseline IMT. The reproducibility of IMT measurements were acceptable (CV 6.4%) and comparable with other reports (Johnson et al., 2007; Salonen, Haapanen, & Salonen, 1991).

6.2.2. Carotid distensibility

Decreased carotid artery distensibility and stiffness is associated with risk factors and has been implicated as a predictor for cardiovascular events (Vlachopoulos, Aznaouridis, & Stefanadis, 2010; Blacher et al., 1998; Haluska et al., 2010; Boutouyrie et al., 1995). The reduction in Cdist is affected by an increase in blood pressure and intrinsic changes in the artery wall (Laurent, 1995; Reneman et al., 2005). Accurate assessment of Cdist requires the determination of several variables, including arterial diameter and concomitant blood pressure measurements. Thus, in part, the long-term variation for Cdist was somewhat high (CV= 14.3 %). Similar variations have been observed in previous reports (Arnett, Chambless, Kim, Evans, & Riley, 1999). The reproducibility of distensibility measurements may be improved by the use of computerized edge-detection analysis of sequential image frames (Selzer, Mack, Lee, Kwong-Fu, & Hodis, 2001). However, small variation in the carotid artery diameter measurements suggests that much of the long-term variation in Cdist is due to physiological fluctuation and not to measurement error (Juonala et al., 2005). Another limitation in measuring Cdist by the

ultrasound method used in the present thesis is the lack of a method to determine central blood pressure i.e. pulse pressure at the site of measurement. Atherosclerotic changes affect the brachial and carotid arteries differently, thus this approach is subject to error (Waddell, Dart, Medley, Cameron, & Kingwell, 2001).

6.2.3. Brachial flow-mediated dilatation

Brachial FMD is a marker of endothelial function that reflects nitric oxide release from vascular endothelial cells (Mullen et al., 2001). Impaired brachial FMD has been shown to predict cardiovascular events in patients with coronary disease (Chan et al., 2003). However, the integrity of FMD is complex (Bonetti et al., 2003). FMD response is greatly affected by balance between CVD risk factors, vasculoprotective elements, genetic predisposition and some unknown variables. Previously, we (Juonala et al., 2004b) and others (Higashi et al., 2003) have shown that the association between body size and FMD is curvilinear in the population of young adults. Furthermore, FMD status may modify the relations between risk factors and atherosclerosis (Juonala et al., 2004b; Järvisalo, Lehtimäki, & Raitakari, 2004). In the present thesis, considerable long-term variation existed in FMD measurements (26 %). However, these values are in line with previous reports (Herrington et al., 2001; Lind et al., 2000). The major sources of variation in FMD studies are artefacts and patient movements (Bartoli et al., 2008). In addition, an automated analysis in measuring FMD to minimize measurement error may offer better reproducibility compared to manual measurements (Bartoli et al., 2008; Frangi, Laclaustra, & Lamata, 2003). Due to relatively large long-term variation in FMD measurements, the non-significant differences in FMD and FMD change should be interpreted cautiously. On the other hand, it may be argued that the observed associations for FMD would have been stronger if the variations between measurements had been smaller.

6.2.4. Liver enzymes

Elevated ALT and GGT are markers of liver damage that have been associated with fat accumulation in liver and CVD (Angulo, 2002; Westerbacka et al., 2004). Currently, the available chemical markers of fatty liver in clinical practice are somewhat limited (Kotronen et al., 2008). In the present study, liver fat content was not able be measured with more ascertainable methods such as magnetic resonance spectroscopy or biopsy. Liver enzyme levels fluctuate over time, therefore one time measurement may underestimate liver fat content. Data on liver enzymes was not available from the baseline (in 2001) examination and therefore measuring, whether there were baseline differences between study groups, was not available. In the present study the study subjects are relatively young, and only few have developed biochemically diagnosed non-alcoholic fatty liver (for men ALT >40 U/l, for women ALT >30 U/l) (Prati et al., 2002). In addition, the cut-off for diagnosing non-alcoholic fatty liver is arbitrary. Previous data suggest that elevated levels of liver enzymes, even within the normal range, may indeed be prognostic with respect to liver fat content and the development of NAFLD. Kotronen et al. have shown that ALT levels correlate linearly with the magnetic

resonance spectroscopy (MRS) proven liver fat, already within the normal range. Similarly, GT has been shown to correlate strongly with ultrasound proven liver fat content (Kotronen et al., 2008; Mofrad et al., 2003; Chang, Ryu, Sung, & Jang, 2007; Patel, Srinivasan, Xu, Chen, & Berenson, 2007). However, because the mean values of increased liver enzymes were in the upper limit of normal, the results should be interpreted with caution.

6.3. RESULTS

6.3.1. Cardiovascular risk factors predicting incident MetS

Study I demonstrated that circulating apoB levels were predictive of 6-year incidence of MetS even after adjusting for risk factors included in the MetS criteria. The odds ratios of apoB predicting incident MetS was higher than those of systolic blood pressure, glucose, non-HDL, OxLDL, HDL cholesterol and apoA1.

Study I demonstrated an association between apoB and the incidence of MetS in young adults independent of risk factors included in the criteria. In line with these data, Onat et al. recently demonstrated in 2,348 middle-aged to elderly men and women that apoB predicts the incidence of MetS independent of waist circumference and CRP over a 6-year follow-up (Onat et al., 2007). In addition, several studies have reported strong association between apoB and cardiometabolic disorders (Ginsberg et al., 2006; Walldius et al., 2006; Sniderman & Faraj, 2007). The present results show that ApoB had somewhat better odds ratios in predicting incident MetS compared to total cholesterol, LDL cholesterol, non-HDL, HDL cholesterol and OxLDL. This is in line with several studies that have shown the superiority of apoB over conventional lipids in identifying subjects with MetS and CVD (Bamba et al., 2007; Walldius et al., 2006). Therefore, apoB may be used in identification of young adults who are at particularly high risk of developing MetS. Patients with elevated levels of apoB are often hypertriglyceridemic or hypercholesterolemic (Ayyobi et al., 2003). Indeed, according to the present results, apoB was predictive of incident hypertriglyceridemia and had a tendency to predict incident low-HDL but also central obesity and hypertension. In addition, apoB was strongly associated with other lipid parameters. Moreover, in multivariable models apoB was consistently associated with incident MetS even when adjusted for the risk factors included in the MetS criteria.

Previous study (Juonala et al., 2008; Mattsson et al., 2010) in the Young Finns study population demonstrated that apoB associates with increased carotid intima-media thickness. Mattsson et al. (Mattsson et al., 2010) showed that those with MetS and high apoB were 3.4 times the risk of 6-year incident high cIMT (>90 percentile) compared with subjects without MetS and normal apoB levels. In the present study, we demonstrate that part of the atherogenicity associated with apoB may be partly explained by the increased oxidation in LDL particles. MetS is often paralleled with many lipid and lipoprotein abnormalities characterized by elevated very low density lipoproteins (VLDL), intermediate density lipoproteins, and chylomicrons (Bamba et

al., 2007; Ginsberg et al., 2006). The use of only LDL cholesterol ignores the significant contribution of atherogenic VLDL-, IDL cholesterol and the composition of LDL particles to development of metabolic derangement (Kontush & Chapman, 2006). ApoB accounts for the number of oxidized, intermediate, low-density- and triglyceride-rich lipoproteins. Therefore, apoB may be used to estimate the number of atherogenic particles in plasma (Sniderman et al., 2007).

These data suggest that the prospective association of apoB with incident MetS and its components may be a reflection of an early metabolic derangement that is also highly atherogenic (Ayyobi et al., 2003; Vekic et al., 2009) but may also suggest that dyslipidemia characterized by elevated apoB levels have a predisposing role in the development of MetS.

Previous studies have shown that high OxLDL concentrations are related with MetS (Holvoet et al., 2008; Holvoet et al., 2004). Further, Holvoet et al recently found a significant association between $OxLDL_{prot}$ and incident MetS during their 5-year follow-up among 1,889 men and women (ages 33-45 years) independent of age, sex, LDL cholesterol and BMI suggesting a causal role for OxLDL in the development of MetS (Holvoet et al., 2008). In the present study, OxLDL was measured using baseline diene conjugation based assay for oxidized LDL lipids as well as using similar technique used in the study by Holvoet et al. based on enzyme-linked immunosorbent assay for oxidized LDL lipids, thus allowing us to comprehensively determine oxidative modification of LDL particles. On the basis of the present results, the association between OxLDL and incident MetS was diluted after adjustments. The results were similar for $OxLDL_{prot}$ and $OxLDL_{lip}$. The discrepancy between results shown here and those of Holvoet et al. may be explained by differences in study populations, measurement protocols and statistical methods. Most importantly, however, apoB was not included as parameter in the study of Holvoet et al (Holvoet et al., 2008). Although studies in animal models and in vitro have suggested that OxLDL may have an important role in the pathogenesis of obesity and insulin resistance (Masella et al., 2006; Maddux et al., 2001), the evidence in clinical studies have not been consistent (Park et al., 2009). Moreover, the study by Holvoet et al did not consider apoB in their analysis, but rather focused on LDL cholesterol (Holvoet et al., 2008).

6.3.2. Pediatric MetS as a risk factor for adult outcomes

Study II showed that despite instability in the diagnosis of youth MetS over a mean 24-year period, dichotomous definitions of MetS in youth predict important disease outcomes, such as adult MetS, high IMT, and T2DM in early to middle adulthood. High BMI alone was as good and in some cases superior to dichotomous pediatric MetS definitions in predicting adult MetS, high IMT and T2DM.

From a pediatric perspective, the American Heart Association has declined provision of a consensus definition on MetS partly due to unclear potential of youth with MetS to maintain the diagnosis even over relatively short periods (Steinberger et al., 2009; Goodman et al., 2007;

Gustafson et al., 2009). Using three different definitions to diagnose MetS in 15-year olds from a population-based sample, Goodman et al. showed that despite consistent risk factor clustering, the diagnosis of MetS was stable in only 50% of cases over a 3-year period (Goodman et al., 2007). Among obese youth aged 6-17 years, Gustafson et al found that only 30% and 45% of those with baseline MetS were confirmed after 60-day and 1.5-year follow-ups (Gustafson et al., 2009). This observation is somewhat surprising considering the short duration of follow-up but also because namely obese youth would expect MetS to be maintained at a comparatively high level. The estimates of mean 24-year stability were in the same order of magnitude compared to previous studies ranging from 40-60% depending on the youth MetS definition employed. This suggests that while long-term stability is low, it does not appear to be substantially worse than short-term stability. This finding is not surprising given the known influence of pubertal stage on a number of MetS components (Frontini, Srinivasan, & Berenson, 2003) that may have contributed to the low short-term stability observed in both prior studies. In addition, while tracking of risk factor levels is known to decrease as the interval between measurements becomes longer (Chen & Wang, 2008; Porkka, Viikari, Taimela, Dahl, & Åkerblom, 1994), the ability to predict future values from baseline levels tends to decrease substantially in the first days to weeks followed by a more modest decline over several years (Porkka et al., 1994).

Given the instability in the categorical diagnosis and poor clinical prediction the clinical utility of categorical pediatric MetS definition is limited in clinical setting (Schubert, Sun, Burns, Morrison, & Huang, 2009; Huang, Nansel, Belsheim, & Morrison, 2008). However, while sensitivity and positive predictive values were low, specificity and negative predictive values were consistently high across the criteria examined. Rather than considering identification of youth with MetS as a means of identifying those who will develop important outcomes in adulthood, it may be more suitable to use these definitions, as a basis for identifying those not at risk so that further attention could be focused on those with unclear potential for developing MetS, high IMT, or T2DM at early-middle adulthood (Schubert et al., 2009). This interpretation appears relevant given the findings that those with youth MetS, irrespective of instability in the categorical diagnosis and poor clinical prediction, were at significantly increased risk of MetS, high IMT, and T2DM in adulthood.

An important finding from this study was that high BMI predicts each outcome as well as, or better than the categorical MetS definitions considered in this study. This finding has clinical relevance. At pediatric visits for health care, BMI can easily and accurately be determined using minimum equipment, which would allow the immediate identification of youth at heightened risk (using e.g. Coles' international tables) that might benefit from therapeutic lifestyle intervention aimed at weight control. Other benefits include the need not to subject a child to a blood draw, and aversion of costs and time associated with laboratory analysis. A caveat to the clinical application of these findings is that a substantial number of contemporary youth will be identified as at-risk. The results of the present thesis are in line with another large scale study done in men by Tirosh et al. who showed that BMI at the adolescence is an independent

predictor of CVD in young adulthood suggesting that obesity has long term consequences. The 17 year risk of CVD was increased by 12.0% for each increment in 1 BMI unit at adolescence. BMI also predicted increased risk of developing T2DM later in life. However, the risk was not independent of that predicted by BMI in adulthood (Tirosh et al., 2011).

One explanation for why the additional measures incorporated into MetS did not improve prediction may be because one measurement of BMI is more accurate than one measurement of the laboratory components of MetS. Pediatric guidelines concerning blood pressure (Falkner & Daniels, 2004) and lipids (Daniels & Greer, 2008) require multiple measurements before elevated levels are diagnosed owing to laboratory and biologic variation. It is possible that multiple laboratory-based and blood pressure measures collected over a period of weeks/months may improve the observed estimates for pediatric MetS, and is a limitation of this study. In line with this hypothesis, Gaziano and colleagues have recently shown a non-laboratory-based risk score (including BMI, blood pressure, smoking status, and reported diabetes status) to predict CVD events as accurately as a risk score that additionally included laboratory-based methods (Gaziano, Young, Fitzmaurice, Atwood, & Gaziano, 2008). Another explanation may be that overweight and obesity precedes the clustering of MetS components such that it may be a more sensitive marker in the pediatric setting. While the specific etiology of MetS is unknown, potential mechanisms posit obesity and insulin resistance as initiating factors. Study II showed that overweight and obesity to remain an independent predictor of adult outcomes in multivariable models but the corresponding association with insulin disappeared. While our study cannot establish causality, these data are consistent with reports from the Bogalusa Heart Study showing a temporal association between degree of baseline adiposity and incidence of hyperinsulinemia in youth and young adults independent of baseline insulin levels and independence of childhood obesity, but not insulin or insulin resistance, in predicting adult MetS (Ravussin & Gautier, 1999; Srinivasan, Myers, & Berenson, 2002).

6.3.3. MetS in predicting vascular changes

Study III demonstrate that MetS was significantly associated with accelerated IMT progression. However, no evidence that MetS would predict IMT progression more than would be expected from the sum of its risk components was found.

Exposure to atherogenic lipid profile in early life may induce changes in arteries that contribute to the development of atherosclerosis. Study III showed that by diagnosing MetS in young adults, it is possible to identify groups of individuals that have accelerated progression of carotid IMT suggestive of increased atherosclerosis development. The present results in over 2000 young adults are in agreement with previous studies in elderly or hypertensive subjects. Hassinen et al. demonstrated in 102 women (baseline age 60-70 years) that MetS/NCEP predicted IMT progression during a 12-year follow-up (Hassinen et al., 2006). In the European Lacidipine Study on Atherosclerosis (ELSA), MetS was associated in a bivariate model with the 4-year change in IMT among 1,734 hypertensive subjects aged 45-75 years at baseline

(Zanchetti et al., 2007). However, in the ELSA study the association between MetS and IMT progression became non-significant after adjustments for covariates.

The association between MetS and IMT progression was independent of the main cardiovascular risk factors. However, inclusion of the dichotomous MetS variable in the multivariable model did not improve the overall predictive value. Moreover, the relation between the number of MetS components and IMT progression was linear, i.e. no evidence to suggest that MetS constellation would predict IMT progression more than would be expected from the sum of its risk components was observed. These results are in line with recent observations suggesting that MetS does not increase cardiovascular morbidity and mortality (Mozaffarian, Kamineni, Prineas, & Siscovick, 2008; Wang et al., 2007) over and above its individual components.

In the present study, MetS/EGIR predicted and MetS/Harm tended to predict IMT change after adjusting for its components. The reason for MetS/EGIR in predicting IMT progression in the present longitudinal analysis may have been the influence of hyperinsulinemia as a diagnostic component in the MetS/EGIR definition. Insulin had a strong effect on IMT progression when all MetS risk factor components were included in a multivariable analysis simultaneously. Various mechanisms have been hypothesized by which hyperinsulinemia could directly promote atherosclerosis (Goke & Fehmann, 1996; Pyörälä et al., 2000). Insulin can increase the formation and decrease the regression of lipid lesions. It stimulates the proliferation of arterial smooth muscle cells and collagen synthesis, and promotes production of various growth factors in the arterial wall. However, hyperinsulinemia may only be a surrogate marker of insulin resistance which was not available in the present study (Reaven & Laws, 1994). Studies have also supported the view that the combination of insulin resistance and compensatory hyperinsulinemia may synergistic effect on the progression of CVD as it has been shown that insulin resistance mainly affects only certain metabolic pathways in insulin transduction leaving other pathways intact (Wang, Goalstone, & Draznin, 2004). These population-based data do not allow us to determine whether insulin has a direct role in promoting atherosclerosis or whether high insulin is marker of underlying insulin resistance.

6.3.4. Arterial function and structure after recovery from the MetS

In Study IV it was demonstrated that recovery from the MetS over a 6-year period had a positive effect on vascular properties. Subjects in the MetS recovery group had smaller IMT and higher Cdist after the follow-up compared to subjects with persistent MetS, although, at baseline there were no differences. In addition, those in the recovery group had decreased rate of IMT progression compared to subjects who had persistent MetS and decreased rate of Cdist regression compared to subjects with incident MetS over the 6-year period.

The findings in Study IV are in line with earlier reports demonstrating the effects of MetS exposure on vasculature. All current MetS definitions identify individuals with evidence of

increased IMT and decreased Cdist (Juonala et al., 2005). It has been established that preclinical atherosclerosis is not an irreversible, but rather a dynamic process. Different interventions on cardiovascular risk factors (dyslipidemia, hypertension, diabetes and obesity) have been shown to slow or even regress the progression of atherosclerosis (Salonen et al., 1995; Nissen et al., 2006; Girerd et al., 1998; Nathan et al., 2003; Raitakari et al., 2004; Meyer et al., 2006). Pathological data from deceased subjects have shown that in young adults (age 30-39 years) preclinical atherosclerotic lesions in the common carotid artery were dominated by foam cell formations (Dalager, Paaske, Kristensen, Laurberg, & Falk, 2007). The internalization of LDL particles (which may be enzymatically and oxidatively modified) by macrophages results in the formation of foam cells which are known to predispose to fatty-streak formation in atherosclerosis (Dalager et al., 2007; Ross, 1999). Foam cell formation is an early manifestation of atherosclerosis and modified LDL particles may still be subsequently cleared from the artery wall to the liver by HDL cholesterol (Dattilo et al., 1992). The reversibility of foam cell lesions may provide an explanation for the dynamic ultrasonographic changes in common carotid artery in subjects with MetS observed in this study. On the basis of these data, favorable changes in vasculature can be achieved in adulthood by improving metabolic risk factors.

The difference in Cdist change between recovery group and incident group was diluted after adjusting for changes in weight or waist circumference suggesting that favorable changes in Cdist may have been mediated by weight loss alone. In addition, subjects who recovered from MetS diagnosis had reduced their adiposity levels compared to other groups. In addition, decrease in physical activity and attentions paid to health habits were independently associated with increase in waist circumference. Further, paying less attention to health habits was independently associated with increase in systolic blood pressure and increase in physical activity associated with increase in HDL cholesterol. Previous studies conducted in samples of overweight adults suggest that weight loss induced by nutritional and exercise intervention is associated with correction of arterial structure and function (Raitakari et al., 2004; Meyer et al., 2006). As reported in other studies (Raitakari et al., 2004; Dattilo et al., 1992; Dengel et al., 2006), weight change observed in the recovery group was associated with favorable changes in blood pressure, lipids, glucose and insulin. All these metabolic changes have been confirmed as determinants of vascular structure and function across all age groups (Tzou et al., 2005; Hassinen et al., 2006; Juonala et al., 2005; Juonala et al., 2004b; Dengel et al., 2006). In addition, insulin has been hypothesized partly responsible for changes in sympathetic stimulation (Vollenweider et al., 1994) which may be accompanied by a reduction of Cdist (Mangoni, Mircoli, Giannattasio, Mancia, & Ferrari, 1997).

When comparing baseline risk factors in recovery and persistent MetS groups, Study IV shows that subjects in the recovery group had more favorable levels of metabolic variables. It is therefore possible that less severe MetS may be more likely to recover. However, at baseline, no differences in vascular parameters were observed between the MetS study groups.

6.3.5. Association of liver enzymes and MetS and their interaction on arterial structure

Study V showed that MetS was independently associated with increased liver enzyme activity both cross-sectionally and prospectively. Recovery from the MetS over a 6-year period was associated with lower activities of ALT at the follow-up compared to those with incident or persistent MetS. Liver enzyme activity did not modify the association between MetS and IMT.

Subjects with the MetS had increased levels of ALT and GGT (compared to those without MetS) independent of obesity and alcohol intake suggestive of liver changes that may indicate increased liver fat content (Kotronen et al., 2008; Bellentani et al., 2000). Patel et al. showed in a large group of young adults that elevation of ALT and GGT, as biomarkers of liver dysfunction and NAFLD, associated adversely to MetS and its components as well as to history of coronary artery disease (Patel et al., 2007). These data are in line with previous studies in which the association of MetS with biopsy, ultrasound and magnetic resonance spectroscopy proven NAFLD was observed (Kotronen et al., 2008). Kotronen et al (Kotronen et al., 2008) reported that in subjects (aged 20-65 years) with MetS, liver fat content was four-fold higher (8.2 % vs. 2.0 %) compared to subjects without MetS. Although fatty liver has been associated strongly to alcohol abuse and obesity, Study V shows that when subjects within a narrow range of BMI and with similar alcohol drinking habits were studied, the association between MetS and liver enzyme levels remained significant. This is in agreement with studies showing that not all morbidly obese individuals develop NAFLD (Clark, 2006) and that some individuals with a fatty liver are not obese (Pratt & Kaplan, 2000). Thus, obesity does not seem to have a direct role in promoting liver fat but may intensify the effects of other factors predisposing liver fat accumulation (Bellentani et al., 2000). Further, increasing trend in serum liver enzyme activities with increasing number of metabolic components was observed. A similar trend has been observed in a small scale clinical study that examined the association between MetS components and liver fat assessed by magnetic resonance spectroscopy (Kotronen et al., 2007). These observations suggest that in the presence of MetS, elevated liver enzyme activity even within the normal range, may indicate the severity of the syndrome in-turn accelerating the development of clinical NAFLD.

In the present study, MetS at baseline correlated independently with ALT and GGT at follow-up. However, when adjusting for BMI or insulin the association between MetS and ALT 6-years later was diminished in women. This observation may be explained by female reproductive hormones that could contribute to lower serum ALT concentrations, but may also indicate that increased ALT activity in women is associated with insulin resistance. Insulin resistance is considered as a key underlying factor in the development of MetS (Reaven, 1988). Suppression of the inhibitory effect of insulin on lipolysis in adipose tissue may lead to an increased production of free fatty acids and glucose production from the liver thus inducing accumulation of fat in liver (Cornier et al., 2008).

Study V also showed that a spontaneous recovery from the MetS over a 6-year period to be associated with lower serum ALT levels compared to those who had incident or persistent MetS

at follow-up. In line with this observation, several intervention studies have reported reduced levels of liver fat deposits after thiazolidinedione treatment and favorable developments in lifestyle habits (Kotronen et al., 2008). However, data on liver enzymes available from the baseline (in 2001) examination was lacking and therefore were not able to evaluate whether there were baseline differences between study groups.

MetS and NAFLD frequently coexist (Brea et al., 2005). In cross-sectional settings NAFLD has been found to correlate with IMT even independent of MetS (Sookoian et al., 2008). Pacifico et al. showed that obese children with NAFLD had significantly increased carotid IMT than obese children without liver involvement and controls. The severity of fatty liver as assessed by ultrasound was independently associated with IMT (Pacifico et al., 2008). In addition, two large longitudinal studies that included middle aged adults have demonstrated a dose-response relationship between liver enzymes and cardiovascular events (Schindhelm et al., 2007; Ruttmann et al., 2005). Fatty liver and atherosclerosis may share common molecular mediators (Targher & Arcaro, 2007). However, it is somewhat unclear whether NAFLD is merely a marker or an early mediator in the development of atherosclerosis (Targher et al., 2007). In the present study, the association between liver enzymes and IMT was diluted when taking MetS into account suggesting that liver changes assessed by ALT and GGT may not indicate an increased risk of CVD over and above what would be expected due to the increased risk of the MetS. Our results are in line with previous literature regarding CVD risk in subjects with rigorously determined NAFLD (Ghouri, Preiss, & Sattar, 2010). However, our findings were limited to cross-sectional design and therefore the causal association between elevated liver enzymes and IMT could not be studied. Further, Kim et al. recently reported that after adjusting for conventional cardiovascular risk factors, the independent association between NAFLD and IMT was limited to subjects with MetS or multiple metabolic abnormalities (Kim, Kim, & Huh, 2009). However, no MetS*ALT or MetS*GGT interaction on IMT was observed. These data suggests that increased liver enzyme activities in young adults may not be indicative of additive risk of atherosclerosis among subjects with MetS.

6.4. CLINICAL IMPLICATIONS

MetS and the dynamics of atherosclerosis are complex processes. Observations from large population based cohorts provide an insight into these processes, thus having critical clinical relevance. The results addressed in the present thesis are important and have direct clinical relevance in early prevention and control of CVD at the population- and high-risk individual level.

The present thesis demonstrates that high levels of apoB may predict individuals with early metabolic derangement thus having increased risk of incident MetS. In addition, childhood MetS and high BMI predicted important disease outcomes in early to middle adulthood, such as MetS, high IMT, and T2DM. MetS in young adulthood was significantly associated with

accelerated IMT progression and markers of early liver damage but recovery from the MetS over a 6-year period had a positive effect on vascular properties and liver function.

This study suggests that apoB may provide useful information in predicting atherosclerosis development early in adulthood. Our findings do not lessen the clinical importance of assessing total-, LDL- and HDL cholesterol but rather emphasize the role of apoB in early metabolic derangement and the importance of prevention and treatment of atherogenic dyslipidemia characterized by apoB. In addition, this study suggest that in the clinical setting, efforts to identify youth with heightened future risk of meaningful outcomes can be minimally achieved with the use of BMI only, thus avoiding cost and other barriers associated with testing and classification of youth MetS. However, clinicians who use high BMI to identify youth at increased future risk need to keep in mind that a large proportion of contemporary youth will be classified as at risk and that those analyses presented here are unable to discount that youth MetS may be useful in identifying and possibly treating other cardiometabolic disorders. As shown in our study, adverse developments of lifestyles leading to obesity are translated into accelerated atherosclerosis already early in adult life. However, based on results in the present study, arterial structure and function may be restored after spontaneous recovery from the MetS. Furthermore, recovery form MetS was associated with weight loss. Therefore, in addition to identification of individuals who are at particularly high risk, a major effort is needed for the reduction of overweight in the whole population of children, adolescents and young adults to reduce the burden of CVD later in their life. In addition, the results of the present thesis suggest that individuals with a past history of MetS are in need for continued vigilance for adverse liver outcomes even after apparent recovery from MetS. Finally, the present thesis demonstrates that the ability of the MetS to identify individuals at risk for accelerated atherosclerosis is no better than its competent parts. These findings suggest that the clinical utility of the MetS as a short term risk assessment tool is limited. Ultrasound methods assessing subclinical atherosclerosis are inexpensive and reliable means to study preclinical atherosclerosis in ambulatory individuals. The results of this study indicate that in addition to evaluating conventional cardiovascular risk factors, carotid ultrasound measures may be used in clinical setting to monitor the progression of atherosclerosis.

6.5. LIMITATIONS

One potential limitation of our study was selection bias due to lost-to follow-up. However, as demonstrated in the present thesis, childhood characteristics in 1980 were essentially similar between participants and non-participants, and study cohort seems to be representative of the original study population. In addition, no difference in MetS prevalence in 2001 among subjects who were lost-to follow-up between 2001 and 2007 compared to participants at both time-points was observed. When comparing risk factors in 2001 in recovery and persistent MetS groups, it was observed that subjects in the recovery group had more favorable levels of metabolic variables. It is therefore possible that less severe MetS may be more likely to recover.

However, in 2001, no differences in vascular parameters were observed between the MetS study groups. Because our study cohort was racially homogenous, the generalizability of our results is limited to white European subjects. In addition, because participants in the Young Finns and Bogalusa studies are still of relative young age, it was not possible to study associations between risk factors and clinical outcome of cardiovascular events. Instead, vascular ultrasound measure was used as an indicator of an atherogenic process. Increased oxidative stress was assessed by measuring OxLDL, which reflects only one aspect of increased oxidative burden. In addition, due to the heterogeneous nature of the chemistry of LDL oxidation, proper determination of OxLDL is problematic (Ahotupa et al., 1999). In the present study, however, two different methods were used to evaluate oxidative modification of LDL particles. $OxLDL_{prot}$ measure is based on the enzyme-linked immunosorbent assay with antibodies (mAb-4E6) directed against a conformational epitope in oxidized apoB-100 molecules in serum (Holvoet et al., 2004; Holvoet et al., 2008). However, the specificity of the monoclonal antibodies against apoB epitopes in various apoB-containing lipoprotein particles is unclear. The apoB-lipoprotein metabolism is a continuum with no definite borderline between small very-low-density and intermediate-density (and low-density) lipoprotein particles. Thus, antibodies against conformational epitopes in apoB are likely to recognize modified apoB in all similar apoB-containing lipoprotein particles. In fact, these triglyceride-enriched apoB-containing lipoprotein particles have also been demonstrated to possess atherogenic effects similar to LDL particles (Oörni, Posio, Ala-Korpela, Jauhiainen, & Kovanen, 2005). Nevertheless, triglyceride-rich large apoB-containing particles contribute generally only around 15% of total apoB in serum, the LDL and IDL particles containing the rest of circulating apoB (Barter et al., 2006).

The original baseline surveys were performed in the 1980s before the importance of abdominal adiposity to clustering of metabolic related risk factors was known. Thus data on waist circumference in childhood was missing. While BMI is considered a reasonable alternative to waist circumference, it may be a less sensitive measure predicting CVD risk. The low numbers with T2DM and use of fasting glucose levels and self-report data to indicate adult T2DM mean that associations with T2DM should be interpreted cautiously. Currently, the available chemical markers of fatty liver in clinical practice are somewhat limited. In the present thesis, the measurement of liver fat content with ascertainable methods such as magnetic resonance spectroscopy or biopsy was not available. Because liver enzyme levels fluctuate over time, measurement as a single time-point may underestimate liver fat content. Data on liver enzymes was not available from the baseline (in 2001) examination and therefore were not able to evaluate whether there were baseline differences between study groups. In addition, exclusion was not made for subjects with viral (hepatitis B and C), toxic, autoimmune or other rare causes of elevated liver enzymes (Wilson disease, hemochromatosis) from the cohort, which may have influence the results. Because the mean values of increased liver enzymes were in upper limit of normal range, the results should be interpreted with caution.

6.6. FUTURE RESEARCH PROSPECTIVES

There is no consensus on how to define MetS. Present MetS definitions represent a simple dichotomous way in clinical practice to identify high risk individuals. However, the use of dichotomous variables results in loss of crucial information concerning the magnitude of risk factors. Thus, future studies are needed to define the MetS to better reflect the continuous nature of the cardiometabolic risk. Further support is needed for the clinical utility of the MetS as a diagnostic category. It is not clear that MetS performs better in predicting increased risk of atherosclerosis than the sum of its individual components. Confirmation in this regard in other populations and with clinical events is needed. Better understanding of the pathophysiology involved in the dynamics of MetS is also further needed. Although, abdominal obesity and insulin resistance are considered as the underlying cause of the MetS, it is still unclear whether all of the components are directly related to these conditions and whether all components deserve an equal importance in risk prediction. In addition, the role of environmental and gene interaction in promoting the MetS needs further focus. More studies to determine metabolic derangement in children and adolescents are needed and whether MetS in youth can predict future diseases. Although, there is agreement that lifestyle changes to induce weight loss are the first-line approach in treating MetS, the ideal diet and form of exercise for the treatment of the MetS remains uncertain. In addition, studies with longer follow-up periods are needed to judge the clinical utility of MetS with respect to cardiovascular outcomes. Due to inherent complexities in lipoprotein physiology and molecular detection, further studies on the specific role of apoB in various lipoprotein particles and oxidation of these lipoprotein particles are needed. The implications of ultrasound measurement in population based samples or especially in clinical setting are not entirely clear and more studies are needed to confirm the role of ultrasound markers as an indicator of clinical atherosclerotic disease. Finally, NAFLD in cardiovascular risk stratification and interaction with the MetS remains unclear. Nevertheless, the strong association between NAFLD and cardiovascular risk deserves particular attention in due to its potential implications in clinical practice. Thus, future follow-ups of the Cardiovascular Risk in Young Finns cohort have been planned, that will allow the associations of risk factors and ultrasound markers assessed in adulthood with clinical atherosclerotic disease and fatty liver assessed by more ascertainable methods to be studied.

7. SUMMARY

1. In addition to MetS components, high levels of apoB predict individuals with increased risk of incident MetS. These data suggest that apoB is a marker of early metabolic derangement and that it may provide additional mechanistic information on the complexity of MetS. No clear evidence was observed to suggest that increased OxLDL would facilitate the development of MetS.

2. Youth with MetS are at increased risk of several adult cardio-metabolic outcomes. However, the simplicity of screening for high BMI or overweight and obesity in the pediatric setting offers a simpler, equally accurate alternative to identifying youth at risk of developing adult MetS, high IMT, or T2DM.

3. MetS may be helpful in identifying groups of individuals who have accelerated progression of IMT indicative of atherosclerosis development. In addition, MetS may induce liver enzyme changes that indicate increased risk of non-alcoholic fatty liver disease. However, weight reduction and maintaining or increasing physical activity may induce favorable changes in metabolic profile to recover from the MetS leading to positive effects on vascular properties and liver function.

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