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Hanna Pohjantähti-Maaroos

Markers of Subclinical Atherosclerosis in Metabolic Syndrome and Erectile Dysfunction

Arterial Elasticity, Oxidized LDL, Fibrinogen and Resting Heart Rate

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HANNA POHJANTÄHTI-MAAROOS

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ABSTRACT

Patients with metabolic syndrome (MetS) are at increased risk for cardiovascular disease (CVD). As the prevalence of MetS continues to increase, a better understanding of the connections between MetS and CVD would be of great benefit. Erectile dysfunction (ED) is considered to be an early clinical sign of systemic atherosclerosis. In addition, impaired arterial elasticity, increased levels of circulating oxidized LDL (oxLDL) and fibrinogen, as well as elevated resting heart rate (RHR) seem to be associated with increased risk of CVD events. The aim of this thesis was to assess whether these markers are associated with MetS, and high estimated 10-year CVD risk, number of MetS variables and ED in MetS. In addition, the effect of cold-pressed turnip rapeseed oil (CPTRO) on these markers of subclinical atherosclerosis was evaluated in comparison with butter.

Altogether 120 men with MetS and 59 physically active (PhA) counterparts without MetS participated in the study. Circulating levels of oxLDL were assessed by a capture ELISA immunoassay and arterial elasticity by a non-invasive radial artery tonometer. 10-year risk of CVD events and death were calculated by FINRISK and SCORE risk models. The presence of ED was assessed by the International Index of Erectile Function (IIEF) questionnaire. The intervention study with a randomized cross-over design consisted of CPTRO and butter periods lasting for 6 to 8 weeks, and separated from each other by an 8-week washout period.

MetS subjects had impaired large arterial elasticity (C1), increased oxLDL and fibrinogen and elevated RHR compared with PhA subjects. MetS subjects at high estimated 10-year CVD risk had impaired large and small arterial elasticity, increased oxLDL and fibrinogen and elevated RHR, compared with those at medium risk. There was no difference between MetS subjects with three, four or five MetS variables. OxLDL was significantly lower after the CPTRO period than after the butter period. MetS subjects with ED had impaired C1, increased fibrinogen and elevated RHR compared with those without ED. Also among MetS subjects with SCORE risk score < 5%, C1 was lower among those with ED compared with those without. In addition, decreased C1 and increased fibrinogen levels were associated with the presence of ED among MetS subjects, independently of traditional CVD risk factors. Physical activity was associated independently with the presence of normal erectile function among MetS subjects.

In conclusion, impaired arterial elasticity, increased oxLDL and fibrinogen and elevated RHR may contribute to the increased CVD risk connected to MetS. 10-year CVD risk score calculation seems to differentiate MetS subjects with respect to markers of subclinical atherosclerosis. However, in the presence of suspected vasculogenic ED, aggressive primary prevention should be considered for MetS patients, irrespectively of the estimated 10-year CVD risk. Dietary intake of CPTRO may delay the atherosclerotic process in MetS. Physical exercise, on its part, may protect against ED in MetS.

National Library of Medical Classification: WG 550, WJ 709, WG 510, QU 85
Medical Subject Headings: Arteriosclerosis/prevention & control; Erectile Dysfunction; Lipoproteins, LDL; Heart Rate; Fibrinogen; Elasticity; Metabolic Syndrome X; Cardiovascular Diseases; Risk Factors; Plant Oils.

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Varhaisen valtimotaudin merkit metabolista oireyhtymää ja erektiohäiriötä sairastavilla – valtimoiden elastisuus, hapettunut LDL, fibrinogeeni ja leposyke
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TIIVISTELMÄ

Metabolista oireyhtymää (MBO) sairastavilla on suurentunut riski sairastua ja kuolla sydän- ja verisuonisairauksiin. Mikäli valtimotauti todettaisiin oireettomassa alkuvaiheessa, tehokas preventio olisi mahdollista. Erektiohäiriö on yleisen valtimonkovettumataudin varhainen kliininen ilmentymä. Hapettuneen LDL:n (oxLDL) suurentunut pitoisuus, valtimoiden heikentynyt elastisuus sekä kohonneet fibrinogeeni- ja leposyke-tasot ovat yhteydessä suurentuneeseen sydän- ja verisuonitapahtuma-riskiin. Väitöskirjatyön tarkoituksena oli selvittää ovatko nämä varhaisen valtimotaudin merkit yhteydessä MBO:ään, sekä korkeaan laskennalliseen valtimotautiriskiin, MBO-osatekijöiden lukumäärään ja erektiohäiriön esiintymiseen MBO-potilailla. Lisäksi tutkittiin, onko rypsiöljy-lisällä näihin muuttujiin suotuisaa vaikutusta.

Tutkimukseen osallistui 120 MBO:ää sairastavaa ja 59 liikunnallista miestä. OxLDL-pitoisuus mitattiin plasmasta ELISA-menetelmällä ja valtimoiden elastisuus kajoamattomalla tonometrilla värttinävaltimosta. Potilaan riski sairastua ja kuolla valtimotautitapahtumaan 10 vuoden aikana laskettiin FINRISK- ja SCORE- riskilaskureilla. Erektiohäiriö diagnosoitiin International Index of Erectile Function (IIEF) – kyselyn perusteella. Interventiotutkimuksessa 37 MBO-miestä käytti vastavuoroisesti rypsiöljy- ja voilisää 6-8 viikon jaksoissa.

MBO-potilaiden suurten valtimoiden elastisuus (C1) oli huonompi ja oxLDL-, fibrinogeeni- ja leposyketasot korkeammat kuin liikunnallisilla verrokeilla. Valtimoiden elastisuus oli alentunut ja oxLDL-, fibrinogeeni- ja leposyketasot kohonneet erityisesti niillä MBO-potilailla, joilla oli suuri laskennallinen valtimotauti-riski tai erektiohäiriö. Niillä MBO-potilailla, joilla oli erektiohäiriö, C1 oli alentunut, vaikka laskennallinen valtimotautiriski oli pieni. MBO-osatekijöiden määrällä ei ollut vaikutusta tutkittuihin markkereihin. Rypsiöljy-jakson jälkeen oxLDL-pitoisuudet olivat selvästi matalammat kuin voi-jakson jälkeen. Alentunut C1 ja kohonnut fibrinogeeni olivat erektiohäiriön itsenäisiä riskitekijöitä, fyysinen aktiivisuus oli puolestaan yhteydessä normaaliin erektiotoimintaan MBO-potilailla.

Valtimoiden heikentynyt elastisuus ja kohonneet oxLDL-, fibrinogeeni ja leposyke-tasot voivat selittää MBO:n yhteyttä valtimotauteihin. Valtimotauti-riskin arvioiminen riskilaskureilla auttaa löytämään ne MBO-potilaat, joilla valtimotauti on jo kehittymässä. Niillä MBO-potilailla, joilla epäillään verisuoniperäistä erektiohäiriötä, tehokasta primaaripreventiota tulisi kuitenkin harkita matalasta laskennallisesta valtimotautiriskistä huolimatta. Rypsiöljylisä voi olla hyödyksi valtimotautien kehittymisen ehkäisyssä. Liikunta puolestaan voi auttaa MBO-potilaita normaalin erektiotoiminnan säilyttämisessä.

Luokitus: WG 550, WJ 709, WG 510, QU 85
Yleinen Suomalainen asiasanasto: seksuaalihäiriöt, miehet, valtimonkovetustauti, lipoproteiinit, syke, rypsiöljy, metabolinen oireyhtymä, riskitekijät, ennaltaehkäisy, sydän- ja verisuonitaudit.

To my three musketeers

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Hanna Pohjantähti-Maaroos

List of the original publications

This dissertation is based on the following original publications.

I Pohjantähti-Maaroos H, Palomäki A, Kankkunen P, Laitinen R, Husgafvel S, Oksanen K. Circulating oxidized low-density lipoproteins and arterial elasticity: comparison between men with metabolic syndrome and physically active counterparts. *Cardiovascular Diabetology 9: 41, 2010.*

II Pohjantähti-Maaroos H, Palomäki A, Kankkunen P, Husgafvel S, Knuth T, Vesterinen K, Oksanen K. Arterial elasticity and oxidized LDL among men with metabolic syndrome and different 10-year cardiovascular risk estimated by FINRISK and SCORE models. *Annals of Medicine, Epub ahead of print, 2011.*

III Palomäki A, Pohjantähti-Maaroos H, Wallenius M, Kankkunen P, Aro H, Husgafvel S, Pihlava JM, Oksanen K. Effects of dietary cold-pressed turnip rapeseed oil and butter on serum lipids, oxidized LDL and arterial elasticity in men with metabolic syndrome. *Lipids in Health and Disease 9: 137, 2010.*

IV Pohjantähti-Maaroos H, Palomäki A. Comparison of metabolic syndrome subjects with and without erectile dysfunction – levels of circulating oxidised LDL and arterial elasticity. *International Journal of Clinical Practise 65: 274-80, 2011.*

V Pohjantähti-Maaroos H, Palomäki A, Hartikainen J. Erectile dysfunction, physical activity and metabolic syndrome: differences in markers of atherosclerosis. *BMC Cardiovascular Disorders 11: 36, 2011.*

 This dissertation also contains unpublished data.

Contents

Abbreviations

ACEi	angiotensin converting entzyme inhibitor
AHA/NHLBI	American Heart Association/National Heart, Lung, and Blood Institute
AIx	augmentation index
ALA	alpha-linolenic acid
ANOVA	analysis of variance
ApoB	apolipoprotein B
ApoB-100	apolipoprotein B-100
ATRb	angiotensin receptor blocker
BMI	body mass index
BP	blood pressure
Ca	calcium
C1	large arterial elasticity
C2	small arterial elasticity
CI	confidence interval
CHD	coronary heart disease
CPTRO	cold-pressed turnip rapeseed oil
CV	cardiovascular
CV%	coefficient of variation %
CVD	cardiovascular disease
DBP	diastolic blood pressure
DHA	docosahexaenoic acid
DPCA	diastolic pulse-contour analysis
ED	erectile dysfunction
ESH	European Society of Hypertension
EPA	eicosapentaenoic acid
ESC	European Society of Cardiology
ESRD	end-stage renal disease
FINRISK60	FINRISK at the projected age of 60
FMD	flow-mediated dilation
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
HMS	Hämeenlinna Metabolic Syndrome research program
HRT	hormone replacement therapy
IDF	International Diabetes Federation
IGT	impaired glucose tolerance
IIEF	International Index of Erectile Function questionnaire
IMT	intima-media thickness
JIS	Joint Interim Societies
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
MET	metabolic equivalent of task
MetS	metabolic syndrome
MRI	magnetic resonance imaging
MUFA	monounsaturated fatty acid
NCEP	National Cholesterol Education Program
NHANES	National Health and Nutrition Examination Survey
NO	nitric oxide
NS	non-significant
OR	odds ratio

OxLDL	oxidized LDL
PhA	physically active
PP	pulse pressure
PUFA	polyunsaturated fatty acid
PCI	percutaneous coronary intervention
PWV	pulse wave velocity
RHR	resting heart rate
SBP	systolic blood pressure
SCORE60	SCORE at the projected age of 60
SD	standard deviation
SEM	standard error of mean
SFA	saturated fatty acid
SPCA	systolic pulse-contour analysis
TG	triglycerides
WHO	World Health Organization

1 Introduction

Metabolic syndrome (MetS), a clustering of metabolic abnormalities (visceral obesity, hypertension, dyslipidemia and glucose intolerance) comprises an increased risk for cardiovascular diseases (CVD). As the prevalence of overweight people continues to increase, a better understanding of the connections between MetS and increased CVD risk would be of great benefit.

The atherosclerotic process begins with an accumulation of oxidatively modified LDL (oxLDL) in the intima of the arteries. This in turn disturbs the function of the arterial wall. Increased levels of circulating oxLDL and impaired arterial elasticity have been found to be associated with CVD. In addition, increased fibrinogen concentrations and elevated resting heart rate (RHR) are associated with an increased risk of CVD events. Despite active research on these and other markers of subclinical CVD, the clinical estimation of increased CVD risk, and thus, the need for primary prevention is for now based on classical CVD risk factors. Whether MetS subjects with different estimated CVD risk or different number of MetS variables differ with respect to the above-mentioned markers of subclinical atherosclerosis is not known.

The pathophysiology and risk factors of erectile dysfunction (ED) are the same as of CVD. Since ED often precedes the clinical manifestations of coronary heart, ischemic brain and peripheral arterial diseases, it is believed to be an early clinical sign of systemic atherosclerotic process. ED is highly prevalent among MetS. However, arterial elasticity, oxLDL, fibrinogen and RHR have not previously been reported in comparison between MetS subjects with and without ED.

Mediterranean type diet, rich in monounsaturated fatty acids (MUFA), has been reported to improve both oxidative stress and arterial function, as well as decrease the risk of CVD events. Previous results on the effects of polyunsaturated fatty acids (PUFA), on their part, are controversial. Turnip rapeseed oil is an important source of both MUFA and PUFA in the Nordic countries. However, the effect of turnip rapeseed oil on markers of subclinical atherosclerosis among MetS subjects is not known.

The aim of this thesis was to assess whether arterial elasticity, circulating oxLDL, fibrinogen and resting heart rate are associated with MetS, and with high estimated 10-year CVD risk, different number of MetS variables and ED among MetS subjects. In addition, the effect of dietary intake of cold-pressed turnip rapeseed oil (CPTRO) on these markers of subclinical atherosclerosis was assessed among subjects with MetS.

2 Review of the Literature

2.1 ATHEROSCLEROTIC PROCESS

The principles of the atherosclerotic process are presented in Figure 1. The low-density lipoprotein particle (LDL) is the major source of accumulating lipids in the process (Stocker&Keaney 2004). LDL particles are transferred to the intimae of the arterial wall by endothelial transsytotic vesicles (Kovanen 2008). Since the proteoglycan layer, rich in collagen, is tight and lacks lymphatic vessels, LDL particles begin to accumulate in the intimae. LDL, entrapped in this layer of the arterial wall, is destroyed by lipolytic and proteolytic enzymes and oxidized by reactive oxygen species (Kovanen 2008). The oxidatively modified LDL particles (oxLDL) become negatively charged and unstable, which in turn leads to fusion of the particle cores. As a result, the larger lipid drops attach even more tightly to the glycoproteins and collagen fibers of the subendothelial space (Kovanen 2008).

The oxidative modification of apolipoprotein B-100 (ApoB-100) lysines on the surface of the LDL particle, predispose it to uptake by macrophages via their scavenger receptors (Stocker&Keaney 2004). The modified LDL particles are then destroyed in the macrophage lysosomes, and the cholesterol from the LDL is released to the cytoplasm of the macrophage (Kovanen 2008). The unregulated uptake of LDL, and the accumulation of cholesterol in the macrophages leads to formation of ester-laden foam cells and a fatty streak (Stocker&Keaney 2004).

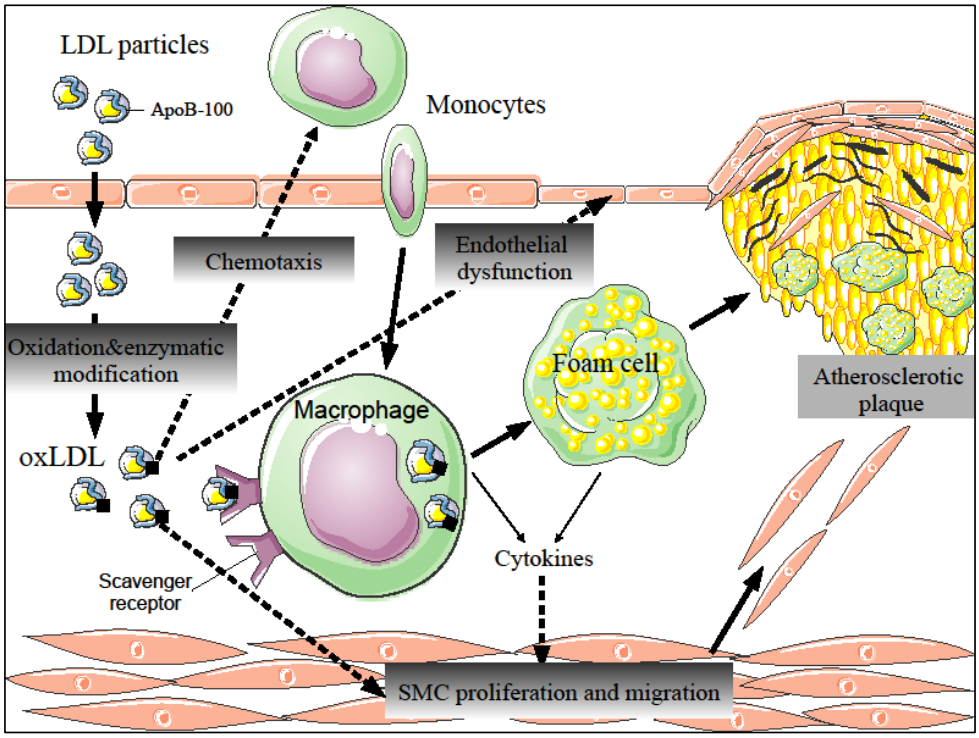

Figure 1. The principles of the atherosclerotic process. Figure was produced using Servier Medical Art. oxLDL = oxidized low-density lipoprotein, SMC = smooth muscle cells

High-density lipoprotein (HDL) particles are able to attach the surface of the foam cells and receive the non-esterificated cholesterol and phospholipids. Since the HDL particles are smaller than LDL and their apolipoproteins do not attach the proteoglycan layer, they transfer cholesterol rapidly from the intima to the lymphatic vessels in the media layer of the arterial wall. If the macrophages are not able to transfer all cholesterol from oxLDL to HDL particles, LDL-derived lipids begin to accumulate, which in turn leads to formation of an atheroma.

The proliferation of arterial smooth muscle cells leads to further synthesis and deposition of collagen, elastin and glycoproteins in the intimae, and thus a formation of a fibro-fatty atheroma (Schoen&Cotran 1999). Stable lesions are characterized by a thick fibrous cap (Stocker&Keaney 2004). In the presence of constant oxidative stress and certain cytokines, the matrix-degrading activity increases (Stocker&Keaney 2004). As the lipid core of this fibro-fatty atheroma continues to enlarge, the fibrous cap gets thinner. The disruption of the fibrous cap leads to exposure of the thrombogenic material beneath the endothelium. The formation of a thrombus may be associated with potentially fatal clinical events such as unstable angina or acute myocardial infarction.

In case of a minor endothelial disruption, the formation of a micro-thrombus and followed proliferation of the fibrous cap, may be asymptomatic. However, the growth of the fibroatheroma as a result of recurrent disruptions eventually leads to an obstructive stenosis and clinical symptoms (Kovanen 2008, Schoen&Cotran 1999, Stocker&Keaney 2004).

2.2 OXIDIZED LDL

2.2.1 Proatherogenic effects

Oxidative modification of LDL can generate minimally modified or fully oxidized LDL (Tanigawa 2006, Ylä-Herttuala 1998). In addition to its role in the formation of foam cells, oxLDL has other proatherogenic effects as well (Stocker&Keaney 2004). Minimally modified LDL stimulates the synthesis of chemotactic products that facilitate the influx of monocytes and T-cells into the arterial wall (Stocker&Keaney 2004). Fully oxidized LDL inhibits the mobilization of macrophages from the intimae and increase the expression of macrophage scavenger receptors, which further stimulates the formation of fatty streaks (Schoen&Cotran 1999, Stocker&Keaney 2004). Accumulation of these cells accelerates arterial inflammation, a phenomenon believed to be essential in the atherosclerotic process. The immunogenity of oxLDL, in turn, induce the production of autoantibodies (Stocker&Keaney 2004). Since oxLDL also impairs the activity of endothelium-derived nitric oxide and is mitogenic to smooth muscle cells and cytotoxic to endothelial cells, the accumulation of oxLDL in the arterial wall impairs the endothelial function and elastic properties of the arteries (Schoen&Cotran 1999, Stocker&Keaney 2004).

2.2.2 Assessment of circulating oxidized LDL

There are individual differences in the susceptibility of LDL to oxidation. For example, LDL antioxidant level, particle size and fatty acid composition, hyperglycemia and hyperinsulinemia have all been reported to contribute to the oxidative process (de Graaf et al. 1991, Esterbauer et al. 1992, Holvoet et al. 2004, Sigurdardottir et al. 2002). Thus, at the same level of LDL concentration, a wide variation may be detected in the amount of LDL oxidation (Ylä-Herttuala 1998). Therefore, assessment of oxLDL might be superior to assessment of LDL in detecting those at high risk for CVD.

LDL in the atherosclerotic lesions is oxidatively modified (Ylä-Herttuala 1998). In plasma, however, antioxidants protect the lipoproteins against oxidation (Stocker&Keaney 2004). In addition, highly oxidized particles in plasma would be rapidly degraded either in the liver or in

the arterial wall (de Rijke 1994). Therefore, it is believed that LDL oxidation occurs mainly in the arterial wall, and that fully oxidized LDL does not exist in the circulation (Stocker&Keaney 2004). However, minimally oxidized LDL has been detected in plasma (Stocker&Keaney 2004). Thus, assays for circulating oxLDL presumably measure the minimally oxidized LDL. The origin of this circulating oxLDL is unknown. As some of the LDL particles are transferred from the intimae to circulation, some oxLDL might be transferred as well (Stocker&Keaney 2004). In addition, oxLDL accumulated in a plaque may be released to circulation after plaque rupture (Itabe 2009).

Circulating oxLDL may be assessed by different immunoassays that use monoclonal antibodies against oxidation-dependent epitopes of LDL (Itabe&Ueda 2007). Some assays use antibodies against oxidized phospholipids (Holvoet et al. 2006). The assay developed by Holvoet et al (2006&2008) uses a monoclonal antibody 4E6 against an oxidation-dependent epitope in the apoB-100 moiety of LDL particle (Holvoet et al. 2008, Itabe&Ueda 2007). Two assays using the antibody 4E6, competitive and sandwich-type ELISA, are commercially available (Mercodia, Sweden). The longitudinal stability and accuracy of circulating oxLDL measurements (developed by Holvoet et al. and the commercial assay by Mercodia), are similar to those of total cholesterol and high-sensitive CRP determinations (Holvoet et al. 2006).

2.2.3 Oxidized LDL and increased cardiovascular risk

A number of studies have assessed oxLDL levels or markers of oxLDL in association with other indicators of subclinical atherosclerosis (Morishita et al. 2009, Toikka et al. 1999, Wallenfeldt et al. 2004, Woodman et al. 2005). Increased oxLDL has been found to be associated with endothelial dysfunction among type 2 diabetic patients, with impaired large artery elastic properties as assessed by magnetic resonance imaging (MRI) or ultrasound among young men and with an increase in pulse wave velocity (PWV) among coronary heart disease (CHD) patients (Morishita et al. 2009, Toikka et al. 1999, Woodman et al. 2005). In addition, increased oxLDL was a prognostic marker of carotid artery intima-media thickness (IMT) progression in a population based study by Wallenfeldt et al (2004). However, there are no reports on the association between increased oxLDL and impaired arterial elasticity in MetS.

Circulating oxLDL levels seem also to be associated with established CVD risk factors and high estimated CVD risk according to the Framingham risk score (Holvoet et al. 2003&2007). In addition, there are number of cross-sectional studies reporting increased oxLDL levels among patients with subclinical and stable CHD and among patients with acute coronary syndrome or cerebral infaction (Holvoet et al. 1998&2001, Hulthe&Fagerberg 2002, Uno et al. 2003).

Nevertheless, the prognostic effect of increased oxLDL on CVD outcomes is less clear. Reduction in plasma oxLDL level has been reported to attenuate atherosclerotic lesion development (Ishigaki et al. 2008). Among patients with acute myocardial infarction and percutaneous coronary intervention (PCI), increased oxLDL level predicted the risk for in-stent restenosis (Naruko et al. 2006). The value of oxLDL in predicting CHD development has also been shown among patients with heart transplantation (Holvoet et al. 2000). In addition, Holvoet et al (2004) reported an independent association between increased oxLDL level and incident myocardial infarction among MetS subjects. Increased oxLDL was not, however, an independent predictor of total CHD risk (Holvoet et al. 2004). Accordingly, in a population-based, large follow-up study among 50966 subjects, oxLDL was significantly related to total CHD risk, but the significance did not remain after adjustment for LDL-cholesterol (LDL-C), HDL cholesterol (HDL-C) and triglycerides (TG) (Wu et al. 2006). On the contrary, in a study by Meisinger et al (2005), increased circulating oxLDL was a stronger predictor of CHD events than conventional lipoprotein profile and risk factors.

2.3 ARTERIAL DYSFUNCTION

2.3.1 Pathophysiology

OxLDL impairs the bioactivity of endothelium-derived nitric oxide (NO) and is cytotoxic to endothelial cells (Cotran et al. 1999, Stocker&Keaney 2004). In addition, classical CVD risk factors, obesity, physical inactivity and some novel risk factors, such as homocysteine or inflammation, cause an increase in oxidative stress, which damages endothelial cells (Kasprzak et al. 2006, Widlansky et al. 2003). This results in endothelial dysfunction, which in turn causes vasoconstriction and acceleration of atherogenesis by enhancement of inflammatory and oxidative processes and an increase in smooth muscle cell proliferation and migration (Bonetti et al. 2003, Kasprzak et al. 2006, Widlansky et al. 2003). Since dysfunctional endothelium increases the risk of plaque rupture and enhances thrombogenesis, it is also important in the late atherosclerotic process. Thus, endothelial dysfunction is a key element in atherosclerosis (Bonetti et al. 2003, Kasprzak et al. 2006, Widlansky et al. 2003).

The media layer of the arterial wall is the principal contributor to the mechanical properties of the artery. Stiffening of arteries, resulting from fracture and fragmentation of the elastic properties and an increase in collagen deposition within the media, is a physiological process associated with aging (O'Rourke 1999). In addition, increased smooth muscle tone and smooth muscle cell hypertrophy contribute to the stiffness of arteries (Glasser et al. 1997). The stiffening process is accelerated by traditional CVD risk factors, oxLDL, endothelial dysfunction, and the accompanying atherosclerosis (Bonetti et al. 2003, Gibbons&Dzau 1994, Toikka et al. 1999).

As large arteries become stiffer, their normal buffering function is altered, which leads to impaired pulsatile function, increase in pulse pressure (PP) and thus, alteration in the normal smooth blood flow at the capillary level (Glasser et al. 1997, McVeigh et al. 2002). An increase in arterial stiffness leads to a premature return of reflected waves in late systole. As a result, central and thus, systolic blood pressure (SBP) rise. Increased SBP increases left ventricular load, which in turn results in left ventricular hypertrophy and increased myocardium oxygen demand. This further damages the endothelial layer of the arteries and enhances the atherosclerotic process (Laurent et al. 2006).

2.3.2 Assessment of arterial function

In addition to a number of laboratory markers, assessment of endothelial dependent vasodilation has emerged as a useful method to study the function of the endothelium (Kasprzak et al. 2006, Widlansky et al. 2003). Vasodilation, resulting from stimulation by increased blood-flow or receptor-dependent agonists, can be detected invasively by catheterization of the brachial artery and measurement with venous occlusive plethysmography (Widlansky et al. 2003). Since invasive methods have significant risks, non-invasive ultrasound imaging of brachial artery flow-mediated dilation (FMD) has become the most used technique in assessing endothelial function (Kasprzak et al. 2006). Although this method measures the function of a peripheral artery, the results seem to correlate with the endothelial function of coronary arteries (Anderson et al. 1995). However, brachial FMD did not correlate with function of resistance arteries in the microvascular circulation in a study by Eskurza et al (2001). Thus, vascular tone regulation in conduit and resistance vessels may be different, which supports the relevance of different arterial function measurements (Widlansky et al. 2003).

There are several non-invasive methods to assess the elastic properties of the artery beyond the endothelium (Laurent et al. 2006). The arterial pressure waveform is derived from the interactions of stroke volume, physical properties of the arteries and blood in the circulation (Glasser et al. 1997). It is composed of the forward blood flow, initiated by the ejection of stroke volume to the aorta, and backward blood flow from the reflectory sites at the peripheral circulation. Changes in the elastic properties of the arterial wall are reflected as alterations in the

PP, the velocity of the pulse wave as well as in the morphology of the pressure waveform (Glasser et al. 1997).

A reduction in arterial elasticity or an increase in systemic resistance leads to an increase in SBP. Diastolic blood pressure (DBP) also rises if systemic resistance increases, but falls if large arterial elasticity decreases (McVeigh et al. 2002). This pathophysiological phenomenon explains the paradox of a positive association between increased CVD risk and DBP, when DBP is considered alone, but a negative association when considered together with SBP (McVeigh et al. 2002). PP, calculated as SBP minus DBP, is the simpliest method to estimate the pulsatile function of large arteries. Brachial PP should, however, not be confused with central PP, as it overestimates the central PP (Laurent et al. 2006).

A pulse wave travels faster in a stiff artery than in a distensible one (McVeigh et al. 2002). Carotid-femoral PWV, the golden standard of assessing arterial stiffness, estimates the regional stiffness by determining the time delay between two recorded measurements at a known distance apart from each other (Laurent et al. 2006, McVeigh et al. 2002). Precise measurement of the distance between two pressure transducers may be problematic and thus, cause errors especially among subjects with abdominal obesity (McVeigh et al. 2002, Van Bortel et al. 2002). In addition, femoral pressure waveform may be difficult to record accurately in patients with MetS, diabetes and peripheral artery disease (Van Bortel et al. 2002). Since distal arteries are stiffer than proximal arteries, PWV is higher in the distal artery (Laurent et al. 2006). In addition, peripheral sites are closer to the reflection sites that amplify the pressure wave (Laurent et al. 2006). Therefore, it is inaccurate to use brachial pulse pressure as a surrogate for aortic or carotid pulse pressure. Since the aorta is the main contributor to the arterial buffering function, it is the main interest when determining the regional stiffness (Latham et al. 1985). Aortic PWV measurement is a non-invasive and direct measurement of arterial stiffness which can be assessed by using mechanotranscuder, tonometer, echotracking or Doppler devices (Laurent et al. 2006).

As described earlier, PWV rises in stiff arteries. As a result, the reflected waves from the peripheral sites arrive back to the central arteries already during end-systole. This, in turn, causes augmentation of the systolic pressure (Laurent et al. 2006, Oliver&Webb 2003). Systolic pulse-contour analysis (SPCA) of the wave reflections of the common carotid or radial artery can be used to derive the augmentation index (AIx) (Oliver&Webb 2003, O'Rourke et al. 2001). AIx is defined as the difference between the first and second systolic peaks (Chen et al. 1996). Although radial tonometry with a transfer function is popular since it is simple to perform, AIx is recommended to be obtained at the central level (Laurent et al. 2006). Similar pulse wave analysis can also be obtained from a peripheral waveform by finger plethysmography (Laurent et al. 2006).

Another method of pulse waveform analysis concentrates exclusively on the diastolic part of the arterial pressure wave (McVeigh et al. 2002). This diastolic pulse-contour analysis (DPCA) uses a modified Windkessel model, an assumed model of the circulation, to identify the reflections in diastole as a decaying wave (Cohn et al. 1999, Laurent et al. 2006). It determines a proximal capacitive (C1) and a distal oscillatory compliance (C2) (Cohn et al. 1999, Laurent et al. 2006). C1 identifies the elasticity of the aorta and other large arteries, C2 the elasticity of small arteries (Cohn et al. 1999). Pulse-contour analysis has been criticised since the model is based on a number of theoretical approximations (Laurent et al. 2006). However, values obtained by this method seem to correlate tightly with those assessed invasively (Cohn et al. 1995). In addition, C1 has been reported to correlate with MRI-determined aortic distensibility, and C2 with endothelial function assessed by FMD (Resnick et al. 2000, Wilson et al. 2004). Although C2 is believed to reflect the endothelial function of the microvascular circulation, it is influenced by the structures of the arterial wall and thus, is not a measurement of solely endothelial function (Cohn et al. 1999, Widlansky et al. 2003). Because of this and the fact that DCPA estimates the systemic stiffness, it has been thought to provide a more complete understanding of arterial stiffness (Woodman et al. 2005).

In addition to the above-mentioned techniques to assess regional and systemic arterial stiffness, increased stiffness can be assessed by ultrasound or MRI locally (Laurent et al. 2006). Carotid IMT and stiffness are the main measures determined by these techniques (Laurent et al. 2006). Although the direct determination of arterial function is considered a major advantage of these methods, the measurements require a high degree of technical expertise and time, which has limited their use (Laurent et al. 2006).

2.3.3 Arterial dysfunction and increased cardiovascular risk

Endothelial dysfunction seems to predict increased risk of CVD events both among patients with established CVD and among high-risk subjects (Martin&Anderson 2009, Widlansky et al. 2003). In addition, hyperemic velocity, the stimulus for flow-mediated dilation, was a significant risk marker of CVD among healthy men in a recent prospective study (Anderson et al. 2011). Brachial FMD is the most often used method to assess endothelial function in prospective studies (Martin&Anderson 2009, Widlansky et al. 2003). It has been reported to predict CVD events above and beyond traditional risk factors (Martin&Anderson 2009). However, since the addition of prognostic accuracy by FMD has been only approximately 1%, its importance in clinical practise is still unclear (Martin&Anderson 2009).

Increased arterial stiffness as assessed by different non-invasive methods has been reported to be associated with traditional and novel CVD risk factors as well as with hypertension, microalbuminuria, MetS, diabetes and ED, all high-risk conditions for CVD (Cohn et al. 1995, Ge et al. 2008, Laurent et al. 2006, Li et al. 2007, Li et al. 2011, Prisant et al. 2006). In addition, many pharmacological and non-pharmacological treatments targeting to CVD risk factors relate to a decrease in arterial stiffness (Laurent et al. 2006) (Table 1).

Table 1. Risk factors, clinical conditions and interventions influencing arterial stiffness. (Modified from Duprez et al. 2001, Kals et al. 2006, Laurent et al. 2006, Li et al. 2007, Prisant et al. 2006)

Arterial stiffness ↑		**Arterial stiffness ↓**	
Risk factors	**Clinical conditions**	**Pharmacological**	**Other**
Aging	Hypertension	Diuretics	Physical exercise
Smoking	Hypercholesterolemia	Beta-blockers	Weight loss
Obesity	Diabetes, IGT	ACE-inhibitors	Low-salt diet
Physical inactivity	Metabolic syndrome	ATR-blockers	Alcohol (moderate)
Microalbuminuria	Coronary heart disease	Ca-antagonists	Garlic
Genetic backround	Congestive heart failure	Statins	Alpha-linoleic acid
Menopausal status	Fatal stroke	Nitrates	Fish oil
Hyperhomocysteinemia	Peripheral arterial disease	Thiazolidinediones	
High CRP level	ESRD	HRT	
Low birth weight	Erectile dysfunction		
	Rheumatic diseases		

ACE = angiotensin converting enzyme; ATR = angiotensin receptor; Ca = calcium; ESRD = end-stage renal disease; HRT = hormone replacement therapy; IGT = impaired glucose tolerance

More importantly, many studies have elucidated the importance of arterial stiffness in predicting CVD events and all-cause and cardiovascular mortality among diabetic and hypertensive individuals, the elderly and the general population (Laurent et al. 2006, Thomas et al. 2008, Vlachopoulos et al. 2010) (Table 2).

Table 2. Arterial stiffness as an independent predictor of clinical endpoints.

	Patients (N)	**Outcome**
Aortic PWV		
Blacher et al. 1999	ESRD (241)	CV mortality
Laurent et al. 2001	Hypertension (1980)	CV mortality
Meaume et al. 2001	Elderly > 70 y (141)	CV mortality
Shoji et al. 2001	ESRD (265)	CV mortality
Boutouyrie et al. 2002	Hypertension	CHD events
Cruickshank et al. 2002	IGT (571)	All-cause mortality
Laurent et al. 2003	Hypertension (1715)	Fatal strokes
Pannier et al. 2005	ESRD (305)	CV mortality
Sutton-Tyrrell et al. 2005	Elderly (2488)	CV events and mortality
Shokawa et al. 2005	General population (492)	CV mortality
Willum-Hansen et al. 2006	General population (1678)	CV mortality
Mattace-Raso et al. 2006	Elderly (2835)	CV mortality and CHD
Choi et al. 2007	Chest pain patients (497)	CV events and CHD
Zoungas et al. 2007	ESRD (207)	CV events
Terai et al. 2008	Hypertension (676)	CV events
Othamane et al. 2009	Hemodialysis (98)	CV mortality
Mitchell et al. 2010	General population (2232)	CV events
Aorta invasive		
Stefanadis et al. 2000	Acute CHD (54)	Recurrent acute CHD
Carotid distensibility		
Blacher et al. 1998	ESRD (79)	All-cause mortality
Barenbrock et al. 2002	ESRD (68)	CV events
Central pulse pressure		
Safar et al. 2002	ESRD (180)	All-cause mortality
Williams et al. 2006	Hypertension (2073)	CV events
Roman et al. 2009	General population (2405)	CV events
Brachial pulse pressure		
Thomas et al. 2008	General population (69989)	CV mortality
Carotid AIx		
London et al. 2001	ESRD (180)	All-cause and CV mortality
Weber et al. 2005	Undergoing PCI (262)	CV events
Williams et al. 2006	Hypertension (2073)	CV events

CHD = coronary heart disease; CV = cardiovascular; ESRD = end-stage renal disease; IGT = impaired glucose tolerance; PCI = percutaneous coronary intervention; PWV = pulse wave velocity; AIx = augmentation index

The predictive value of arterial stiffness seems to remain even after adjustment for classical CVD risk factors, including brachial PP. Therefore, it has been accepted as an intermediate endpoint for CVD events (Laurent et al. 2006). The largest amount of prognostic evidence has been achieved by assessing aortic stiffness using carotid-femoral PWV (Laurent et al. 2006, Vlachopoulos et al. 2010) (Table 2). Data concerning arterial stiffness measured at other arterial sites or methods is less consistent (Laurent et al 2006). For example, carotid stiffness predicted CV events among small number of patients with end-stage renal disease (ESRD) but not in a larger study among patients with established CVD (Dijk et al. 2005). In addition, the predictive value of central AIx and PP has been established among patients with ESRD, hypertension and those undergoing PCI, (Table 2), but the data among the general population or other patient groups is discordant (Laurent et al. 2006). There are no prospective end-point studies on the possible predictive value of DPCA.

In a study by Boutouyrie et al (2002), the independent predictive value of PWV was better among low- and medium-risk subjects than among high-risk subjects according to the Framingham risk score. This finding and the established prognostic value of arterial stiffness implicate that measuring of arterial function detect high-risk subjects that might be classified as low- to medium-risk subjects according to traditional risk assessment. Whether improvement of arterial function predicts a concomitant decrease in CVD events, independently of normalization of traditional CV risk factors, needs to be established.

2.4 OTHER INDICATORS OF INCREASED CARDIOVASCULAR RISK

2.4.1 Fibrinogen

Fibrinogen is one of the major coagulation proteins in blood. It is a precursor of fibrin and an important contributor to blood viscosity and platelet aggregation (Fibrinogen Studies Collaboration 2005). Hyperfibrinogenemia has been shown to directly promote thrombosis and increase the resistance to thrombolysis in mice (Machlus et al. 2011). The important role of fibrinogen in thrombosis has raised an interest in its contribution to CVD outcomes (Stec et al. 2000).

Increased fibrinogen levels have been reported to be associated with traditional CVD risk factors and high estimated CVD risk, but also with the presence and extent of coronary calcium among those with low risk according to the Framingham score (Okwuosa et al. 2011, Park et al. 2010, Stec et al. 2000). In addition, increased fibrinogen levels have been reported among subjects with a high-risk condition for CVD, such as ED and MetS, as well as among subjects with diagnosed CVD (Ford 2003, Ma et al. 2010, Stec et al. 2000, Vlachopoulos et al. 2006).

The predictive value of increased fibrinogen for CVD events and mortality has been established in a number of prospective studies (Fibrinogen Studies Collaboration 2005, Salomaa et al. 2002, Shi et al. 2010). In a large meta-analysis of 154 211 subjects in 31 prospective studies there was a moderately strong association between increased fibrinogen and risk of CHD, stroke and CVD- and all-cause mortality (Fibrinogen Studies Collaboration 2005). In a prospective study in a Finnish cohort, a 1-SD increase in fibrinogen was associated with a 1.23-fold increase in all-cause mortality, although the association with CHD events was not significant (Salomaa et al. 2002). In another study, increased fibrinogen was associated with worse long-term outcomes in patients with acute coronary syndrome (Shi et al. 2010).

2.4.2 Resting heart rate

RHR reflects cardiac morbidity. Elevated RHR increases peak coronary flow during diastole. This enhances pulsatile and shear stress on the endothelium, which leads to endothelial dysfunction and loss of arterial elasticity (Arnold et al. 2008, Chen et al. 2008, Park et al. 2010,

Tomiyama et al. 2010). Arterial stiffness results into a greater pulsatile load on the heart, which in turn causes a mismatch in oxygen supply and demand in the presence of CHD. This imbalance promotes ischemia, arrhytmias and ventricular dysfunction, leading to acute coronary syndromes and sudden death (Arnold et al. 2008).

RHR is an emerging CVD risk factor. Especially hypertension, insulin resistance and obesity have been reported to be associated with adrenergic overdrive (Mancia et al. 2007). Accordingly, elevated RHR has been reported among MetS (Pannier et al. 2006, Rana et al. 2010). Elevated RHR has also been associated with increased arterial stiffness, assessed by PWV (Chen et al. 2008, Park et al. 2010, Tomiyama et al. 2010). In addition, a predictive value of RHR has been reported in number of studies among patients with CHD, hypertension and heart failure as well as among the general population (Arnold et al. 2008). Also in the National FINRISK Study, elevated RHR was an independent and strong predictor of incident CVD in healthy subjects (Cooney et al. 2010). However, addition of RHR to risk score models already containing lipids and blood pressure, did not markedly improve risk estimation among the same FINRISK population (Cooney et al. 2010).

2.4.3 Risk score models

The interaction of several CVD risk factors is crucial in the development of atherosclerosis. Therefore, the decision to take preventive actions against CVD should be based on patient's total risk burden rather than on the level of any particular risk factor (Conroy et al. 2003). Risk score models have been developed to assess the total risk of CVD composed by the individual risk burden (Conroy et al. 2003, Vartiainen et al. 2007&2010). Since younger subjects with notable risk factors have low absolute risk, European Society of Cardiology (ESC) has recommended the extrapolation of the risk to age 60 to assess a possible high relative risk among younger subjects (Fourth Joint Task Force of the European Society of Cardiology 2007). SCORE and FINRISK models are the most often used risk estimates in Finland. In addition to these, the Framingham model, an estimation of 10-year risk of CV events based on a data among Americans, has been used in clinical practise (Anderson et al. 1991).

The SCORE model is based on data from 12 European cohort studies. It estimates the 10-year risk of fatal CVD (Conroy et al. 2003). Separate estimation equations are provided for high- and low-risk European regions (Conroy et al. 2003). The predictive effect of the SCORE risk charts were applied to persons aged 45 to 64 years (Conroy et al. 2003).

The FINRISK model has been constructed from series of prospective studies among a Finnish cohort aged 30 to 64. It estimates the 10-year risk of lethal and non-lethal CVD events (Vartiainen et al. 2007). Since HDL-C, the presence of diabetes, and family history of CVD are included in the FINRISK estimation in addition to gender, age, SBP and smoking, it takes into account more CV risk factors than SCORE (Conroy et al. 2003, Vartiainen et al. 2007&2010).

In a recently published Finnish study comparing the SCORE, FINRISK and Framingham risk estimations in the Finnish population, the absolute risk estimated by these scores differed signifigantly from each other (Vartiainen et al. 2010). The different definitions of outcomes and included risk factors were considered to explain the difference. The threshold for high risk is 20% according to the Framingham and 5% according to the SCORE. A threshold of 10% according to the FINRISK seems to correspond to the high risk thresholds estimated by the Framingham and SCORE (Vartiainen et al. 2010). The SCORE function seems to underestimate the risk among those with diabetes, low HDL-C and CVD in the family (Vartiainen et al. 2010).

2.5 ERECTILE DYSFUNCTION

2.5.1 Prevalence

ED, a consistent inability to achieve or maintain sufficient erection for sexual performance, has been estimated to affect 150 million men worldwide (Ayta et al. 1999). Since the risk of ED increases with age, obesity and in the presence of other cardiovascular risk factors and CVD, the prevalence is predicted to increase to 300 million by the year 2025 (Ayta et al. 1999, Selvin et al. 2007). In a Portuguese study, age-adjusted prevalence of ED was 48.1%. The prevalence increased with age being 29% among those aged 40 to 49, 50% among those aged 50 to 59, and 74% among those aged 60 to 69 (Teles et al. 2008).

ED is often prevalent among men with MetS (Bal 2007, Corona et al. 2006, Esposito et al. 2005). In the study by Esposito et al (2005), 26.7% of MetS subjects had ED compared to a control group with an ED prevalence of 13%. In a study by Corona et al (2006), MetS was diagnosed in 29.4% of men attending health care for sexual dysfunction whereas as many as 96.5% of those with diagnosed MetS had ED. The prevalence of ED seems to increase with increasing number of metabolic abnormalities and especially in the presence of insulin resistance or obesity (Bacon et al. 2006, Bansal et al. 2005, Esposito et al. 2005). In addition, the presence of ED may be predictive of MetS in men without obesity (Kupelian et al. 2006). Although MetS has been reported as an independent risk factor for ED (Heidler et al. 2007), the presence of diabetes but not MetS, was associated with ED among men aged < 45 years in a study by Wang et al (2010). In another study among obese non-diabetic subjects, MetS was not associated with ED, whereas the degree of obesity was (Gatti et al. 2009).

The prevalence of ED in the presence of diabetes is extremely high. In a study by Al-Hunayan et al (2006), almost one third of men aged 21-65 and diagnosed with type 2 diabetes within the previous year was also diagnosed with ED. Among diabetic patients the risk of ED further increases with duration of diabetes and poor glycemic control (Kalter-Leibovici et al. 2005). It has been estimated that over 50% of all men with diabetes in the U.S. suffer from ED (Selvin et al. 2007). In addition, the prevalence of ED is higher among diabetic patients with silent CHD than among those without (Gazzaruso et al. 2004).

Also other CV risk factors are highly prevalent among men with ED. Conversely, ED is highly prevalent among men with clustering of CV risk factors (Roumeguére et al. 2003, Selvin et al. 2007) In the study by Roumeguére et al (2003), increased 10-year CVD risk according to the Framingham model was present in 56.6% of men with and in 32.6% of men without ED. In addition to traditional CV risk factors, lower attained education and physical inactivity also seem to be associated with a higher prevalence of ED (Selvin et al. 2007).

An increased prevalence of ED has been reported in the presence of established CVD (Montorsi et al. 2003&2005, Solomon et al. 2003). ED was prevalent in 58% of men with CHD, 65% of men with cerebrovascular disease and 73% of men with peripheral arterial disease in a study by Montorsi et al (2005). In addition to clinical manifestations of CVD, the prevalence of ED seems to differ with respect to the severity of CVD (Montorsi et al. 2006). ED prevalence was 18% in men with a recent acute myocardial infarction and one-vessel disease, but as high as 67% among men with chronic angina pectoris and multivessel disease (Montorsi et al. 2006).

2.5.2 Pathophysiological connection to cardiovascular diseases

ED is believed to be an early clinical manifestation of systemic atherosclerosis (Jackson et al. 2010, Shin et al. 2011). In addition to the same risk factors, ED and other CVDs share the same pathophysiology (Jackson et al. 2010). Increased oxidative stress and low-grade inflammation have been reported to be associated both with ED and CVD (Barassi et al. 2009, Stocker&Keaney 2004, Vlachopoulos et al. 2006). As presented earlier, oxidative stress and inflammation together with other CV risk factors damage the endothelium of the arteries (Ganz 2005, Kirby et al. 2005, Stocker&Keaney 2004). Since maintenance of satisfactory erection is a

result of endothelium-derived release of nitric oxide and vasodilation, endothelial dysfunction is also a common denominator of vasculogenic ED (Billups 2005, Ganz 2005, Kirby et al. 2005). As the atherosclerotic process, initialized by oxidative stress and endothelial dysfunction, proceeds, a stenotic plaque may cause mechanical occlusion in the cavernosal arteries - a continuum similar to that of other CVDs (Billups 2005).

The penis is a highly vascularized organ with high amount of endothelium (Vlachopoulos et al. 2007). In addition, a marked dilation of penile arteries is required for normal erection (Ganz 2005). For these reasons, endothelial dysfunction is believed to manifest as ED before clinical symptoms from other cardiovascular beds (Kirby et al. 2005, Montorsi et al. 2005, Shin et al. 2011). Another theory, supporting ED as an early sign of the same atherosclerotic process, is the arterial size hypothesis presented by Montorsi et al (2005). According to this theory, the same level of plaque burden affects blood flow more in the smaller penile (1-2 mm in diameter) than in larger coronary (3-4 mm), carotid (5-7 mm) and femoral (6-8 mm) arteries (Montorsi et al. 2005). This theory is supported by studies reporting an increase in the prevalence of ED with respect to clinical manifestations from arteries with larger diameter, as well as by studies reporting ED to preceed symptoms of other CVDs (Jackson et al. 2010, Montorsi et al. 2005).

Increased oxidative stress, low-grade inflammation and endothelial dysfunction have also been proposed as possible links between MetS and ED (Esposito et al. 2005, Vlachopoulos et al. 2007). In addition, decreased testosterone levels are associated especially with obesity and insulin resistance and thus, may partly explain the connection between MetS and ED (Guay 2009, Laaksonen et al. 2005, Shabsigh et al. 2008).

2.5.3 International Index of Erectile Function questionnaire

Cavernous blood flow can be evaluated by penile doppler and pharmacologic duplex ultrasonography in men with suspected vasculogenic impotence (Golijanin et al. 2007). The measurement is time-consuming and invasive, which has restricted its use in larger clinical studies.

The International Index of Erectile Function (IIEF) questionnaire was developed and validated in 1996-1997 for a sildenafil clinical trial (Rosen et al. 1997). Since then it has been recommended and used as the golden standard outcome measure in different types of ED trials (Rosen et al. 2002). The IIEF questionnaire has been accepted by scientific journals and clinical practioners as a valid and reliable method to assess sexual functioning in men (Rosen et al. 2002). It has also been reported to have high sensitivity and specificity for ED (Rosen et al. 1997). In addition to its ability to distinguish men with and without ED, IIEF questionnaire has been reported to properly classify the severity of ED (Cappelleri et al. 1999).

Despite the wide use of the IIEF questionnaire, some limitations have also been reported. The questionnaire does not provide information about the partner relationship or sexual functioning of the partner, which may be crucial for normal erectile function (Rosen et al. 2002). In addition, in a study comparing a modified version of the questionnaire with penile Doppler blood flow measurement and clinical evaluation, the questionnaire was not able to differentiate between vasculogenic and non-vasculogenic ED (Blander et al. 1999). Since the questionnaire does not provide information on the etiology of the ED, a detailed clinical history and examination should be conducted among patients with ED.

The IIEF questionnaire consists of 5 different domains of sexual functioning (Rosen et al. 1997). Questions 1-5 and 15, dealing with the ability to achieve and maintain erection during the past four weeks, are considered as the ED domain of the questionnaire (Table 3). According to the IIEF guidelines, subjects with score ≤ 25, calculated as a sum of the questions 1-5 and 15, are considered to have ED. The severity of ED is classified as mild if score is 22-25, mild to moderate if score is 17-21, moderate if score is 11-16 and severe if score is 6-10 (Rosen 1998).

Table 3. Questions and response options of the erectile dysfunction domain of the IIEF questionnaire.

Q1: How often were you able to get an erection during sexual activity?
Q2: When you had erections with sexual stimulation, how often were your erections hard enough for penetration?
Response options:
0 = No sexual activity
1 = Almost never/never
2 = A few times (much less than half the time)
3 = Sometimes (about half the time)
4 = Most times (much more than half the time)
5 = Almost always/always

Q3: When you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?
Q4: During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?
Response options:
0 = Did not attempt intercourse
1 = Almost never/never
2 = A few times (much less than half the time)
3 = Sometimes (about half the time)
4 = Most times (much more than half the time)
5 = Almost always/always

Q5: During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?
Response options:
0 = Did not attempt intercourse
1 = Extremely difficult
2 = Very difficult
3 = Difficult
4 = Slightly difficult
5 = Not difficult

Q15: How do you rate your confidence that you can get and keep an erection?
Response options:
1 = Very low
2 = Low
3 = Moderate
4 = High
5 = Very high

A single-question assessment of ED has also been studied. Derby et al (2000) found a good correlation between a single item self-assessment question and the IIEF questionnaire. In addition, the response rate of the single-question assessment was higher. In another study, a single-item question (no, moderate or complete ED) predicted accurately the ED diagnosed by an urologist (O'Donnell et al. 2005). Although the single-question assessment may be useful in epidemiological studies, it is worth to remember that it does not provide information on the specific components of ED (the ability to achieve or maintain erection), and the information on the severity of ED is limited (Rosen et al. 2002).

2.5.4 Erectile dysfunction and other markers of subclinical atherosclerosis

OxLDL particles have been detected in the cavernosal biopsies of men with ED (Boudjeltia et al. 2007). In addition, increased oxidative stress has been reported among ED compared with non-ED subjects (Barassi et al. 2009). There are no previous reports on circulating oxLDL levels among subjects with ED. Neither are there reports on the association of increased fibrinogen and ED in MetS. However, Vlachopoulos et al (2006) have reported fibrinogen as an independent predictor for the presence of ED both among men with and without coronary artery disease.

Endothelial dysfunction as assessed by a regional measurement of brachial FMD or coronary endothelial function testing has been reported to be associated with ED in several studies (Elesber et al. 2006, Kaiser et al. 2004, Uslu et al. 2006, Yavuzgil et al. 2005). Accordingly, in a study assessing arterial elasticity by the DPCA, small but not large arterial elasticity was associated with ED among hypertensive men (Prisant et al. 2006). On the other hand, in studies assessing large arterial stiffness by ultrasound or PWV, impaired large arterial elasticity has been associated with ED (Kaya et al. 2007, Uslu et al. 2006, Vlachopoulos et al. 2008). Besides these measurements of large arterial function, also higher carotid IMT values have been reported among ED than non-ED subjects (Ucar et al. 2007, Vlachopoulos et al. 2008). There are, however, no previous reports on the association between arterial elasticity and ED among MetS subjects.

2.5.5 Erectile dysfunction and increased cardiovascular risk

A high prevalence of both silent and clinical CVD has been reported among men with ED (Lee et al. 2008, Min et al. 2006, Polonsky et al. 2009, Rogers et al. 2010, Vlachopoulos et al. 2005). In the study by Rogers et al (2010) angiographically proven CHD correlated significantly with stenoses in the pudendal arteries. Vlachopoulos et al (2005) reported that 19% of men with vasculogenic ED have silent CHD on coronary angiography. In addition, in a study among 9150 men referred for coronary computed tomography, ED was significantly associated with abnormal calcification of the coronary arteries (Lee et al. 2008). ED has also been reported as an independent predictor of severe CHD and peripheral arterial disease among men referred for stress testing (Min et al. 2006, Polonsky et al. 2009). Furthermore, the presence and severity of ED seems to be associated with the severity of CVD (Min et al. 2008, Montorsi et al. 2006, Polonsky et al. 2009, Solomon et al. 2003).

Approximately 70% of men with angiographically proven CHD have reported that ED symptoms preceeded the symptoms of CHD (Montorsi et al. 2003). The time interval between ED and CHD symtoms and ED and CVD events has been reported at 2-3 years and 3-5 years, respectively (Hodges et al. 2007, Montorsi et al. 2006). This finding supports the arterial size hypothesis by Montorsi et al (2005).

A number of studies has reported ED as a risk marker for incident CVD (Araujo et al. 2010, Blumentals et al. 2004, Dong et al. 2011, Guo et al. 2010, Inman et al. 2009, Ponholzer et al. 2005, Thompson et al. 2005). The predictive value of ED seems to be independent of established CVD risk factors and Framingham risk score (Araujo et al. 2010). In a retrospective analyzis of 12 825 patients, the presence of ED conferred a two-fold increased risk for developing acute myocardial infarction (Blumentals et al. 2004). In a large meta-analysis of seven cohort studies among a total of 45 558 men, the adjusted relative risk for CVD events was 1.47 for ED compared with non-ED subjects. Similarly, in a study by Thompson et al (2005), the adjusted risk of cardiovascular event during a five-year follow-up was increased by 1.45 times in men with ED compared with those without. This is comparable with a factor 1.4 for a family history of myocardial infarction and 1.1 for a 0.52 mmol/L increase in total cholesterol (Thompson et al. 2005). In a prospective study among 2495 men, the 10-year risk to develop CHD was 65% higher and the risk to develop stroke 43% higher among those with moderate to severe ED compared with those without ED (Ponholzer et al. 2005). In addition, ED is associated with increased all-

cause mortality primarily through an increase in CV mortality (Araujo et al. 2009, Guo et al. 2010).

Differences in the predictive value of ED have been reported with respect to other risk factors (Corona et al. 2010, Inman et al. 2009). In the study by Corona et al (2010), the predictive effect of ED on incident CVD was better among obese than lean subjects. In another study by Corona et al (2011), PP was an independent predictor of major CVD events among young but not in older ED subjects. Especially among men aged 40-69 years, ED seems to be associated with a marked risk of incident CVD, whereas among men > 70 years, the prognostic importance is limited (Inman et al. 2009) (Figure 2). It is noteworthy that among men aged < 49 years, the presence of ED may be associated with a 50-fold increase in CHD incidence (Inman et al. 2009).

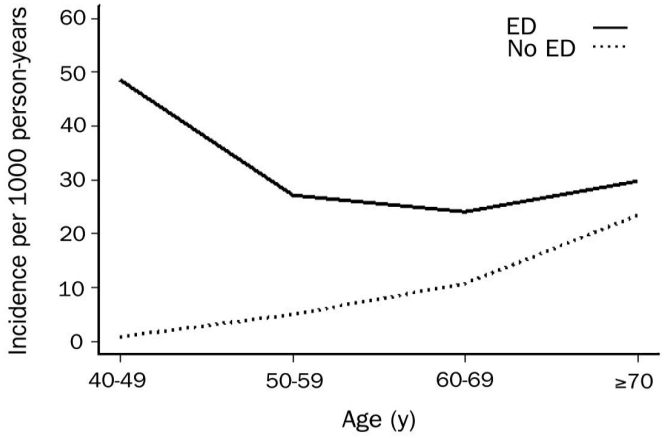

Figure 2. Incidence of CHD with respect to age and the presence of ED (Inman et al. 2009). Reprinted with kind permission of Mayo Proceedings.

2.6. METABOLIC SYNDROME

2.6.1 Definition and prevalence

MetS, a clustering of even mild metabolic abnormalities including visceral obesity, hypertension, dyslipidemia and insulin resistance, has become a leading health concern due to its increasing prevalence and connection to type 2 diabetes and CVD (Mottillo et al. 2010). A number of different MetS definitions have been presented. In 1999, World Health Organization (WHO) defined MetS as the presence of impaired glucose regulation or insulin resistance with at least two of the following criteria: blood pressure ≥ 140/90 mmHg or treatment, dyslipidemia (TG ≥ 1.7 mmol/L and/or HDL-C < 0.9 mmol/L (men) and < 1.0 mmol/L (women)) or treatment, central obesity (waist-hip ratio > 0.90 (men) and > 0.85 (women)) and microalbuminuria. The WHO definition has been widely used in epidemiological studies, whereas the complexity of the definition has restricted its use in clinical practice. More simple definitions have been proposed by organizations such as the National Cholesterol Education Program (NCEP), International Diabetes Federation (IDF), American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) and Joint Interim Societies (JIS). The diagnostic criteria of these recommendations are presented in Table 4.

Table 4. MetS definitions according to NCEP (Third report of the National Cholesterol Education Program 2001), AHA/NHLBI (Grundy et al. 2005), IDF (Zimmet et al. 2005), ESC/ESH (Mancia et al. 2007) and JIS (Alberti et al. 2009) recommendations.

	NCEP (2001)	**AHA/NHLBI (2005)**	**IDF (2005)**	**ESC/ESH (2007)**	**JIS (2009)**
Waist, cm	> 102 (♂) > 88 (♀)	≥ 102 (♂) ≥ 88 (♀)	≥ 94 (♂) ≥ 80 (♀)	> 102 (♂) > 88 (♀)	≥ 94 (♂) ≥ 80 (♀)
Gluc,mmol/L	≥ 6.1	≥ 5.6 or T2DM	≥ 5.6 or T2DM	≥ 5.6	≥ 5.6 or T2DM
BP, mmHg	≥ 130/85	≥ 130/85 or treatment	≥ 130/85 or treatment	> 130/85	≥ 130/85 or treatment
TG, mmol/L	≥ 1.7	≥ 1.7 or treatment	≥ 1.7 or treatment	> 1.7	≥ 1.7 or treatment
HDL-C, mmol/L	< 1.03 (♂) < 1.30 (♀)	< 1.03 (♂) < 1.30 (♀) or treatment	< 1.03 (♂) < 1.30 (♀) or treatment	< 1.00 (♂) < 1.20 (♀)	< 1.03 (♂) < 1.30 (♀) or treatment
Definition	≥ 3 of the components	≥ 3 of the components	Waist + ≥ 2 of the components	≥ 3 of the components	≥ 3 of the components

Gluc = fasting plasma glucose; BP = blood pressure; TG = triglycerides; HDL-C = high-density lipoprotein cholesterol

The NCEP recommendation, presented in 2001, has emerged as the most often used definition in studies assessing the connection between MetS and CVD (Guize et al. 2008, Mottillo et al. 2010). In 2005, the NCEP definition was revised by the AHA/NHLBI Scientific Statement (Grundy et al. 2005) by lowering the cut-off for fasting glucose. In addition, the AHA/NHLBI - definition includes patients with medications for hypertension, dyslipidemia or hyperglycemia (Grundy et al. 2005). The IDF recommendation, also presented in 2005, requires a fulfillment of a lowered threshold for waist circumference together with at least two of the criteria presented also by the AHA/NHLBI definition. The ESC/ESH definition includes a combination of NCEP criteria and new thresholds for HDL-C in women and glycemia since it does not include treatment for diabetes (Mancia et al. 2007). In order to unify the different definitions, the JIS definition was proposed by several major organizations such as the IDF Task Force on Epidemiology and Prevention, AHA/NHLBI, American Heart Association, World Heart Federation, International Atherosclerosis Society, and International Association for the

Study of Obesity in 2009 (Alberti et al. 2009).

In addition to the above-mentioned definitions, different modifications of these have been used in number of studies. Therefore, there is a marked variation in the reported prevalences of MetS, which complicates the interpretation of the results of several trials (Guize et al. 2008). Since the most recent MetS definitions have lowered the thresholds of the criteria, and the number of overweight people is increasing, the number of MetS patients is increasing as well (Guize et al. 2008, Mozumdar&Liguori 2011).

MetS, defined by the NCEP recommendation, affected approximately one third of middle-aged men participating in the third National Health and Nutrition Examination Survey (NHANES 1998-1994) in the US (Ford et al. 2002). There was a significant increase in the prevalence of MetS in the NHANES 1999-2006 (Mozumdar&Liguori 2011). In a population-based study of 1209 Finnish men without CVD aged 42 to 60 years, the prevalence of MetS in the 1980s ranged from 8.8-14.3% depending on the definition (Lakka et al. 2002).

In a recent study, the prevalence of MetS, according to the AHA/NHLBI definition, was 20.0% in men and 13.5% in women. According to the IDF definition, the prevalences for men and women were 26.0% and 18.4%, and according to the NCEP definition 11.7% and 7.5%, consecutively (Guize et al. 2007). The prevalence of MetS seems to be approximately 50% higher according to the IDF definition than according to the NCEP definition, whereas the AHA/NHLBI definition provides intermediate prevalences (Guize et al. 2008).

2.6.2 Increased risk of type 2 diabetes and cardiovascular disease

Subjects with MetS, regardless of the definition, are at 3- to 5-fold risk for type 2 diabetes compared to non-MetS subjects (Ford et al. 2008, Mancia et al. 2010). Since the newer MetS definitions have lowered the thresholds for glycemia and waist circumference, they may be better in predicting the incidence of diabetes (Ford et al. 2008). However, in a recent prospective study, there was no difference between the IDF and NCEP definitions in the prediction of incident diabetes (Mancia et al. 2010).

There are number of longitudinal studies evaluating the predictive value of MetS for CVD events and mortality (Bonora 2006, Isomaa et al. 2001, Lakka et al. 2002, Luksiene et al. 2011, Mancia et al. 2010, Mottillo et al. 2010). In the Botnia study of Finland and Sweden, cardiovascular mortality was six-fold higher and the risk of CHD and stroke three-fold higher among subjects with MetS, defined by the WHO definition, compared to those without (Isomaa et al. 2001). In a recent large review and meta-analysis of 87 studies and 951 083 patients, the presence of MetS, defined by the NCEP or AHA/NHLBI recommendations, was associated with a two-fold increase in cardiovascular outcome and 1.5-fold increase in all-cause mortality (Mottillo et al. 2010). The risk of CVD events is particularly high in the presence of diabetes or previously diagnosed CVD (Bonora 2006). Although the new definitions have increased the prevalence of MetS, their impact on morbidity is similar or lower compared to the NCEP definition (Guize et al. 2008, Mancia et al. 2010). In some studies among both diabetic and non-diabetic individuals the IDF definition did not predict the risk of CHD at all although the risk according to the NCEP definition was significant (Nilsson et al. 2007, Tong et al. 2007).

The predictive value of the MetS seems to decrease with increasing age (Mozzafarian et al. 2008, Thomas et al. 2011). In a prospective study among 129 655 subjects, MetS failed to predict all-cause mortality among patiens above 65 years (Thomas et al. 2011). Among older patients, CVD risk factors, especially hypertension and glycemia, seem to be more important than the syndrome itself (Mozzafarian et al. 2008).

The data concerning the effect of increasing number of metabolic abnormalities is discordant (Ford 2004, Hamburg et al. 2008, Kuk&Ardern 2010, Lapointe et al. 2007, Liu et al. 2007, Nakanishi et al. 2003). The clustering of MetS variables has been reported to be associated with increased large arterial stiffness, endothelial dysfunction and increased oxLDL (Hamburg et al. 2008, Lapointe et al 2007, Li et al. 2011, Nakanishi et al. 2003, Scuteri et al. 2010). However,

Koskinen et al (2009) found no difference in carotid IMT progression between subjects with three or four to five MetS components. In addition arterial stiffness was associated with an increasing number of MetS variables only among women in a study by Ferreira et al (2007). In addition, in a large follow-up study, there was no clear association between the number of MetS variables and mortality among general population (Kuk&Ardern 2010). On the contrary, increasing number of MetS variables has been reported to be associated with increased CVD risk in other studies both among diabetic patients and the general population (Ford 2004, Liu et al. 2007). The different MetS definitions and modifications of the definitions, used in the studies, may explain the discrepancy of the results.

In addition, several studies have suggested that the impact of certain metabolic abnormalities and especially combinations of MetS variables are more deleterious than others (Guize et al. 2008, Moebus et al. 2010). For example, combination of altered glucose tolerance and elevated BP together with either elevated TG or obesity seem to have the greatest effect on large arterial stiffness (Scuteri et al. 2010, Stehouwer et al. 2008). Microalbuminuria of the MetS components, included only in the WHO definition, and a combination of increased blood pressure, obesity and increased glucose levels have been reported to be associated with the greatest mortality risk (Isomaa et al. 2001, Guize et al. 2007). On the other hand, in a study comparing the predicitive effect of 16 different combinations of the AHA/NHLBI definition, some combinations presented with equal risk of CHD events as non-MetS subjects (Moebus et al. 2010). Thus, the wide variation in the number and combinations of individual metabolic abnormalities with respect to different definitions may also explain the inconsistency of the results (Guize et al. 2007).

Despite the extensive evidence on the connection to increased CVD risk, the importance of MetS has been questioned (Kahn et al. 2005, Mottillo et al. 2010). Since diabetic patients are at a two- to five-fold increased risk of CVD (Ginsberg&MacCallum 2009, Mancia et al. 2010), it has been suggested that the presence of diabetes among most MetS patients completely explains the increase in CVD risk (Kahn et al. 2005). However, in a recent meta-analysis, MetS maintained its predictive value for CVD outcomes even in the absence of diabetes (Mottillo et al. 2010). In addition, in a study among diabetic patients the increasing number of other MetS variables further increased the CVD risk (Liu et al. 2007).

Nevertheless, the predictive effect of MetS on CVD may be inferior or provide only limited advantage as an additional variable to the Framingham risk score (McNeill et al. 2005, Stern et al. 2004). In addition, the prognostic importance of the syndrome compared to the sum of its individual components has repeatedly been challenged (Bayturan et al. 2010, Corona et al. 2011, Ford et al. 2008, Johnson &Weinstock 2006, Kahn et al. 2005, Koskinen et al. 2009). In a review of seven clinical trials, MetS did not associate with atherosclerotic plaque progression after adjustment for its individual components (Bayturan et al. 2010). In a study by Koskinen et al (2009), MetS was associated with accelerated IMT progression but not more than expected from the sum of its components. Similarly, in a longitudinal study among ED subjects, the MetS was only little if at all superior to the sum of its individual components in predicting CVD incidence (Corona et al. 2011). In addition, only five of the studies on the predictive effect of MetS on CVD outcomes in the large meta-analysis by Mottillo et al (2010), reported risk estimates that were adjusted for at least one MetS component. Furthermore, MetS does not seem to improve the prediction of incident diabetes over that provided by impaired fasting glucose (Cameron et al. 2008, Ford et al. 2008).

2.6.3 Additional abnormalities associated with increased cardiovascular risk

Subjects with MetS have a wide variety of other abnormalities that are not included in the definitions but are associated with increased CVD risk (Bonora 2006, Guize et al. 2008). For instance, the presence of small, dense LDL particles, dysfunctional, dense HDL particles and increased oxidative stress are associated with the MetS (Hansel et al. 2004, Holvoet et al. 2008, Sigurdardottir et al. 2002). These factors as well as hyperglycemia and hyperinsulinemia, often

present in MetS subjects, seem to correlate with higher susceptibility of LDL to oxidation (Stocker&Keaney 2004, Holvoet et al. 2008). Correspondingly, increased level of circulating oxLDL has been detected among MetS in number of studies (Holvoet et al. 2004&2008, Hulthe&Fagerberg 2002, Sigurdardottir et al. 2002). It has also been hypothesized that increased oxLDL might contribute to the development of MetS but the results have been discordant (Holvoet et al. 2008, Koskinen et al. 2011). However, increased oxLDL among MetS have been reported to be associated with increased risk of myocardial infarction (Holvoet et al. 2004). Except for this study among the elderly (Holvoet et al. 2004), there are no longitudinal studies on the predictive value of increased oxLDL for CVD events in MetS.

Despite some contradicting reports (Levisianou et al. 2009, Tentolouris et al. 2008), also increased arterial stiffness, assessed by different methods, seems to be associated with MetS (Cernes et al. 2008, Czernichow et al. 2010, Ferreira et al 2007, Fjeldstad et al. 2007, Ge et al. 2008, Guize et al. 2008, Stehouwer et al. 2008). Most of these studies have assessed arterial stiffness by PWV, whereas only two previous studies have used the DPCA (Fjeldstad et al. 2007, Ge et al. 2008). The results of these two studies cannot, however, be generalized to Caucasian MetS subjects since MetS was not defined according to definitions in the study by Fjeldstad et al (2007), and the study by Ge et al (2008) was conducted among Chinese subjects. Whether MetS affects arterial function at central, peripheral or both sites is still unclear (Czernichow et al. 2010, Ferreira et al. 2007, Ge et al. 2008, Plantinga et al. 2008, Stehouwer et al. 2008). In addition, the pathophysiological mechanisms connected to arterial stiffness in Mets, for example the possible relation with increased LDL oxidation, need to be established. Furthermore, there are no prospective endpoint studies on the predictive value of arterial dysfunction in MetS (Laurent et al. 2006, Stehouwer et al. 2008, Vlachopoulos et al. 2010).

In addition to the presence of increased oxidative stress and impaired arterial function, a wide spectrum of biochemical abnormalities, all associated with increased CVD risk, have been reported in MetS (Bonora 2006). These include markers of increased thrombosis and coagulation, such as fibrinogen, markers of enhanced inflammation, atherogenic dyslipidemia (i.e. small and dense LDL particles, increased apolipoprotein B (ApoB)), homocysteinemia, hyperuricemia and microalbuminuria (Bonora 2006).

Besides these biochemical markers, ED, an early clinical manifestation of atherosclerosis, is highly prevalent among MetS (Bal et al. 2007). In addition to being a result of the atherosclerotic process, ED among MetS may be a consequence of decreased testosterone levels associated with obesity (Corona et al. 2009). In addition, increased stimulation of sympathetic nervous system and an accompanying increase in RHR have been reported to occur in MetS (Mancia et al. 2007, Pannier et al. 2006).

2.7 FACTORS AFFECTING LDL OXIDATION, ARTERIAL FUNCTION AND ERECTILE FUNCTION

2.7.1 Dietary interventions

Dyslipidemia is an essential factor in the atherosclerotic process (Stocker&Keaney 2004). Therefore, different interventions to improve lipid values or accompanying abnormalities have been studied widely. A beneficial dietary intervention should result in a decrease in total and LDL-C without reductions in HDL-C (Nicolosi et al. 2001). Most dietary approaches have focused on the alteration of fat composition and quantity in diet. It is well known that an increase in dietary intake of saturated fatty acids (SFA) has adverse effects on total and LDL-C levels (Buyken et al. 2010, Mensink et al. 2003). An intervention focusing in the reduction of SFA and total fat intake reduces total and LDL-C, but also HDL-C (Nicolosi et al. 2001). Intake of unsaturated fatty acids, on the other hand, seems to improve lipid profile without affecting HDL-C (Buyken et al. 2010, Mensink et al. 2003).

Unsaturated fatty acids are classified as monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Depending on the location of the first double bond, PUFAs are further classified as n-6 or n-3 PUFA. Linoleic acid, an n-6 PUFA, is an essential fatty acid which cannot be synthetisized endogeneously. N-3 PUFA can be derived from marine sources, (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), or from plants, (alpha-linolenic acid (ALA)) (De Caterina 2011).

MUFAs seem to diminish the susceptibility of LDL to oxidation (Cicero et al. 2008, Kratz et al. 2002, Moreno et al. 2008). Accordingly, a MUFA rich diet has been reported to improve oxidative stress measures and decrease circulating oxLDL levels among healthy non-obese men but also among MetS and other high CVD risk subjects (Egert et al. 2011, Fitó et al. 2007, Jones et al. 2011, Perez-Martinez et al. 2010). This beneficial impact on the oxidative process may explain why a Mediterranean type diet, rich in MUFA, decreases cardiovascular morbidity and mortality (Trichopoulou et al. 2003).

The results concerning the effect of PUFAs on oxidative process are inconsistent. Enhanced LDL oxidation has been assumed to result from an increased amount of PUFA in the small and dense LDL particles (de Graaf et al. 1991). Accordingly, in a study by Schwab et al (1998), PUFA-rich diet increased the susceptibility of LDL to oxidation compared with a MUFA-rich diet among subjects with IGT. On the other hand, in a study by Egert et al (2007) ALA-enriched rapeseed oil did not enhance LDL oxidizability. Similarly, in another study, moderate amounts of rapeseed oil-derived n-3 PUFA did not enhance LDL oxidation when supplemented to a diet rich in MUFA (Kratz et al. 2002). Although the results of these studies are contradicting, it has been concluded that n-3 PUFA in large amounts (eg 20 g/day) or in a diet rich in PUFA increase the susceptibility of LDL to oxidation (Mata et al. 1996, Nestel et al. 1997).

Interventions with unsaturated fatty acids have also been reported to affect arterial function. In a recent large meta-analysis, plant-derived n-3-PUFA was reported to improve endothelial function among healthy individuals, and markers of endothelial function among overweight dyslipidemic patients and type 2 diabetic patients (Egert&Stehle 2011). In a study by Cortés et al (2006), a meal supplemented with n-3 PUFA rich walnuts improved endothelial function as assessed by FMD, but the same effect was not found after supplementation with MUFA. In addition to improvement in endothelial function, n-3 PUFAs from marine and plant sources have been reported to improve arterial compliance among MetS, diabetic, hypertensive and overweight patients (Nestel et al. 1997, Pase et al. 2011).

N-3 PUFAs from marine sources have consistently been reported to decrease CV events and all-cause mortality both in primary and secondary prevention (De Caterina 2011, Marchioli et al. 2002, Wang et al. 2006). The results regarding the plant-derived n-3 PUFA, ALA, are less clear (De Caterina 2011, Wang et al. 2006). Although several studies have reported beneficial effects of ALA on CVD outcomes, the heterogeneity and even validity of some trials as well as background consumption of marine-derived n-3 PUFA are thought to have interfered with the results (De Caterina 2011). In addition, in other studies ALA intake did not have effect on CVD outcomes (De Caterina 2011, Wang et al. 2006).

The results concerning the effects of the n-6 PUFA, linoleic acid, also a major constituent of turnip rapeseed oil, are also discordant. Intake of linoleic acid has been reported to decrease the risk of CHD whereas some studies have not found any relation between linoleic acid intake and outcome measurements (Djoussé et al 2001, Dolecek 1992, Yam et al. 1996).

Turnip rapeseed oil consists of high amounts of MUFA, a composition similar to that of olive oil. It is also the main dietary source of plant-derived n-3 PUFA in the Northern Europe. In addition, it contains a considerable amount of n-6 PUFA. However, the small amount of bioactive compounds with a potent antioxidant activity, such as tocopherols, phytosterols and phenols, are not optimally preserved in the industrial process (Attorri et al. 2010).

Rapeseed oil has been reported to reduce total and LDL cholesterol level whereas its effect on HDL-C seems to be neutral (Adamsson et al. 2011, Gulesserian&Widhalm 2002, Seppänen-Laakso et al. 1993, Södergren et al. 2001, Valsta et al. 1992, Vermunt et al. 2001). In a study by

Attorri et al (2010), micronutrient-enriched rapeseed oil, obtained by optimizing the processing of oil, reduced markers of oxidative stress in rats. Södergren et al (2001), however, did not find a difference in the degree of lipid peroxidation between traditionally produced rapeseed oil- and SFA-rich diets. The effect of turnip rapeseed oil on circulating oxLDL levels or arterial function is not known.

2.7.2 Physical activity

Regular aerobic exercise may attenuate the age-related reduction in large arterial elasticity (Sugawara et al. 2004) and decrease circulating oxLDL (Elosua et al. 2003, Ziegler et al. 2006). However, resistance training at high intensities may have the opposite effects (Miyachi et al. 2003&2004, Muñoz et al. 2010). An age-related reduction in arterial compliance has been reported to be greater among resistance-trained than sedentary subjects (Miyachi et al. 2003). In addition, several months of resistance training led to a greater decrease in arterial elasticity than in controls (Miyachi 2004). Furthermore, even a short bout of exercise at high intensity has been reported to induce oxidative stress (Muñoz 2010). On the contrary, lifetime vigorous but not light-to-moderate habitual physical activity was found to have beneficial effects on carotid stiffness in the study by Van de Laar et al (2010).

Physical activity decreases the risk of ED and even seems to improve sexual function among those with established ED (Bacon et al. 2006, Esposito et al. 2004, Kratzik et al. 2009). The effects of physical activity on ED may be mediated at least via decrease in weight, decrease in oxidative stress, improvement in endothelial function and an increase in testosterone levels (Elosua et al. 2003, Esposito et al. 2009, Revnic et al 2007, Sugawara et al. 2004). In the study by Kratzik et al (2009) physical exercise of 1000 kcal/week was enough to reduce the risk of ED. The risk of ED was reported to decrease even further with the increase of weekly energy expenditure of exercise up to 4000 kcal. In another study, the adjusted incidence of ED was significantly lower among men with physical activity >200 kcal/day than among those with <200 kcal/day (Feldman et al. 2000). On the other hand, sedentary behaviour seems to increase the risk of ED (Bacon et al. 2006). Whereas midlife lifestyle changes may be too late to reverse the adverse effects of smoking, obesity and alcohol consumption on ED, an increase in physical activity seems to be effective even then (Derby et al. 2000).

In addition to the above-mentioned effects, physical activity and cardiorespiratory fitness (Lakka 1993, Myint 2008) have been reported to be associated with decreased fibrinogen levels. The effect of regular physical exercise on lowering the RHR is also well established.

2.7.3 Medication

Statins lower LDL-C levels and decrease inflammation and oxidative stress (Sowers 2003). Thus, statin use may decrease circulating levels of oxLDL (Hofnagel et al. 2007). In addition, statins have been reported to improve endothelial dysfunction in different clinical conditions except in advanced diabetic disease (Sowers 2003). In addition, statins seem to have direct effects on the endothelial and smooth muscle cells, which in turn lead, for instance, to an increase in active endothelim-derived nitric oxide level and decrease in smooth muscle cell proliferation (Sowers 2003). In a recent meta-analysis, statins were reported to improve both peripheral and coronary endothelial function (Reriani et al. 2011). Accordingly, in a study by Leibovitz et al (2001) atorvastatin treatment improved small, but not large arterial elasticity, as assessed by DPCA, among hypercholesterolemic patients.

Calcium antagonists, ACE-inhibitors and ATR-blockers seem to improve especially the compliance of large arteries, independently of the effect on blood pressure (Glasser et al. 1998, Nashar et al. 2004). In the study by Nashar et al (2004), losartan use was followed by an increase in large but not small arterial elasticity, also assessed by the DCPA measurement. Beta-blockers, however, do not seem to affect arterial compliance, nor are there reports on a beneficial effect of

the antihypertensive medications on oxidative stress or circulating oxLDL levels (Glasser et al. 1998, Nashar et al. 2004).

Certain drugs are thought to affect erectile function and thus, worsen the ED. The effect of beta-blockers on ED has been studied widely. Increased risk of ED has been reported to be associated with the use of non-selective beta-blockers but not with the use of selective ones (Shiri et al. 2007). In addition, in a systematic review of randomized trials, the risk of sexual dysfunction caused by beta-blockers was low, much lower than generally thought (Ko et al. 2002). The results regarding thiazide diuretics, ACE-inhibitors and calcium-channel blockers are contradictory. Whereas some studies have reported no effect or even reduced risk of ED, some studies have found a significant association with these medications and increased risk of ED (Blumentals et al. 2003, Grimm et al. 1997, Shiri et al. 2007). On the contrary, ATR-blockers and statins may even improve sexual function (Doğru et al. 2008, Düsing et al 2003). Nevertheless, ED seems to be a consequence of the underlying vascular disease rather than a result of medications (Jackson et al. 2010). Unless ED has developed within four weeks of intitiation of certain medication, changing the drug will probably not alleviate the symptoms (Jackson et al. 2010).

3 Aims of the Study

The purpose of this thesis was to study whether arterial elasticity, circulating oxLDL, fibrinogen and RHR are associated with the presence of MetS, and the presence of high estimated 10-year cardiovascular risk, an increasing number of MetS variables and ED among MetS subjects. In addition, the effect of dietary intake of CPTRO on these markers of subclinical atherosclerosis was assessed among subjects with MetS.

The specific aims of the study were to assess, whether these markers

1. differ between MetS and physically active (PhA) subjects **(I)**.

2. differ between MetS subjects with different estimated 10-year cardiovascular risk **(II).**

3. differ between MetS subjects with a different number of metabolic features **(II)**.

4. may be influenced by an intervention period with CPTRO in comparison with butter among MetS subjects **(III)**.

5. differ between MetS subjects with and without ED, and whether the difference is present also among those with a low estimated risk of cardiovascular death **(IV)**.

6. are associated with the presence of ED, and whether physical activity is associated with normal erectile function among MetS subjects **(IV, V).**

4 Subjects and Methods

4.1 SUBJECTS AND STUDY DESIGN

This study is a part of the Hämeenlinna Metabolic Syndrome (HMS) Research Program. It is an entity investigating atherosclerotic risk factors in men with MetS. Study subjects were referred from consecutive patients ascertained to have MetS in primary health care. The total number of subjects participating in the study was 179 of which 120 had MetS and 59 were PhA counterparts. All study subjects were men, aged 30 to 65. MetS was defined according to the NCEP criteria as the presence of at least three of the following five criteria (Third report of the National Cholesterol Education Program 2002):

- waist circumference > 102 cm
- serum triglycerides level ≥ 1.7 mmol/L
- serum high density lipoprotein (HDL) cholesterol level < 1.03 mmol/L
- blood pressure ≥ 130/85 mmHg
- plasma glucose level ≥ 6.1 mmol/L or diabetes

Only PhA subjects who had not been diagnosed with CVD, did not fulfil the criteria of MetS and exercised physically more than three times a week and 30 minutes per exercise without chest pain, dyspnea or fatigue were recruited. Suspicion of non-vascular ED was an exclusion criterion in Studies IV and V.

The study consists of five substudies, referred as I-V in the text. The overview of the number of subjects, specific inclusion criteria, design and main interests of different substudies are presented in Table 5. Some subjects participated in all five studies. The study designs in more particular were as follows:

Study I. 40 MetS and 40 PhA subjects participated in this cross-sectional study. Subjects with diagnosed CVD or use of statin, ACE-inhibitor, or ATR-blocker medications were excluded. The differences in the selected markers of subclinical atherosclerosis were studied between the groups. All MetS subjects in this study also participated in Study II.

Study II. 120 men with MetS participated in this cross-sectional study. 10-year risk of CVD events and death were assessed by SCORE and FINRISK risk score models at the actual and at the extrapolated age of 60. 79 men without diagnosed CVD or statin medication were included in the analyses dealing with the 10-year CVD risk estimates. The differences in the assessed markers of subclinical atherosclerosis were studied between different risk groups. All study subjects were included in the analyses dealing with the effect of different risk and protective factors as well as the effect of different number of MetS variables on the assessed markers of subclinical atherosclerosis.

Study III. 43 men with MetS were recruited from the participants of Study II. 6 men withdrew because they were unable to comply with the dietary regimens. Subjects' usual diet was supplemented with CPTRO and butter in an open, balanced and randomized cross-over design (Figure 3). Lipids and selected markers of subclinical atherosclerosis were assessed after both periods.

Study IV. 70 men with MetS, aged 35-60, participated in this cross-sectional study. 10-year risk of CVD death was assessed by SCORE risk score model at the actual age. Selected markers of subclinical atherosclerosis were compared between ED and non-ED subjects. 53 subjects without diagnosed CVD were included in the analyses among those at low estimated CVD risk. The predictive effect of ED on oxLDL and arterial elasticity was assessed among all study

subjects. All subjects in this study also participated in Study II.

Study V. 57 MetS and 48 PhA subjects participated in this cross-sectional study. Only men with maximal erectile function score or ED according to the IIEF were included. The differences in the assessed markers of subclinical atherosclerosis were studied between ED and non-ED subjects as well as between MetS and PhA subjects. The effect of different risk and protective factors on the presence of ED were assessed among all subjects and separately among MetS and PhA subjects. 17 MetS subjects and 30 PhA subjects in this study participated also in Study I and all MetS subjects in Study II.

Table 5. Number of subjects, specific inclusion criteria, designs and main interests of Studies I-V.

Study	Subjects (N)	Specific criteria	Design	Main interest
I	40 MetS 40 PhA	subjects without CVD, statin, ACEi- or ATRb medication	cross-sectional	oxLDL arterial elasticity
II	120 MetS	subjects without CVD and statin in analyses dealing with risk score models (n=79)	cross-sectional	oxLDL arterial elasticity 10-year CVD risk no of MetS variables
III	37 MetS	-	cross-over, intervention: CPTRO vs. butter	lipids oxLDL arterial elasticity
IV	70 MetS	subjects aged 35-60, subjects without CVD in analyses dealing with risk score model (n=53)	cross-sectional	oxLDL arterial elasticity ED
V	57 MetS 48 PhA	subjects without non-selective beta-blockers, maximal IIEF score or ED	cross-sectional	oxLDL arterial elasticity ED physical activity fibrinogen resting heart rate

ACEi = angiotensin converting enzyme inhibitor; ATRb = angiotensin receptor blocker; CPTRO = cold-pressed turnip rapeseed oil; CVD = cardiovascular disease; ED = erectile dysfunction; IIEF = International Index of Erectile Function; MetS = metabolic syndrome; oxLDL = oxidized LDL; PhA = physically active

The basic and clinical characteristics of the subjects participating in Studies I-IV are presented in Tables 6 and 7, and of subjects participating in Study V in Tables 8 and 9, respectively. There were no significant differences in medications known to affect the markers of subclinical atherosclerosis, between the compared groups of Studies I, II and IV. In Study V, subjects with ED were significantly more often on selective beta-blocker medication than those without ED, and MetS subjects were more often on ASA, selective beta-blocker, ATR-blocker, diuretic and statin medications than PhA subjects. There was no significant difference in the use of any other medication between the groups in Study V.

Table 6. Basic characteristics of the subjects in Studies I-IV.

	Study I		**Study II**		**Study III**	**Study IV**	
	PhA (n=40)	MetS (n=40)	Medium (n=35)	High (n=44)	MetS (n=37)	non-ED (n=43)	ED (n=27)
Age, y	50.8 ± 8.1	49.8 ± 7.1	46.5 ± 8.1	50.5 ± 8.0	53.7 ± 7.3	48.4 ± 6.4[§]	53.2 ± 5.6
Smokers							
- current	2.5%[§]	30.0%	11.4%	31.8%	16.2%	18.6%	18.5%
- former	30.0%	40.0%	54.3%	34.1%	56.8%	51.2%	51.9%
- never	67.5%	30.0%	34.3%	34.1%	27.0%	30.2%	29.6%
Diabetics	2.5%[#]	40.0%	5.7%[#]	59.1%	51.4%	34.9%	44.4%
Hypertension	5.0%*	22.5%	42.9%	52.3%	56.8%	46.5%	59.3%
CVD	-	-	-	-	16.2%	7.0%	18.5%

Study I: Physically active (PhA) and metabolic syndrome (MetS) subjects
Study II: MetS subjects with medium (5-14.99%) and high (≥15%) estimated 10-year cardiovascular disease (CVD) risk according to FINRISK at the projected age of 60
Study III: MetS subjects, CPTRO vs. butter intervention
Study IV: MetS subjects with and without erectile dysfunction (ED)
[*] $p < 0.05$, [§] $p < 0.01$, [#] $p < 0.001$

Table 7. Clinical characteristics of the subjects in Studies I-IV.

	Study I		**Study II**		**Study III**	**Study IV**	
	PhA (n=40)	MetS (n=40)	Medium (n=35)	High (n=44)	MetS (n=37)	non-ED (n=43)	ED (n=27)
SBP	127.6±9.3$^{\#}$	139.6±15.8	131.5±10.6$^{\#}$	145.2±14.9	145.5±12.3	138.1±13.3	140.2±14.4
DBP	74.3 ±7.2$^{\#}$	82.1 ± 9.0	79.0 ± 7.2§	84.7 ± 8.9	90.8 ± 5.6	82.9 ± 8.2	81.9 ± 7.6
Waist	87.8±6.4$^{\#}$	112.2±12.0	111.0 ± 10.4	116.5±14.7	111.1 ± 13.1	113.9±12.9	113.9±10.8
Chol	5.31 ± 0.8§	5.98 ± 1.0	5.44 ± 0.9§	6.18 ± 1.2	5.15 ± 1.49	5.47 ± 1.2	5.20 ± 1.4
HDL-C	1.68± 0.4$^{\#}$	1.17 ± 0.2	1.10 ± 0.2	1.19 ± 0.3	1.15 ± 0.27	1.17 ± 0.2	1.15 ± 0.4
LDL-C	3.42 ± 0.7*	3.87 ± 1.0	3.53 ± 0.9	3.90 ± 1.0	3.33 ± 1.25	3.36 ± 1.0	3.23 ± 1.1
TG	0.88± 0.4$^{\#}$	2.67 ± 1.5	2.42 ± 1.2	3.09 ± 2.4	2.19 ± 1.13	2.79 ± 2.2	2.45 ± 1.7
Glucose	5.53± 0.6$^{\#}$	6.99 ± 1.8	6.18 ± 1.3§	7.33 ± 2.1	6.62 ± 1.19	6.97 ± 1.9	7.29 ± 2.0

Study I: Physically active (PhA) and metabolic syndrome (MetS) subjects
Study II: MetS subjects with medium (5-14.99%) and high (≥15%) estimated 10-year CVD risk according to FINRISK at the projected age of 60
Study III: MetS subjects, CPTRO vs. butter intervention
Study IV: MetS subjects with and without erectile dysfunction (ED)

* $p < 0.05$, § $p < 0.01$, $^{\#}$ $p < 0.001$

Chol = total cholesterol; CPTRO = cold-pressed turnip rapeseed oil; DBP = diastolic blood pressure; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SBP = systolic blood pressure; TG = triglycerides

Table 8. Basic characteristics of physically active (PhA) and metabolic syndrome (MetS) subjects with and without erectile dysfunction (ED) in Study V.

	PhA		**MetS**		
	no ED	ED	no ED	ED	
	(n=35)	(n=13)	(n=21)	(n=36)	p
Age[1], y	49.4 ± 7.5	56.9 ± 6.7	45.9 ± 4.6	54.1 ± 8.2	< 0.001
Smokers[b]					< 0.01
- current	2.9%	0%	23.8%	16.7%	
- former	31.4%	46.2%	47.6%	50.0%	
- never	65.7%	53.8%	28.6%	33.3%	
Diabetics[2,a]	0%	0%	33.3%[#]	52.8%	< 0.001
Hypertension[2,a]	2.9%*	23.1%	52.4%	55.6%	< 0.001
CVD[b]	**-**	**-**	14.3%	19.4%	< 0.05

[1-2] p value for the difference between men with normal erectile function (n=56) and ED (n=49):
[1] $p < 0.001$, [2] $p < 0.01$, NS if not mentioned.
[a-b] p value for the difference between PhA (n=48) and MetS (n=57) subjects:
[a] $p < 0.001$, [b] $p < 0.01$, NS if not mentioned.

Table 9. Clinical characteristics of physically active (PhA) and metabolic syndrome (MetS) subjects with and without erectile dysfunction (ED) participating in Study V.

	PhA		**MetS**		
	no ED (n=35)	ED (n=13)	no ED (n=21)	ED (n=36)	p
SBP[3,a]	127.2 ± 9.2	126.9 ± 11.2	136.5 ± 12.2	139.8 ± 16.5	< 0.001
DBP[a]	73.9 ± 6.7	74.5 ± 5.6	82.1 ± 7.4	81.7 ± 8.8	< 0.001
Waist[2,a]	88.6 ± 7.1	90.8 ± 8.9	113.5 ± 10.5	114.8 ± 13.3	< 0.001
Chol	5.27 ± 0.7	5.33 ± 0.8	5.26 ± 1.1	5.30 ± 1.6	NS
HDL-C[3,a]	1.61 ± 0.3	1.81 ± 0.4	1.20 ± 0.2	1.16 ± 0.3	< 0.001
LDL-C	3.47 ± 0.7	3.32 ± 0.9	3.24 ± 0.9	3.21 ± 1.0	NS
TG[3,a]	0.94 ± 0.5	0.86 ± 0.4	2.49 ± 1.4	3.19 ± 5.1	< 0.001
Glucose[2,a]	5.49 ± 0.4	5.59 ± 0.6	6.54 ± 1.0	6.99 ± 2.2	< 0.001

[1-3] p value for the difference between men with normal erectile function (n=56) and ED (n=49):
[1] $p < 0.001$, [2] $p < 0.01$, [3] < 0.05, NS if not mentioned.
[a-b] p value for the difference between PhA (n=48) and MetS (n=57) subjects:
[a] $p < 0.001$, [b] $p < 0.01$, NS if not mentioned.

Chol = total cholesterol; DBP = diastolic blood pressure, HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SBP = systolic blood pressure; TG = triglycerides

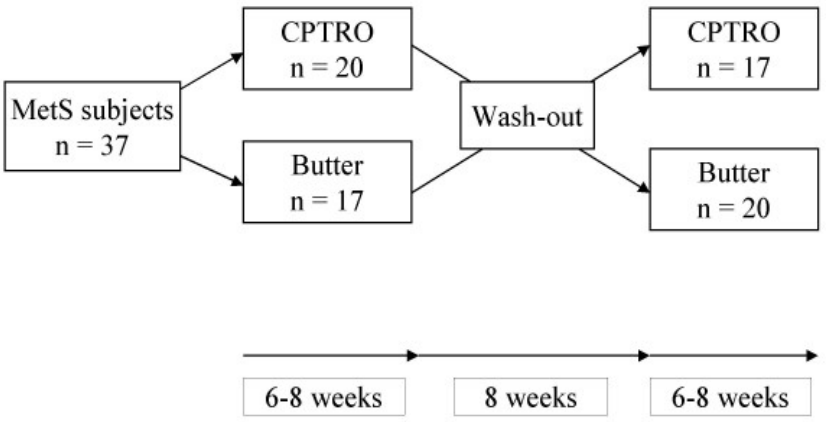

Figure 3. The open, randomized, cross-over design of Study III.

4.2 METHODS

4.2.1 Individual interview and physical examination

Information on the subjects' medical history, smoking, alcohol consumption, physical activity, dietary habits and CVD in family was collected during a standardized interview. Smoking status was categorized as never, former or current. Subjects' weight, height, waist circumference and blood pressure were measured according to general recommendations. PP was calculated as SBP minus DBP. Body mass index (BMI) was calculated as weight (kg) / height² (m^2). The physical examination was conducted after both study periods in Study III.

4.2.2 Physical activity

Subjects filled in a questionnaire on their average times, duration, type and intensity level (four predetermined choices) of physical exercise per week. Physical exercise energy expenditure index (kcal/day) was calculated by multiplying the MET value and exercise times per week and person's weight in kilograms and mean duration of exercise in hours and finally dividing it by 7 (Aittasalo et al. 2004). A compendium of physical activities and subjects' self-rated intensity levels of the exercise sessions were used to estimate the MET value (Ainsworth et al. 2000). In Study V, subjects were divided into three groups according to the energy expenditure on physical activity (kcal/day). Physical activity level was considered low if the energy expenditure was < 200 kcal/day, moderate if 200-400 kcal/day and high if > 400 kcal/day.

4.2.3 Clinical chemistry

Venous blood was drawn after at least 15 minutes of rest in a sitting position between 7:00 and 9:00 a.m. Subjects were asked to fast and not to take any medication, drink or smoke for 12 hours before blood sampling. Blood samples were collected into 10 mL EDTA tubes, 5 mL lithium-heparin gel tubes and 2 mL natrium-fluoride tubes. Lipid, glucose and fibrinogen levels were immediately analyzed according to validated methods. All laboratory tests were analyzed at Kanta-Häme Central Hospital. The laboratory practices strict internal quality control with daily and monthly control samples performed by a national external quality assurance program (Labquality Oy). Clinical chemistry was assessed after both study periods in Study III.

4.2.4 Circulating oxidized LDL

Blood samples for the measurements of oxLDL were stored at -70 to -85° C before the analysis. Plasma levels of oxLDL were determined as duplicates according to a validated monoclonal antibody 4E6-based capture ELISA (Mercodia AB, Uppsala, Sweden) (Hulthe&Fagerberg 2002, Sigurdardottir et al. 2002). The monoclonal antibody mAb-4E6 is the same as in the assays previously described by Holvoet et al (Holvoet et al. 1998&2001). The same experienced researcher at Kanta-Häme Central Hospital performed all determinations of oxidized LDL. The CV% of oxLDL measurement was 7.7%. OxLDL was measured after both study periods in Study III.

4.2.5 Arterial elasticity

Arterial elasticity was measured after at least 15 minutes of rest in a semi-sitting position. The measurement was carried out in a temperature-controlled room. Subjects refrained from eating, smoking, drinking caffeinated drinks and taking medication for 12 hours and drinking alcohol for two days prior to the measurement. Arterial elasticity was assessed non-invasively by recording radial artery pulse wave by an arterial tonometer (HDI/PulseWave™ CR-2000, Hypertension Diagnostics, Inc., Eagan, Minnesota, USA) which uses a modified Windkessel

method (Cohn et al. 1995). During the measurement, individual pulse-waves were cross-correlated. The capacitive elasticity of large arteries (C1) and the reflective elasticity of small arteries (C2) were automatically assessed by the CR-2000 as a mean of the five most similar pulse waves appearing during the measurement. C1 identifies the elasticity of the aorta and other large arteries, C2 the endothelial function of the microvascular circulation (Cohn 1999). The data was automatically displayed on a computer screen from which the uniformity of the waveforms and possible artifacts were easily detected. The mean of four consecutive measurements was assessed to diminish the variability and bias. Blood pressure and RHR were automatically assessed by the CR-2000 during the elasticity measurement. Same experienced nurse performed all measurements. Intraindividual CV% of C1 and C2 measurements were 9.0% and 8.8%, respectively. Arterial elasticity was measured after both study periods in Study III.

4.2.6 10-year cardiovascular risk score calculation

Subjects with CVD and statin medication were excluded from the risk estimate analyses in Study II and subjects without CVD in Study IV. FINRISK and SCORE models were used to calculate the 10-year risk of CVD events and death. Since absolute risk among younger subjects with notable risk factors is low, the risk score of men younger than 60 years was extrapolated to age 60 to assess a possible high relative risk.

The FINRISK 10-year risk of lethal and non-lethal cardiovascular events was calculated as the sum of the risk of a CHD event and the risk of stroke (Vartiainen et al. 2007). SCORE 10-year risk of CVD death was calculated by using coefficients for populations at high CVD risk and the formula presented by the SCORE project (Conroy et al. 2003).

In Study II differences were assessed between low-, medium- and high-risk subjects according to FINRISK and SCORE at the actual age and at the extrapolated age of 60 (FINRISK60, SCORE60). In Study IV differences were assessed between subjects with and without ED among those with SCORE < 5% calculated according to the actual age.

4.2.7 Cold-pressed turnip rapeseed oil and butter supplementation

Subjects were given both verbal and written instructions at the beginning of both study periods. Butter (37.5 g, maximal water content of 16%, Valio Ltd, Finland) and CPTRO (35 mL, 0.92 g/ml, Kankaisten Öljykasvit Ltd) were provided free of charge. The daily fat adjunct comprised approximately 11-12% of daily energy intake. Subjects were recommended to use the fat adjunct as such, i.e. without heating or frying. To ensure that subjects maintained their every-day diet and physical activity similar during the study periods, they were asked to fill in a diet and exercise questionnaire during the periods.

4.2.8 International Index of Erectile Function questionnaire

Subjects participating in the HMS Research Program (120 MetS and 80 PhA subjects) were asked to fill in the IIEF questionnaire (Rosen et al. 1997). Of these subjects, all MetS subjects and 59 PhA subjects were included in this dissertation. The sum of the IIEF questions 1-5 and 15 was calculated to assess the presence of ED. Subjects with an IIEF score ≤ 25, were considered to have ED, whereas subjects with score 26 to 30 were considered to have normal erectile function. The question number 15 (how do you rate your confidence that you can get and keep your erection?) was used to assess the presence of ED in men reporting lack of sexual activity in questions 1-5. In Study V, those with IIEF score 26-29 were excluded to ensure that the subjects participating in the study truly had either completely normal or impaired erectile function. Accordingly, among those reporting lack of sexual activity in questions 1-5, a subject reporting high confidence in the question number 15 was excluded. Those reporting very high confidence

were considered to have normal erectile function and those reporting very low to medium confidence were considered to have ED.

4.2.9 Statistical methods

Statistics were analyzed with SPSS for Windows 17.0 (IBM Corporation, Somers, NY, USA). Data are expressed as mean ± SD if not mentioned otherwise. $P < 0.05$ was considered statistically significant. Associations between continuous and categorical variables were assessed by T-test in case of normality and by Mann-Whitney U-test in case of non-normality. ANOVA was used to analyze the adjusted p values for differences in multi-dimensional variables in case of normality. Kruskall-Wallis test was used in case of non-normality. The Bonferroni post-hoc analysis was used. When Mann-Whitney U-test was used for three-category variables in case of non-normality, the p value was corrected by multiplying by three. Associations between categorical variables were studied by χ^2 test.

In Studies II and IV, linear regression model was used stepwise in assessing the effect of different covariates on oxLDL and arterial elasticity. Correlations between different covariates were assessed and only covariates with correlation coefficients of < 0.5 with each other were included in the analyses. Covariates with evident non-linear relation to oxLDL and arterial elasticity were excluded. Analyses were reassured by automatic forward and backward methods as well as manually by the enter method. β- and p-values are presented for covariates included in the models. Residual analyses were carried out for the models. In Study V a binary logistic regression model was used to analyze the relations of risk factors and markers of atherosclerosis to the presence of ED. Only covariates without a strong correlation with each other were included in the multivariate analysis. Multivariate analysis was conducted with the enter method by removing covariates without association to ED one by one. The result was verified with the forward conditional method and adjusted for the use of medications.

4.2.10 Ethical considerations

The study was carried out at Kanta-Häme Central Hospital, Linna Clinic and Mehiläinen Oy. The Ethics Committee of the Kanta-Häme Hospital District approved the study protocol and the study followed the ethical principles outlined in the Declaration of Helsinki. Subjects were given both oral and written information on the study before they signed an informed consent. All data were analyzed anonymously.

5 Results

The main findings regarding the association of C1, C2, oxLDL, fibrinogen and RHR with MetS, a high estimated 10-year CVD risk (according to FINRISK60), an increasing number of MetS variables, or ED in MetS, and the effect of CPTRO on these markers of subclinical atherosclerosis, are presented in Table 10.

Table 10. The association of MetS as well as high 10-year CVD risk, number of MetS variables, erectile dysfunction and CPTRO intervention among MetS subjects with markers of subclinical atherosclerosis.

	C1	C2	oxLDL	Fibrinogen	RHR
MetS	↓	↔	↑	↑	↑
High 10-year CVD risk	↓	↓	↑	↑	↑
Number of MetS variables	↔	↔	↔	↔	↔
Erectile dysfunction	↓	↔	↔	↑	↑
CPTRO	↔	↔	↓	↔	↔

↓ decreased, ↑ increased, ↔ no difference
C1 = large arterial elasticity; C2 = small arterial elasticity; CPTRO = cold-pressed turnip rapeseed oil; CVD = cardiovascular disease; MetS = metabolic syndrome; oxLDL = oxidized low-density lipoprotein; RHR = resting heart rate

5.1 LARGE ARTERIAL ELASTICITY

C1 was lower among MetS than PhA subjects (Figure 4) **(I, V)**. The difference remained significant after adjustment for physical activity (p=0.021). C1 was significantly lower also among MetS subjects with ED than among those without ED (Figure 4) **(IV)**. After adjustment for age, diabetes, family history of CVD, smoking, physical activity, statin and beta-blocker medication, waist circumference, blood pressure and lipids, or for a 10-year risk of CVD death by the SCORE, the difference in C1 remained significant, p=0.008 and p=0.014, respectively. Among subjects with SCORE < 5%, men with ED had lower C1 than those without ED, 15.9 ± 3.4 ml/mmHgx10 vs. 18.5 ± 3.3 ml/mmHgx10, respectively (p=0.01). The difference remained significant even after adjustment for age, SBP, diabetes, smoking, lipids, physical activity and beta-blocker medication (p=0.048). In the study among PhA and MetS subjects, there was a significant decrease in C1 in the presence of ED and MetS. C1 was highest among PhA subjects without ED and lowest among MetS subjects with ED (Figure 4) **(V)**.

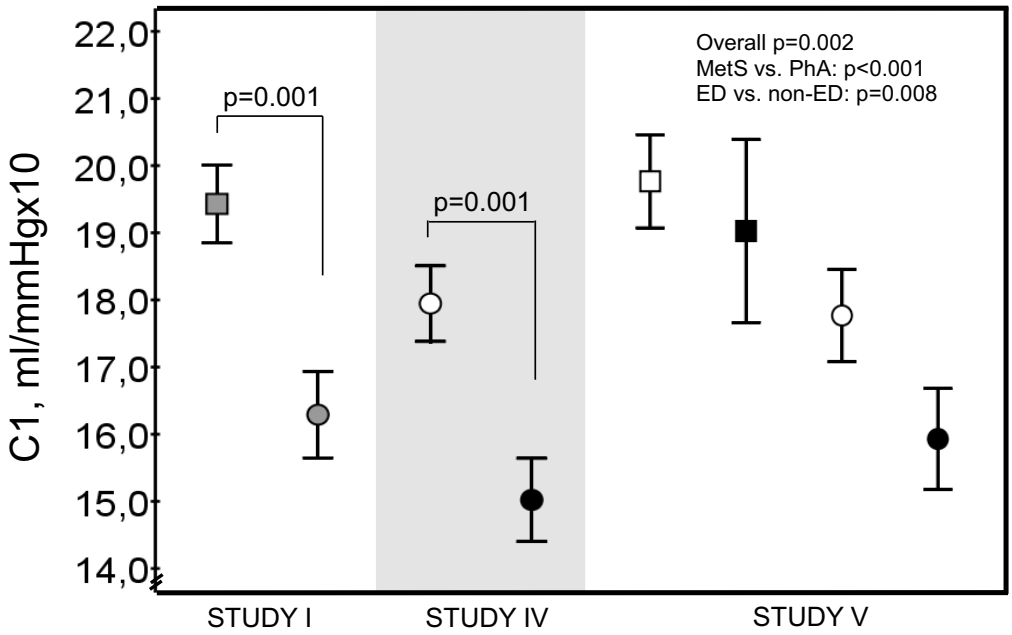

Figure 4. Large arterial elasticity (C1) among physically active (PhA) and metabolic syndrome (MetS) subjects and among MetS and PhA subjects with and without erectile dysfunction (ED). Mean ± SEM are presented.

Study I: PhA MetS
Study IV: MetS without ED MetS with ED
Study V: PhA with maximal IIEF score PhA with ED
MetS with maximal IIEF score MetS with ED

MetS subjects with high estimated 10-year CVD risk according to both FINRISK and SCORE at the actual and at the projected age of 60, had lower C1 than those with lower estimated risk (Figure 5) **(II)**. With an alternative risk score division according to FINRISK at the actual age (low < 5%, medium 5-9.99% and high ≥ 10%), there was still a significant difference between low- and high-risk subjects in C1 (p=0.004).

Diabetes (β = -1.18, p=0.033) and PP (β = -0.33, p<0.001) were inversely and waist circumference directly (β = 0.11, p<0.001) associated with C1 **(II)**. When RHR was added to the analyses, it was selected as a significant covariate in the final model of C1 (β = -0.12, p<0.001) together with the above-mentioned covariates. In the multivariate analysis of Study IV, the presence of ED was associated with lower C1 even after adjustment for age, traditional cardiovascular risk factors and medications (β = -2.05, p=0.005). Also SBP (β = -0.24, p<0.001) and waist circumference (β = 0.10, p=0.003) were significant predictors in the adjusted model. There was no difference in C1 between subjects with three, four or five MetS variables **(II)**, or after oil and butter periods **(III)**.

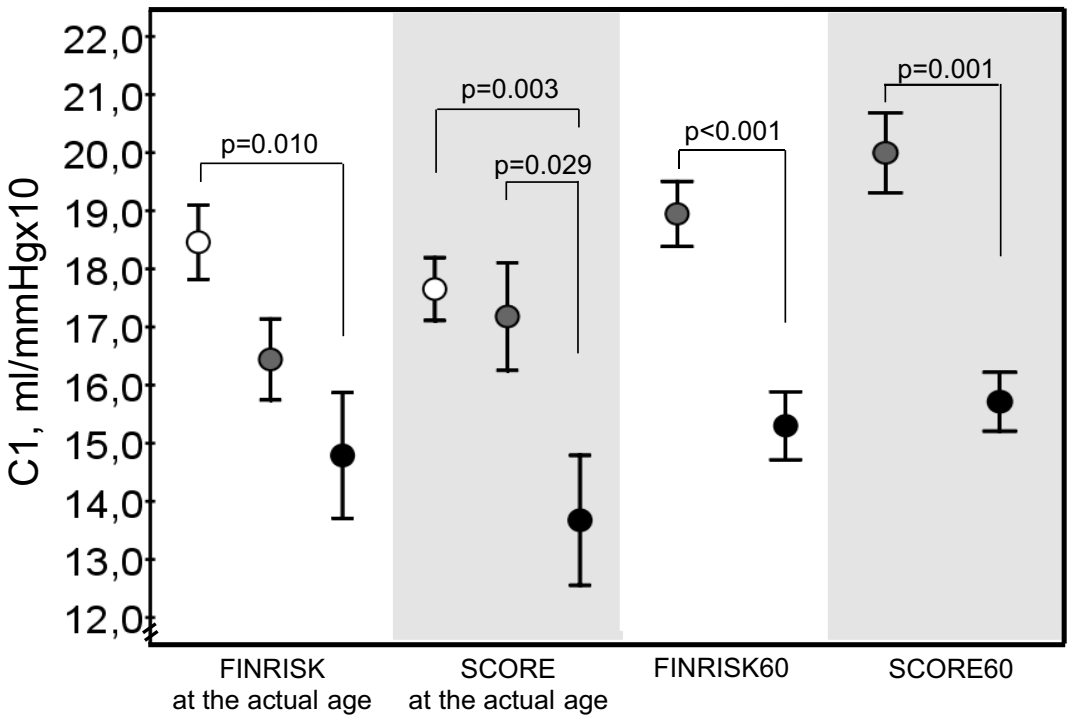

Figure 5. Large arterial elasticity (C1) among metabolic syndrome subjects with different estimated 10-year cardiovascular risk according to FINRISK and SCORE at the actual and at the projected age of 60 (FINRISK60, SCORE60). Mean ± SEM are presented.

○ Low risk (FINRISK < 5%, SCORE < 3%)

● Medium risk (FINRISK 5-14.99%, SCORE 3-4.99%)

● High risk (FINRISK ≥ 15%, SCORE ≥ 5%)

5.2 SMALL ARTERIAL ELASTICITY

There was no significant difference in C2 between MetS and PhA subjects (Figure 6) **(I, V).** MetS subjects with ED had lower C2 than those with normal erectile function, but the difference was not statistically significant (Figure 6) **(IV)**. Neither was there a difference between ED and non-ED MetS subjects among those with SCORE risk score < 5% **(IV)**. Among MetS and PhA subjects, C2 was lower among those with ED than among those with maximal erectile function score (Figure 6) **(V)**. The difference did not, however, remain significant after adjustment for age or beta-blocker medication.

Both high- and medium-risk subjects according to FINRISK at the actual age had significantly lower C2 compared to low-risk subjects (Figure 7) **(II)**. With an alternative risk score division of FINRISK at the actual age (low < 5%, medium 5-9.99% and high ≥ 10%), there was a significant difference between low- and medium-risk subjects in C2 (p=0.001). High-risk subjects according to SCORE at the actual age had significantly lower C2 compared to medium- and low-risk subjects (Figure 7) **(II)**. According to FINRISK60, high-risk subjects had impaired C2 compared with medium-risk subjects (Figure 7) **(II)**. There was no difference in C2 between medium- and high-risk subjects according to SCORE60 (Figure 7) **(II)**.

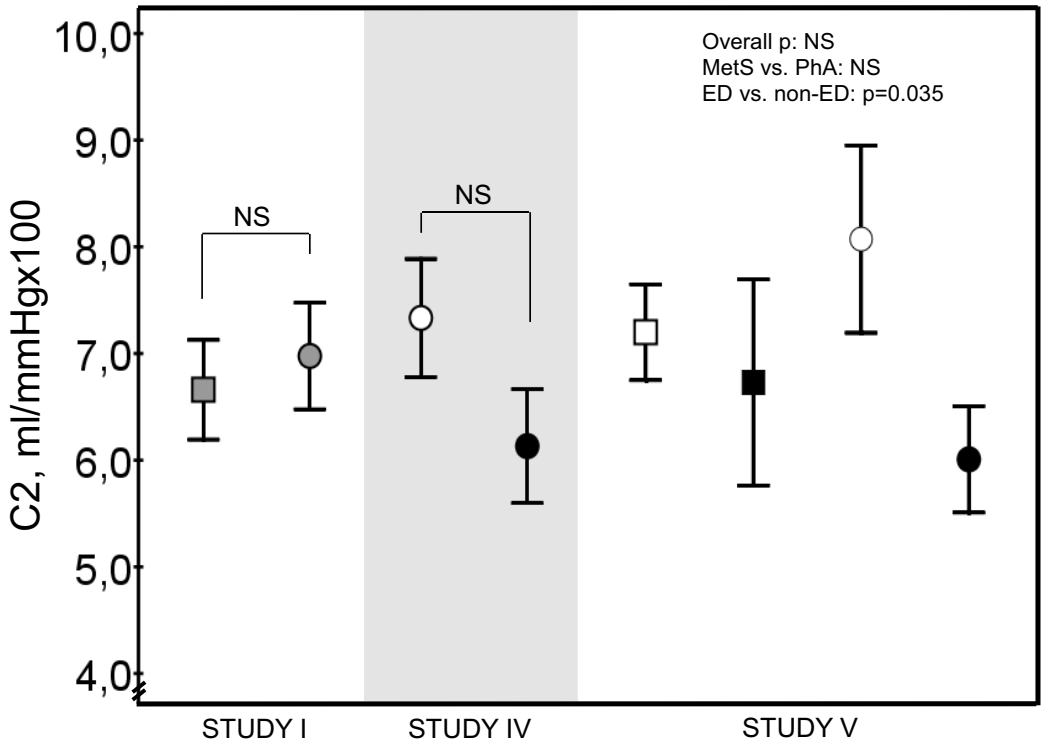

Figure 6. Small arterial elasticity (C2) among physically active (PhA) and metabolic syndrome (MetS) subjects and among MetS and PhA subjects with and without erectile dysfunction (ED). Mean ± SEM are presented.

Study I: PhA MetS
Study IV: MetS without ED MetS with ED
Study V: PhA with maximal IIEF score PhA with ED
MetS with maximal IIEF score MetS with ED

In the univariate analysis, C2 was inversely (OR 0.87, 95% CI 0.76-0.99, p=0.04) associated with the presence of ED, but it was not a significant predictor for the presence of ED in the multivariate analysis **(V)**. In the multivariate analysis of Study II, age (β = -0.22, p<0.001) and PP (β = -0.08, p=0.017) were inversely associated with C2. When SBP was selected separately for the model instead of PP, it was inversely associated with C2 (β=-0.05, p=0.006), whereas DBP was not a significant determinant. The presence of ED did not explain changes in C2 in the multivariate analyses of Study IV. There was no difference in C2 between subjects with three, four or five MetS variables **(II)** or between oil and butter periods **(III)**.

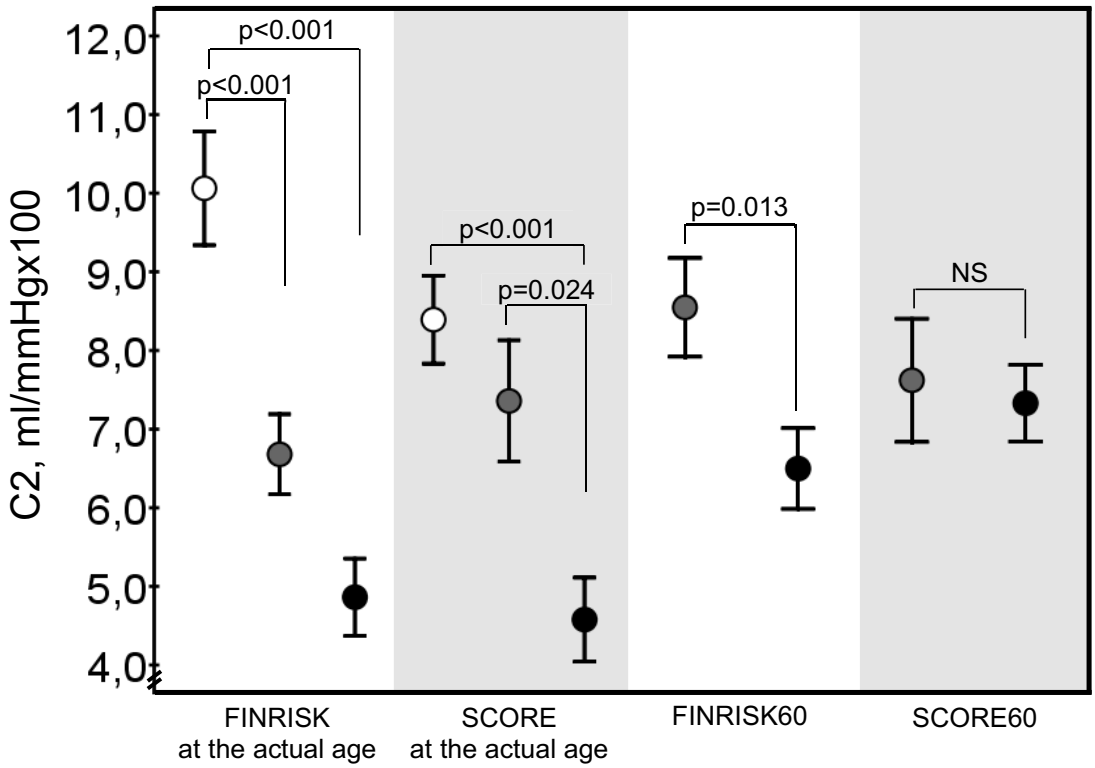

Figure 7. Small arterial elasticity (C2) among metabolic syndrome subjects with different estimated 10-year cardiovascular risk according to FINRISK and SCORE at the actual age and at the projected age of 60 (FINRISK60, SCORE60). Mean ± SEM are presented.

○ Low risk (FINRISK < 5%, SCORE < 3%)

● Medium risk (FINRISK 5-14.99%, SCORE 3-4.99%)

● High risk (FINRISK ≥ 15%, SCORE ≥ 5%)

5.3 OXIDIZED LDL

OxLDL levels were significantly higher among MetS than among PhA subjects (Figure 8) **(I)**. The difference remained significant even after adjustment for LDL-C (p=0.014) and physical activity (p=0.015) and smoking (p=0.004). In addition, oxLDL levels were 16% lower after the CPTRO period than after the butter period (Figure 8) **(III)**.

High-risk subjects according to FINRISK60 had higher oxLDL levels than medium-risk subjects (Figure 8) **(II)**. There was no difference in oxLDL between the risk groups according to either FINRISK and SCORE at the actual age or SCORE60. Although the differences did not reach statistical significance, oxLDL was higher among MetS subjects with ED than among those without (78.7 ± 30.8 U/L vs. 74.9 ± 35.6 U/L) **(IV),** and oxLDL had an increasing trend in the presence of ED and MetS (71.8 ± 21.4 U/L among PhA subjects without ED, 75.5 ± 35.5 U/L among PhA subjects with ED, 78.5 ± 40.1 U/L among MetS subjects without ED and 80.8 ± 33.5 U/L among MetS subjecst with ED) **(V)**.

In the multivariate analyses, LDL-C (β = 17.20, p<0.001) and triglycerides (β = 3.54, p=0.046) were directly and physical exercise (β = -0.04, p=0.016) inversely associated with oxLDL **(II)**.

When fibrinogen was added to the multivariate analyses, it was also a significant covariate in the final model of oxLDL (β = 9.52, p=0.018) together with the above-mentioned covariates. The presence of ED did not explain changes in oxLDL **(IV)**.

Total cholesterol was 8% and LDL-C 11% lower after the CPTRO period than after the butter period (Figure 9) **(III)**.

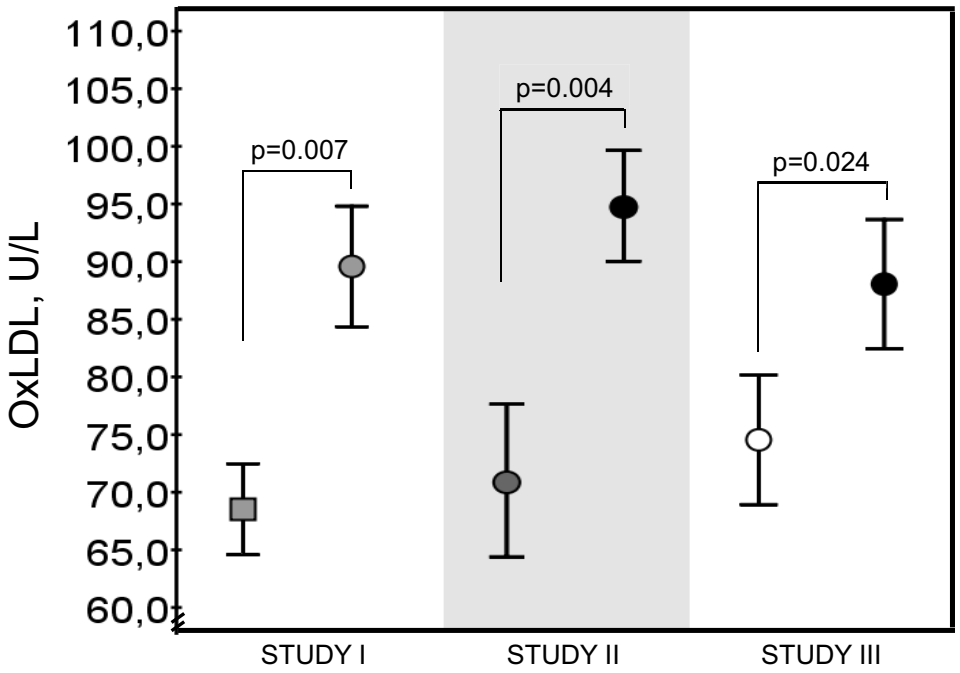

Figure 8. Oxidized LDL (oxLDL) among physically active (PhA) and metabolic syndrome (MetS) subjects, among MetS subjects with different estimated cardiovascular risk according to FINRISK at the projected age of 60 and among MetS subjects after CPTRO and butter periods. Mean ± SEM are presented.

Study I: PhA MetS
Study II: Medium risk (5-14.99%) High risk (≥ 15%)
Study III: After oil period After butter period

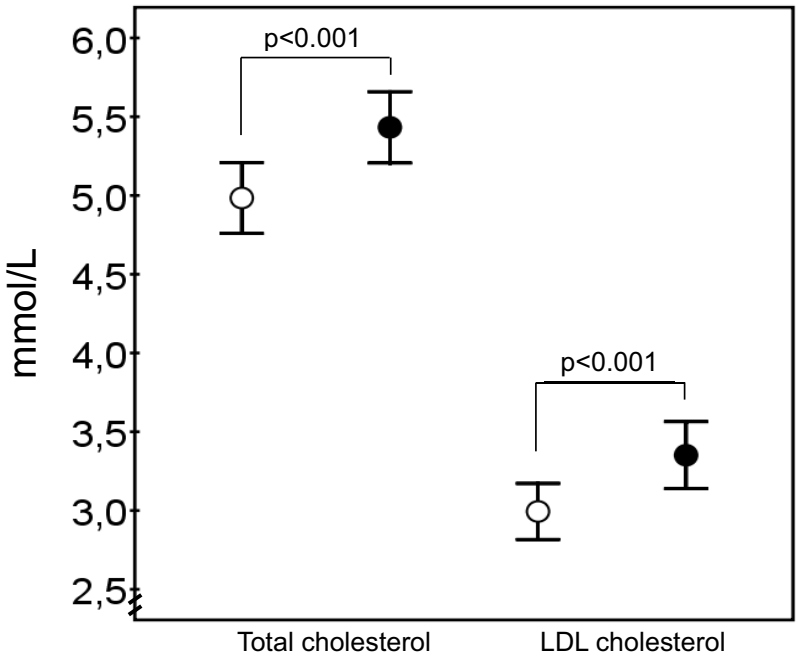

Figure 9. Total and LDL cholesterol among metabolic syndrome subjects after the CPTRO and butter periods. Mean ± SEM are presented.
Study III: ○ After oil period ● After butter period

5.4 PREDICTORS OF ERECTILE DYSFUNCTION

Of the MetS and PhA subjects participating in the HMS Research Program and without a possible non-vascular reason for ED, 73% and 81% completed the IIEF questionnaire. ED was more often present among MetS than among PhA subjects, 63.2% and 27.1%, respectively (p<0.001).

MetS was associated with the presence of ED even after adjustment for age, physical activity, smoking and total cholesterol (OR=5.83, 95% CI 1.730-19.618, p=0.004). The association of MetS and ED did not remain significant after adjustment for individual MetS components. Of the markers of subclinical atherosclerosis, both C1 (OR 0.88, 95% CI 0.79-0.97, p=0.01) and C2 (OR 0.87, 95% CI 0.76-0.99, p=0.04) were inversely associated, whereas fibrinogen (OR 2.83, 95% CI 1.52-5.27, p=0.001) and RHR were directly associated (OR 1.07, 95% CI 1.03-1.11, p=0.001) with the presence of ED.

In the multivariate analysis, age (OR 1.19, CI 95% 1.07-1.32, p=0.001), fibrinogen (OR 4.67, 95% CI 1.17-18.63, p = 0.029) and RHR (OR 1.07, 95% CI 1.003-1.14, p=0.040) were associated directly and physical activity > 400 kcal/day (OR 0.12, 95% CI 0.02-0.78, p=0.027) inversely with the presence of ED. The analysis was adjusted for smoking, family history of CVD, lipids, hypertension, diabetes, CVD, BMI and the use of selective beta-blockers, statins, ACE-inhibitors, ATR-blockers and diuretics.

In the multivariate analysis among MetS subjects only, age (OR 1.23, 95% CI 1.09-1.39, p=0.001) and fibrinogen (OR 4.30 95% CI 1.21-15.2, p=0.024) were associated directly and physical activity > 400 kcal/day (OR 0.05, 95% CI 0.004-0.65, p=0.022) inversely with the

presence of ED. These covariates were significant predictors of ED even after adjustment for smoking, diabetes, hypertension, CVD, LDL and HDL cholesterol, triglycerides, BMI, family history of CVD, and selective beta-blockers. Age was the only significant predictor of the presence of ED among PhA subjects (OR 1.16, 95% CI 1.04-1.30, p=0.008).

5.5 FIBRINOGEN

Fibrinogen levels were significantly higher among MetS than among PhA subjects (Figure 10) **(I, V)**. Subjects with high risk according to FINRISK60 had significantly higher fibrinogen levels than subjects with medium risk (Figure 10) **(II)**. There was no difference in fibrinogen levels between the risk groups according to FINRISK or SCORE at the actual age or SCORE60. MetS subjects with ED had higher fibrinogen levels than MetS subjects with a maximal IIEF score (Figure 10) **(V)**.

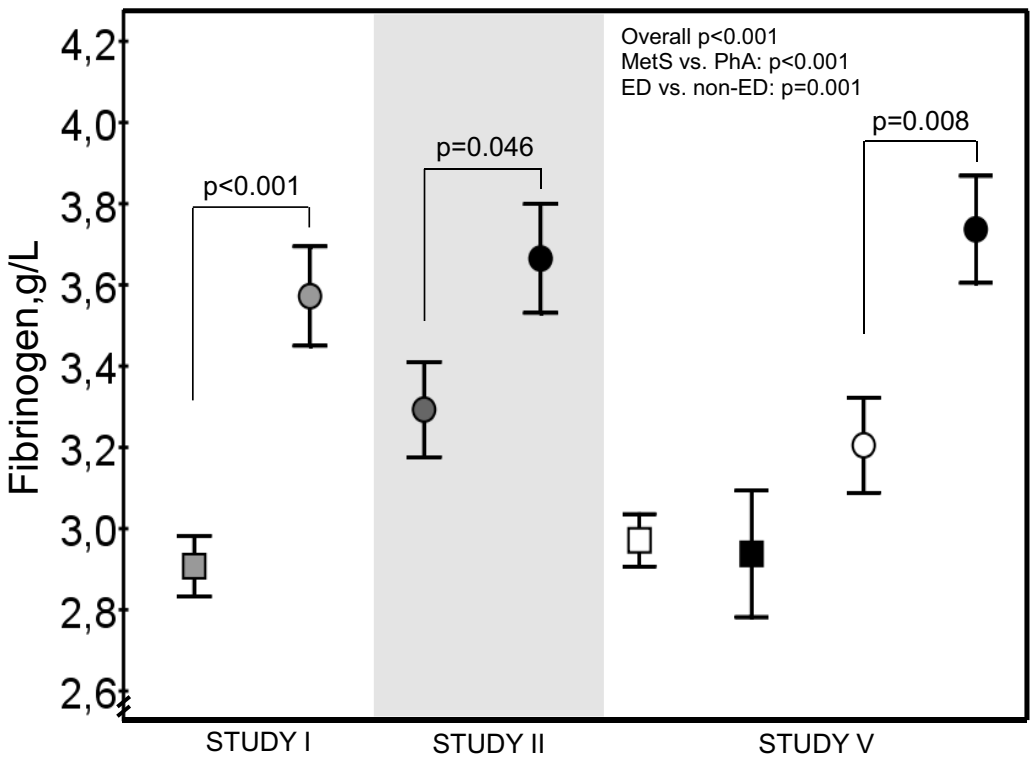

Figure 10. Fibrinogen among physically active (PhA) and metabolic syndrome (MetS) subjects, among MetS subjects with different estimated cardiovascular risk according to FINRISK at the projected age of 60 and among MetS and PhA subjects with and without erectile dysfunction (ED). Mean ± SEM are presented.

Study I: PhA MetS
Study II: Medium risk (5-14.99 %) High risk (≥ 15%)
Study V: PhA with maximal IIEF score PhA with ED
MetS with maximal IIEF score MetS with ED

As presented earlier in the results of oxLDL and ED, fibrinogen was directly associated with oxLDL in the adjusted multivariate analyses among all MetS subjects (β=9.52, p=0.018) **(II)**, and with the presence of ED among PhA and MetS (OR 4.67, p=0.029) and among MetS subjects only (OR 4.30, p=0.024) **(V).**

5.6 RESTING HEART RATE

RHR was higher among MetS than among PhA subjects (Figure 11) **(I, V)**. The difference remained significant even after adjustment for age and selective beta-blocker medication (p<0.001). MetS subjects with high risk according to FINRISK60 had higher RHR than those with medium risk (Figure 11) **(II)**. There was no difference in RHR between the risk groups according to FINRISK or SCORE at the actual age or SCORE60. RHR was also higher among MetS subjects with ED than among those without ED, 68.1 ± 10.3 beats/min *vs.* 63.3 ± 8.7 beats/min, respectively (p=0.004) **(IV)**. However, the difference did not remain significant after

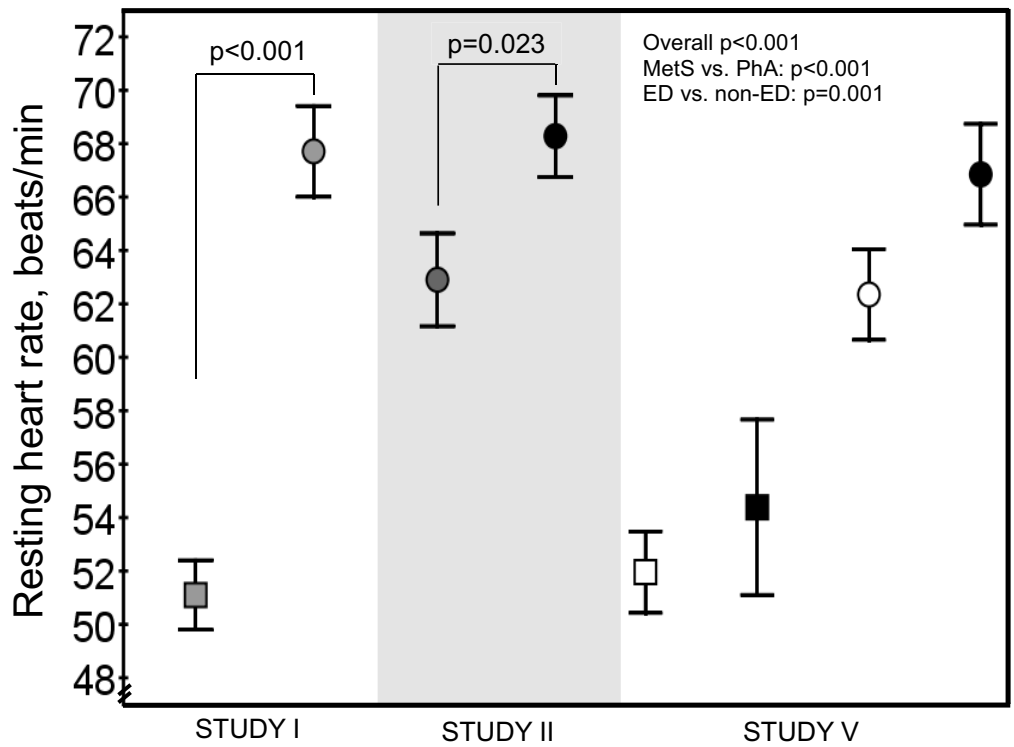

Figure 11. Resting heart rate among physically active (PhA) and metabolic syndrome (MetS) subjects, among MetS subjects with different estimated cardiovascular risk according to FINRISK at the projected age of 60 and among MetS and PhA subjects with and without erectile dysfunction (ED). Mean ± SEM are presented.

Study I: PhA MetS
Study II: Medium risk (5-14.99%) High risk (≥ 15%)
Study V: PhA with maximal IIEF score PhA with ED
MetS with maximal IIEF score MetS with ED

adjustment for beta-blocker medication. RHR was also higher among PhA and MetS subjects with ED than among those with a maximal IIEF score (Figure 11) **(V)**. The difference remained significant even after adjustment for age and selective beta-blocker medication (p=0.001).

As presented earlier in the results of C1 and ED, RHR was associated inversely with C1 in the adjusted multivariate analyses among all MetS subjects (β = -0.12, p<0.001), and directly with the presence of ED (OR 1.07, p=0.040) among PhA and MetS subjects **(V)**. RHR was not associated with C2 or oxLDL levels.

6 Discussion

In this study, markers of subclinical atherosclerosis were associated with the presence of MetS, and the presence of high estimated 10-year CVD risk and ED among MetS. The number of MetS variables was not associated with the markers. In particular, MetS subjects had impaired C1 and increased levels of oxLDL, fibrinogen and RHR compared with PhA subjects. Among men with MetS, those at high estimated 10-year CVD risk according to FINRISK60 had impaired C1 and C2 as well as increased levels of oxLDL, fibrinogen and RHR compared with those at medium risk. FINRISK model at the projected age of 60 was superior to FINRISK at the actual age and SCORE in differentiating MetS subjects with respect to markers of subclinical atherosclerosis. OxLDL was lower after the intervention period with CPTRO than after the butter period. MetS subjects with ED had impaired C1, increased fibrinogen and elevated RHR compared with those not having ED. Also among MetS subjects with SCORE risk score < 5%, C1 was lower among those with ED than among those without. In addition, impaired C1 and increased fibrinogen were associated with the presence of ED in MetS, independently of traditional CVD risk factors. Physical activity, on the other hand, was associated with the presence of normal erectile function among MetS subjects.

6.1 ARTERIAL ELASTICITY AND OXIDIZED LDL IN THE PRESENCE OF METABOLIC SYNDROME AND HIGH 10-YEAR CARDIOVASCULAR RISK

6.1.1 Large arterial elasticity

MetS subjects had significantly lower C1 compared with PhA subjects. This is in agreement with two previous studies reporting impaired C1 among MetS, assessed by the same pulse wave analysis as in the present study (Fjeldstad et al. 2007, Ge et al. 2008). However, the previous results among Chinese MetS subjects cannot be generalized to Caucasians (Ge et al. 2008). In addition, the study by Ge et al (2008) included subjects with ACEi and ATRb medications, possible contributors to arterial elasticity. In our study, the use of these medications was an exclusion criterion. In the study by Fjeldstad et al (2007) impaired C1 was found among Caucasian MetS subjects but the definition of MetS was based only on waist circumference and blood pressure.

Also in line with our finding, a recent study assessing arterial stiffness by PWV reported large arterial dysfunction among MetS compared with healthy controls, even in the absence of diabetes (Czernichow et al. 2010). In another study, arterial stiffness was associated with MetS defined according to the NCEP, which was used also in the present study, but not according to the IDF definition (Lin et al. 2009). On the contrary, in a study among diabetic patients there was a significant difference in PWV between MetS and non-MetS subjects according to the IDF definition, but not according to the NCEP definition (Levisianou et al. 2009). Also in another study among diabetic patients, MetS *per se* was not associated with a reduction in aortic distensibility (Tentolouris et al. 2008). All subjects in the contradicting studies, but only minority of subjects in the present study, had type 2 diabetes. In addition, arterial function was assessed by different methods than in the present study (Levisianou et al. 2009, Tentolouris et al. 2008).

Regular physical exercise at low-intensity may attenuate the age-related reduction in large arterial elasticity (Sugawara et al. 2004). However, a greater reduction in central arterial compliance has been reported among resistance-trained men exercising at vigorous level than among sedentary men (Miyachi et al. 2003 &2004). In the present study, a number of PhA subjects reported regular exercise at a vigorous level. Since the difference in C1 between MetS

and PhA subjects remained significant even after adjustment for physical activity, we believe that the amount and level of exercise among PhA subjects did not markedly interfere with the results.

MetS subjects at a high estimated 10-year CVD risk by both FINRISK and SCORE models at the actual and at the extrapolated age of 60 had impaired C1 compared with those at lower risk. It is intriguing that impairment in elastic properties of large arteries was evident in the presence of statistically estimated high risk, but in the absence of CVD symptoms. Our finding supports a previous study reporting an association between increased PWV and Framingham risk score (Hae Guen Song et al. 2010). There are, however, no previous reports on the association between impaired arterial elasticity and FINRISK or SCORE risk score among MetS.

Obesity, elevated glucose levels and hypertension have previously been reported to correlate with impaired large arterial elasticity (Fjeldstad et al. 2007, Ge et al. 2008). Accordingly, diabetes and PP were associated negatively with C1 in the multivariate analysis of the present study. Since subjects at high estimated 10-year CVD risk according to FINRISK more often had diabetes and higher SBP levels than those at medium risk, these risk factors may explain a great part of the difference in C1 between the groups. However, after adjustment for the presence of prediabetes/diabetes or blood pressure, the difference in C1 remained significant. Thus, an aggressive management of other CVD risk factors in MetS should be considered. Since all our study subjects had MetS, the presence and magnitude of CVD risk factors, in addition to those included in the MetS criteria, must have contributed to the deterioration of large arterial elasticity.

An elevated PP is known to reflect the increased stiffness of large arteries (McVeigh et al 2002). In a large prospective study among healthy subjects, high PP was associated with an increased CVD risk among men aged 45 to 57 years (Domanski et al. 2002). In a study among middle-aged and elderly individuals, CHD risk was associated with lower DBP at any level of SBP ≥ 120 mmHg (Franklin 1999). In agreement, we found a better correlation between elevated PP and impaired C1 than between SBP and C1 among middle-aged men with MetS.

Previously large arterial stiffness has been found to be associated with future CVD events (Boutouyrie et al. 2002, Meaume et al. 2001). The impaired C1 found among the MetS subjects may therefore contribute to the increased risk of CVD connected to MetS. However, the predictive value of MetS with respect to incident CVD has been reported to be inferior to the Framingham score or to provide little information on risk when used as an additional variable to the Framingham risk score (McNeill et al. 2005, Stern et al. 2004). Similarly, our findings suggest that CVD risk calculators are able to identify MetS subjects at high CVD risk, additive to the presence of MetS only. Whether arterial elasticity measurement is superior or of additional benefit to risk score models in predicting future CVD events in MetS, needs to be elucidated in a prospective study.

6.1.2 Small arterial elasticity

C2 is believed to reflect the endothelial function of the microvascular circulation that is more prone to the development of atherosclerosis (Cohn 1999). In addition, hypertension, diabetes and smoking, markedly more often present among MetS than PhA subjects in the present study, have been reported to impair especially C2 (Cohn et al.1995, McVeigh et al. 1991&1993, Ge et al. 2008). Furthermore, subjects with medications that may improve arterial elasticity were excluded from Study I (Cohn 2000, Leibovitz et al. 2001, Nashar et al. 2004). Despite these contributing factors, we found no difference in C2 between MetS and Pha subjects, which disagrees with a study assessing impaired C2 by the same method in Chinese MetS subjects (Ge et al. 2008). However, our study agrees with previous studies reporting MetS to preferentially affect the central rather than the peripheral parts of the arteries (Plantinga et al. 2008, Stehouwer et al. 2008). Similarly, impaired central rather than peripheral arterial elasticity was detected among type 2 diabetic patients with ischaemic CHD in a study by Hatsuda et al (2006). Thus,

our finding further supports the theory of impairment in the elastic properties of particularly large arteries to be the connection between MetS and increased CVD risk.

C2 was significantly lower among MetS subjects at high estimated 10-year CVD risk than among those those at lower risk according to all other risk score estimates except SCORE60. Subjects with statin medication were excluded from the risk estimate analyses, and there was no significant difference in the use of ACEi- or ATRb-medications between the risk groups. Thus, medications that may improve C2 did not affect the results. As there was no difference in C2 between MetS and PhA subjects, the presence and magnitude of CVD risk factors included in the risk score models seem to contribute to the impairment of elastic properties at the level of small arteries.

Especially blood pressure, but also age and gender have been reported to determine peripheral vascular function (Fjeldstad et al. 2007, Ge et al 2008, Plantinga et al. 2006). In agreement, an increase in age and SPB or PP predicted impaired C2 in the present study. Since subjects at high estimated CVD risk were older and had higher SBP than those at lower risk, these factors may explain a great part of the difference in C2 between the risk groups. However, there are also previous reports on the association between plasma glucose or HDL-C concentration and C2, assessed by the same method as in the present study (Ge et al. 2008, Resnick et al. 2000). We excluded subjects with diagnosed CVD and statin from the risk estimate analyses. In addition, since subjects at high estimated CVD risk more often had diabetes and a trend for higher lipid levels than those at lower risk, it cannot be concluded which of the CVD risk factors contributed to the impairment in C2 between different risk groups.

6.1.3 Oxidized LDL

OxLDL levels were significantly higher among MetS than PhA subjects. This finding is in agreement with number of previous studies, although study populations, MetS definitions and laboratory techniques in these reports were different from the ones of the present study (Holvoet et al. 2004, Lapointe et al. 2007, Sigurdardottir et al. 2002, Ueba et al. 2009, Valle Gottlieb et al. 2010). Our finding disagrees with a study by Sjögren et al (2005). They found no difference in oxLDL levels between MetS subjects and healthy controls. A possible reason for this inconsistent finding might be the small number and percentage of MetS subjects in their study.

Smoking and alcohol consumption have been reported to relate to elevated oxLDL levels, whereas physical activity may improve the resistance of LDL against oxidation (Schröder et al. 2006, Talmud et al. 2005, Ziegler et al. 2006). In our study, MetS subjects were more often smokers, less active physically, and they used more alcohol than PhA subjects, which may contribute to the increased oxLDL. However, the difference remained significant after adjustment for the amount of physical activity. In addition, exercise at vigorous level, quite often reported by the PhA subjects in the present study, may actually induce oxidative stress acutely (Muñoz et al. 2010).

MetS subjects at high risk according to FINRISK60 had elevated oxLDL levels compared with subjects at medium risk. This finding supports previous studies reporting an association between increased oxLDL levels and Framingham risk score (Holvoet et al. 2003, Ueba et al. 2009). Since elevated oxLDL levels are associated with type 2 diabetes (Njoujou et al. 2009), the higher number of diabetic subjects among those at high risk may at least partly explain the difference in oxLDL. On the other hand, since SCORE risk model does not take into account the presence of diabetes, it may explain why there was no difference in oxLDL levels between the risk groups according to SCORE.

Triglycerides are known to boost the production of small, dense LDL particles more prone to oxidation, which in turn has previously been implicated as a link between elevated levels of oxLDL and MetS (Sigurdardottir et al. 2002). Regular aerobic exercise, on the other hand, may

reduce the level of oxidative stress (Elosua et al. 2003). In agreement, physical inactivity, LDL-C and triglycerides related positively to increased oxLDL among MetS subjects in Study II.

Oxidative stress is substantial in the development of atherosclerosis (Madamanchi et al. 2005, Stocker&Keaney 2004). Increased oxLDL among MetS subjects in the present study may reflect the increased systemic oxidative stress previously reported to be associated with MetS (Hansel et al. 2004). Since increased circulating oxLDL seems to be associated with established CHD, atherosclerotic plaque growth and acute coronary syndrome (Holvoet et al. 2001&2004, Hulthe&Fagerberg 2002, Meisinger et al. 2005), oxLDL formation may be another mechanism explaining the increased CVD risk in MetS. In MetS, increased oxLDL levels have previously been reported to predict the incidence of myocardial infarction (Holvoet et al. 2004). Whether assessment of circulating oxLDL is superior to or of additional benefit to risk score models in predicting incident CVD events among MetS need to be elucidated in a prospective study.

6.2 ARTERIAL ELASTICITY AND OXIDIZED LDL IN THE PRESENCE OF DIFFERENT NUMBER OF METS VARIABLES

There was no difference in C1, C2 or oxLDL levels between men with three, four or five MetS variables. This finding disagrees with some previous studies. Nakanishi et al (2003) reported an association between clustering of MetS variables and increased PWV. In a study by Hamburg et al (2008) impaired arterial flow-mediated function was associated with increasing number of MetS variables. In addition, oxLDL was higher among subjects with five MetS components than among those with only three in a study by Lapointe et al (2007).

However, Koskinen et al (2009) found no difference in carotid IMT progression between subjects with three and four to five MetS components. This is in line with our study as IMT is also considered to be a marker of subclinical atherosclerosis. In addition, large arterial stiffness, assessed by PWV, was associated with increasing traits of MetS only among women in a previous study by Ferreira et al (2007). Furthermore, in a large follow-up study, there was no clear association between the number of MetS variables and mortality (Kuk&Ardern 2010). The variation of findings may be caused by different study populations, MetS definitions or methods used.

In addition, the presence of different combinations of MetS variables may explain the discrepancy of the results (Guize et al. 2007, Isomaa et al. 2001, Stehouwer et al. 2008). For example, a combination of elevated blood pressure, altered glucose tolerance and obesity has been found to be associated with increased arterial stiffness (Stehouwer et al. 2008). Clustering of these risk factors has also been reported to be associated with the greatest mortality risk, whereas some combinations seem to pose a risk similar to that among non-MetS subjects (Guize et al. 2007, Isomaa et al. 2001, Moebus et al. 2010). Thus, instead of the effect of different number, it might be more appropriate to study the effect of different combinations of MetS variables. We did not assess the different MetS combinations in association with oxLDL and arterial elasticity, in part because of the relatively small number of MetS subjects.

6.3 THE EFFECT OF COLD-PRESSED TURNIP RAPESEED OIL ON OXIDIZED LDL AND ARTERIAL ELASTICITY

OxLDL and total- and LDL-C levels were significantly lower after the intervention period with CPTRO than after the butter period. The lower level of total cholesterol was due to the lower level of LDL-C. However, the concentration of circulating oxLDL was proportionally markedly lower than that of LDL-C after the CPTRO period.

In the Nordic countries, turnip rapeseed oil is an important source of unsaturated fatty acids. In contrast, butter consists mainly of SFA. Compared with olive oil, common in the Mediterranean region, Virgino^{R} turnip rapeseed oil contains less MUFA and SFA but a considerable amount of n-6 PUFA, *i.e.* linoleic acid and plant-derived n-3 PUFA, *i.e.* ALA. Since CPTRO, supplemented in the present study, contained only slight amounts of phenolic compounds, also known to affect CVD risk markers (Attorri et al. 2010, Cicerale et al. 2010), the effects of CPTRO were presumably mediated mainly by the high contents of MUFA and PUFA.

The significant reductions in total and LDL-C levels are in accordance with earlier rapeseed oil studies (Seppänen-Laakso et al. 1993, Södergren et al. 2001, Valsta et al. 1992) as well as with a recent randomized and controlled study on the effects of 6 week Nordic diet rich in rapeseed oil (Adamsson et al. 2011). In addition, in agreement with most of the previous studies (Gulesserian&Widhalm 2002, Södergren et al. 2001, Valsta et al. 1992, Vermunt et al. 2001) the use of CPTRO did not have significant effect on HDL-C levels.

LDL particles enriched with MUFA seem to be resistant against oxidation (Cicero et al. 2008, Kratz et al. 2002, Moreno et al. 2008). Since MUFA are the main fatty acids in CPTRO, their protective effect presumably contribute to the decreased level of oxLDL after the CPTRO-enriched diet in the present study. Although the effects of CPTRO in MetS have not been studied earlier, a high MUFA-rich diet has been reported to improve oxidative stress parameters and decrease oxLDL levels among healthy non-obese men as well as among MetS and other high CVD risk subjects (Egert et al. 2011, Fitó et al. 2007, Jones et al. 2011, Perez-Martinez et al. 2010). Also in line with our study, MUFA-rich virgin olive oil protected LDL against oxidation among type 2 diabetic patients in a study by Perona et al (2009). However, in disagreement with our findings, Södergren et al (2001) did not find a difference in the degree of lipid peroxidation between rapeseed oil-based and SFA-rich diets. However, they reported similar reductions in lipid levels as we did. The smaller study population or shorter intervention period in their study, or different methods used may explain the difference between the findings.

The previous findings regarding the effects of linoleic acid, and ALA, the other main constituents of CPTRO, are discordant (Djoussé et al. 2001, Kratz et al. 2002, Schwab et al. 1998, Yam et al. 1996). LDL susceptibility to oxidation has been assumed to be associated with an increased amount of PUFA in the LDL particles (De Graaf et al. 1991). In a study by Schwab et al (1998) a PUFA-rich diet increased susceptibility of LDL to oxidation when compared to a MUFA-rich diet among subjects with IGT. However, rapeseed oil-derived n-3 PUFA did not increase LDL oxidizability when provided in a context of a diet rich in MUFA (Egert et al. 2007 Kratz et al. 2002). In addition, the adverse effects of n-3 PUFA seem to be associated with large amounts (> 20 g/day), whereas the daily amount of ALA in the present study was only 4.2 g (Nestel et al. 1997). Since CPTRO, used in the present study, consists of considerable amounts of MUFA as well as n-6 and n-3 PUFA, the individual effects of these fatty acids on oxLDL levels cannot be assessed.

Increased circulating oxLDL levels have been reported to be associated with subclinical and clinical atherosclerosis and to predict future CVD events (Holvoet et al. 1998, Hulthe&Fagerberg 2002, Meisinger et al. 2005). Supplementing CPTRO to a habitual diet seems to protect LDL against oxidation compared with butter. CPTRO supplement may thus delay the development of atherosclerotic lesions and decrease the risk of CVD events among men with MetS.

In several recent studies, plant-derived n-3-PUFA, has been found to improve endothelial function among healthy individuals and arterial compliance among MetS, diabetic, hypertensive and overweight patients (Egert&Stehle 2011, Nestel et al. 1997, Pase et al. 2011). In disagreement, there was no difference in C1 or C2 after the intervention periods in the present study. One explanation might be that the intervention period was too short to affect the arterial function. However, even one meal supplemented with walnuts rich in n-3-PUFA has been found to improve brachial FMD as a sign of improved endothelial function (Cortés et al. 2006).

On the other hand, the same effect was not seen after a meal supplemented with MUFA-rich olive oil in the same study (Cortés et al. 2006). Differences in fat type, study groups and methods used to evaluate arterial function may explain the discrepancy between our study and the previous ones.

6.4 ERECTILE DYSFUNCTION

6.4.1 Association with arterial elasticity and oxidized LDL

We found significantly lower C1 among MetS subjects with ED than among those without ED. Similarly, C1 was lower in the presence of ED among both PhA and MetS subjects and was lowest among men with both MetS and ED. To our knowledge, there are no previous reports on the association between ED and impaired large arterial elasticity among MetS subjects. However, our finding is in line with previous studies reporting an association between impaired aortic strain and distensibility indices and ED among men without increased CVD risk or established CVD (Kaya et al. 2007, Uslu et al. 2006). In addition, a higher carotid-femoral PWV has been reported among men with ED and hypertension (Vlachopoulos et al. 2008). Since large arterial stiffness seems to be associated with atherosclerosis and future coronary events (Boutouyrie et al. 2002, Meaume et al. 2001), our findings support the hypothesis of ED being an early sign of underlying CVD.

MetS subjects with ED in Study IV were older, more often diagnosed with type 2 diabetes or hypertension and were more often on beta-blocker medication than those without ED. Although age was the only difference in clinical characteristics between ED and non-ED MetS subjects that reached statistical significance, the above-mentioned risk factors of ED and impaired arterial elasticity may have affected the results. However, the difference in C1 remained significant even after adjustment for these risk factors. In addition, in the multivariate analysis, the presence of ED was associated with impaired C1 independently of traditional risk factors and beta-blocker medication. In line with this finding, Araujo et al (2010) has reported an association between ED and increased CVD incidence, independent of established CVD risk factors.

Aggressive primary prevention is recommended for those with an estimated 10-year risk of cardiovascular death ≥ 5% according to the SCORE risk model (Fourth Joint Task Force of the ESC 2007). In the analyses among MetS subjects at low risk of CVD death, we intriguingly found impaired C1 among those with ED than among those without. Therefore, we suggest that assessment of ED should be part of patient's total CVD risk estimation. Active primary prevention should be considered for MetS subjects with suspected vasculogenic ED, irrespectively of the 10-year CVD risk calculation.

Endothelial dysfunction seems to be a key mechanism in the pathogenesis of ED and atherosclerotic CVD (Kirby et al. 2005, Stocker&Keaney 2004). C2, assessed by pulse wave analysis in our study, reflects the systemic endothelial function of the microvascular circulation (Cohn 1999, Laurent et al. 2006). In the present study, there was a non-significant trend for C2 to be decreased among ED subjects. In addition, C2 was associated with the presence of ED but the difference did not remain significant after adjustments, similar to a study using the same method to assess arterial stiffness (Prisant et al. 2006). However, in other previous studies, a significant association between endothelial dysfunction, assessed by a regional measurement of brachial FMD, and ED has been reported (Elesber et al. 2006, Kaiser et al. 2004, Uslu et al. 2006, Yavuzgil et al. 2005).

C2 is not a direct measure of endothelial function since it is also affected by alterations in the elastic properties of the arterial wall (Cohn et al. 1995, McVeigh et al. 2002). Accordingly, in a study by Eskurza et al (2001) brachial FMD did not correlate with the function of resistance arteries in the microvascular circulation. In addition, penile arteries, which are one to two

millimetres in diameter, are considered to be large arteries in the pulse wave analysis (Cohn 1999). Thus, besides the relatively small number of study subjects, the method used may explain why there was no significant difference in C2 between ED and non-ED subjects in the present study.

Although there are no previous reports on oxLDL levels among ED subjects, increased oxidative stress has been reported to be associated both with ED and MetS (Barassi et al. 2009, Njajou et al. 2009, Sigurdardottir et al. 2002). Since all subjects in Study IV had MetS, they were assumed to have increased oxLDL because of the metabolic abnormalities. Thus, the highly selected study group may explain, why oxLDL levels among MetS subjects with and without ED were similar. In the present study, physical activity at a high level was associated with normal erectile function, but has previously been reported to acutely increase oxidative stress (Muñoz et al. 2010). Thus, the high level of physical exercise among non-ED subjects may partly explain why the increasing trend of oxLDL in the presence of ED among PhA and MetS subjects of the Study V was not significant.

6.4.2 Prevalence of erectile dysfunction and association with physical activity

ED was highly prevalent among MetS, whereas PhA subjects often had a maximal IIEF score. The presence of ED among PhA subjects was lower than expected, although subjects with decreased IIEF score, still considered as normal erectile function, were excluded (Teles et al. 2008). PhA subjects had a healthier lifestyle on the whole. They were more seldom current or ex-smokers, used significantly less alcohol and were markedly more active physically than MetS subjects. Since the MetS definition includes abdominal obesity, PhA subjects also had lower BMI and waist circumference. Both obesity and smoking have been reported to be associated with ED (Bacon et al. 2006). Although the effect of alcohol consumption on the risk of ED is unclear according to previous studies (Horansanli et al. 2008, Kalter-Leibovici et al. 2005), the overall healthy behaviour of the PhA subjects must have contributed to the high prevalence of normal erectile function among them.

A high prevalence of ED in MetS has also been reported previously (Bal et al. 2007). The prevalence of ED among diabetic and CVD patients is known to be even higher (Montorsi et al. 2005, Selvin et al. 2007). Accordingly, diabetes and CVD were more often present among MetS subjects with ED than among those without. ED subjects were also older and more often hypertensive, which may also contribute to the number of ED subjects, and thereby to the results of the study.

MetS subjects with ED were more often on selective beta-blockers, which have been suspected to impair sexual function (Ko et al. 2002). However, the actual risk of sexual dysfunction caused by beta-blockers was reported to be low in a large review of randomized trials (Ko et al. 2002). In addition, the results of the present study were adjusted for the use of selective beta-blockers, whereas subjects on non-selective beta-blockers were excluded. Furthermore, MetS subjects with ED were also more often on medications known to improve sexual function (Doğru et al. 2008, Düsing et al. 2003). Thus, the high use of medications among subjects with both MetS and ED and the high prevalence of ED among MetS, may reflect the presence of metabolic diseases and the underlying atherosclerotic process.

Physical exercise at a level of > 400 kcal/day was associated with a maximal IIEF score among all participants as well as among only MetS subjects. The significance of this finding remained even after adjustment for multiple CVD risk factors and beta-blockers. In addition, men with a maximal IIEF score were physically more active than ED subjects in Study V. There are no previous reports on the positive association between physical activity and the presence of normal erectile function in MetS. However, our finding is in line with previous studies reporting a beneficial effect of regular physical exercise in preserving normal erectile function (Bacon et al 2006, Esposito et al. 2004, Kratzik et al. 2009). Previously, physical exercise has also been reported to protect from developing MetS and type 2 diabetes (Laaksonen et al.

2002&2005). Our findings further support the importance of regular physical exercise and otherwise healthy lifestyle in the management of the increasing number of people with overweight, MetS and concomitant CVD.

6.5 FINDINGS REGARDING FIBRINOGEN AND RESTING HEART RATE

6.5.1 Fibrinogen

In line with previous studies, fibrinogen levels were significantly higher among MetS than PhA subjects (Church et al. 2002, Ford et al. 2003, Ma et al. 2010). Physical activity and cardiorespiratory fitness (Lakka et al. 1993, Myint et al. 2008) have been reported to be associated with decreased fibrinogen levels, whereas increased fibrinogen levels seem to be associated with obesity and smoking (Church et al 2002, Sinha et al 2005). Thus, physical inactivity, higher prevalence of smoking and general physical unfitness among MetS subjects must have contributed to the higher fibrinogen levels.

However, fibrinogen was associated positively with oxLDL levels, independently of traditional CVD risk factors and physical activity. In addition, MetS subjects at high estimated CVD risk according to FINRISK60 had higher fibrinogen levels than those at medium risk, although there was no difference in the above-mentioned risk factors between the groups. This is in agreement with a previous study reporting increased fibrinogen among high risk subjects according to the Framingham risk score (Park et al. 2010).

Among MetS subjects, fibrinogen levels were significantly higher in the presence of ED than in the presence of maximal IIEF score. In addition, increased fibrinogen was associated with the presence of ED, independently of traditional CVD risk factors among MetS but not PhA subjects. There are no previous reports on the association between increased fibrinogen and ED among MetS. However, Vlachopoulos et al (2006) have reported an independent predictive value of fibrinogen for the presence of ED both among men with and without CHD.

In a large individual meta-analysis, there was a moderately strong association between increased fibrinogen levels and incident CVD events and mortality (Fibrinogen Studies Collaboration 2005). Thus, increased fibrinogen may partly explain the increased CVD risk associated both with MetS and with ED. Especially men with MetS and coexisting ED should be considered at high risk for CVD events.

6.5.2 Resting heart rate

In agreement with previous studies, RHR was higher among MetS than PhA subjects (Pannier et al. 2006, Rana et al. 2010). Since MetS subjects were sedentary, their physical unfitness probably contributed to their higher RHR. However, elevated RHR was associated independently with lower C1 among MetS subjects in Study II. Our findings support a previous study reporting a connection between adrenergic overdrive and MetS (Mancia et al. 2007). Adrenergic stimulation causes increased peak blood flow and thus shear stress in the endothelium (Arnold et al. 2008, Mancia et al. 2007). This, in turn, results in endothelial dysfunction and impaired arterial elasticity (Arnold et al. 2008). Although there are no previous reports on the association between RHR and C1, elevated RHR has previously been connected to large arterial stiffness, assessed by PWV, as well as to higher IMT in studies among general population (Chen et al. 2008, Park et al. 2010, Tomiyama et al. 2010).

In the present study, an elevated RHR was detected among MetS subjects with ED compared to those without. The higher use of beta-blockers among these subjects may have contributed to the number of subjects with ED, but would also be expected to lower RHR. However, elevated RHR was associated with the presence of ED, independently of traditional CVD risk factors, physical activity, and beta-blocker medication, which also supports the suggested pathophysiological connection between RHR and endothelial dysfunction (Arnold et al. 2008,

Mancia et al. 2007).

MetS subjects at high CVD risk according to FINRISK60 had a higher RHR than those at medium risk, even though there was no significant difference in factors affecting RHR such as physical activity, waist circumference, BMI or the use of beta-blockers. However, we did not assess the physical fitness of the study subjects, a major contributor to RHR. In a previous study among FINRISK population, inclusion of RHR to risk estimate formulas already containing lipids and blood pressure, did not markedly improve risk estimation (Cooney et al. 2010). Whether the results of that study hold true also among MetS subjects is not known.

Our findings imply that elevated RHR might serve as a sign of subclinical atherosclerosis in MetS. Hence, our results are in line with previous studies reporting elevated RHR as an independent risk factor for CVD events (Cooney et al. 2010).

6.6 METHODOLOGICAL ISSUES

6.6.1 Patient recruitment and study design

Study subjects were not a randomly selected population-based sample, which was a limitation of the study. In addition, since only men were recruited, and Studies II, III and IV included only MetS subjects, the results cannot be generalized to women and subjects without MetS. Furthermore, the relatively small number of study subjects must be taken in account in the evaluation of the non-significant results.

Since there were no healthy control subjects in Studies II, III and IV, the effect of the MetS *per se* could not be assessed in these study settings. However, the main aims of Studies II and IV was to compare the differences among MetS subjects, defined by the presence of different CVD risk or ED, not the differences between MetS and non-MetS subjects. Accordingly, in Study III we wanted to focus on MetS subjects because of their increased CVD risk. However, baseline measurements of lipids and oxLDL or an intervention period with placebo would have been extremely beneficial in evaluating the effect of CPTRO supplement in Study III.

Sedentary subjects with atherosclerotic disease may not experience CVD symptoms because of lack of physical activity. To ensure that PhA subjects did not have obstructive atherosclerotic disease, participation of PhA subjects was only allowed if they exercised physically more than three times a week and more than 30 minutes per bout of exercise. Despite this relatively low minimum demand, many PhA subjects reported regular physical exercise of high amounts at vigorous intensity. Since the previous reports on the effect of different intensity levels of exercise on arterial elasticity and oxidative stress are discordant (Elosua et al. 2003, Miyachi et al. 2004, Muñoz et al. 2010, Sugawara et al. 2004), the amount and level of physical activity among PhA subjects may have bidirectionally influenced the results.

With the exception of Study III, the designs of all other studies in this project were cross-sectional. In Study III, we analyzed the differences in oxLDL and arterial elasticity after a short intervention period, not the incidence of CVD events. Therefore, we cannot obtain any prognostic information regarding future CVD events on the ground of this project. However, the findings of this project suggest that the predictive value of arterial elasticity, oxLDL, fibrinogen and RHR for incident CVD, additional to the presence of MetS, ED or high estimated CVD-risk, might be worth elucidating in a prospective end-point study.

6.6.2 Arterial elasticity

Non-invasive pulse wave analysis is based on a number of theoretical approximations (Laurent et al. 2006). In addition, there are no studies on this method's possible predictive value for incident CVD (Laurent et al. 2006). However, impaired C1 and C2 have been found to be associated with high risk conditions for CVD events such as hypertension, symptomatic CHD,

severity of peripheral vascular disease, microalbuminuria, ED and MetS (Cohn et al. 1995, Duprez et al. 2001, Fjeldstad et al. 2007, Ge et al. 2008, Li et al 2007, Prisant et al. 2006, Zimlichman et al. 2005). In addition, values assessed by non-invasive DPCA have been reported to correlate tightly with those obtained invasively (Cohn et al. 1995). C1 also seems to relate significantly to aortic distensibility assessed by MRI (Resnick et al. 2000). Although C2 is not solely a measurement of endothelial function, a good correlation with endothelial function assessed by FMD has been reported (Wilson et al. 2004). Furthermore, DPCA estimates systemic arterial stiffness, which has been thought to provide a more complete understanding of arterial function (Laurent et al. 2006, Woodman et al. 2005). The golden standard for measuring arterial elasticity, PWV, may be inaccurate among subjects with MetS, abdominal obesity and diabetes (Laurent et al. 2006). For the above-mentioned reasons, DPCA, a non-invasive, reproducible and easily accessible method, was used in the present study (Laurent et al. 2006, Zimlichman et al. 2005).

The commercial HDI/PulseWave CR-2000 apparatus automatically measures a mean of the five most similar pulse waves during 30 seconds of measurement to derive C1 and C2. In most of the previous studies using the same pulse wave analysis, often only one, but a maximum of three consecutive measurements have been conducted to achieve elasticity indices (Duprez et al. 2001, Fjeldstad et al. 2007, Ge et al. 2008, Prisant et al. 2006). We decided to perform four consecutive measurements to minimize the variability and bias caused by one measurement only.

6.6.3 Circulating oxidized LDL

Although oxidation of LDL occurs mainly in the arterial wall, increased circulating oxLDL, determined by a method using the same monoclonal antibody as in the present study, has been reported to be associated with subclinical and clinical atherosclerosis and incident CVD (Holvoet et al. 1998, Hulthe&Fagerberg 2002, Meisinger et al. 2005, Stocker&Keaney 2004, Tanigawa et al. 2006). The oxLDL assay used in the present study has been reported to have an excellent reproducibility (Pai et al. 2002). The intraindividual CV% of oxLDL in our study was in the range reported for the capture ELISA assay by Mercodia. In order to diminish the variability of the measurements, the same experienced researcher performed all oxLDL measurements in duplicate for every subject.

In MetS, LDL-Cl is usually not increased, whereas ApoB levels are high (Bonora 2006). This means that the number of LDL particles is increased in the MetS. These small and dense LDL particles are more susceptible to oxidation (Bonora 2006, Sigurdardottir et al. 2002). Since ApoB is a marker of all atherogenic lipoproteins, it has been proposed as an alternative risk factor for LDL-C (Third report of the National Cholesterol Education Program 2002). Assessment of ApoB levels or the number and size of LDL particles would have been valuable additions to the study.

6.6.4 10-year cardiovascular risk score calculation

The 10-year CVD risk score was calculated only among those without established CVD, since the risk score models are designed to assess the risk for future CVD. In Study II, there was relatively large number of men with statin medication, but without established CVD. These subjects had previously been estimated to be at high risk for CVD events by their physician. In addition, statins affect lipid levels and may also affect arterial elasticity (Leibovitz et al. 2001). Therefore, subjects with statin medication were also excluded from the comparisons of different risk score groups in Study II. In Study IV, statins were more often used among men with ED. Since we did not compare different risk score groups but rather MetS subjects with and without ED among those with SCORE risk score < 5%, subjects with statin medication were included in all analyses of Study IV.

FINRISK60 differentiated MetS subjects with impaired arterial elasticity and increased oxLDL better than FINRISK at the actual age in Study II. This finding supports the ESC recommendation of extrapolating the risk to age 60 to reveal young subjects at high relative risk (Fourth Joint Task Force of the ESC 2007). Differences were most evident between FINRISK60 risk scores 5-14.99% and ≥ 15%. As impaired C2 was detected already among subjects with risk score ≥ 5% according to FINRISK at the actual age, a lower threshold for primary prevention might be considered when actual age is used in estimates. Significant differences in arterial elasticity were also evident between SCORE risk scores < 5.0% and ≥ 5% both at the actual and at the projected age. It supports the recommended threshold of SCORE ≥ 5% in detecting those at high CVD risk (Fourth Joint Task Force of the ESC 2007).

The FINRISK model takes account for more CVD risk factors than SCORE (Vartiainen et al. 2010). In addition, it has been constructed from a series of prospective studies among Finns (Vartiainen et al. 2010). These reasons may explain why FINRISK was superior to SCORE in the present study in differentiating MetS subjects with respect to differences in markers of subclinical atherosclerosis. However, since SCORE at the actual age is the most widely used risk score model in Europe, it was the only risk estimator used in Study IV.

6.6.5 Diet intervention

The balanced crossover design, used to avoid problems caused by the variability between the subjects, was the strength of the intervention study. Whereas most dietary approaches have focused on the alteration of fat composition and quantity in diet, CPTRO was supplemented to the habitual diet in the present study. Since a constant dose of CPTRO and a comparable amount of butter were used, the optimal daily dose of CPTRO cannot be determined based on this study. In addition, arterial elasticity, oxLDL, fibrinogen and RHR were not assessed at the baseline, which was a significant limitation. Because of this, we cannot make conclusions on the possible beneficial effects of CPTRO on markers of subclinical atherosclerosis compared with the habitual diet without supplements. However, whipped cream with a similar dose of SFA as in the butter administered in our study, did not cause a significant increase in postprandial oxidative stress in a previous study (Bloomer et al. 2010). Thus, at least the dose of CPTRO, administered in the present study, may be beneficial as a supplement to a habitual diet.

Since repeated heating may destroy the beneficial compounds of the oils and generate unhealthy effects instead (Leong et al. 2010, Srivastava et al. 2010), study subjects were asked to drink CPTRO as such. However, six men (14%) were unable to comply with the dietary regimens despite the short intervention period. To make long-time use of CPTRO as such more feasible, CPTRO could be advised to be used in salad and dip sauces.

Fat and energy intake were not assessed by dietary records, which may be considered as a limitation. However, the stability of both habitual diet and physical activity was ascertained by comparing the detailed food frequency and physical activity questionnaires filled in at the end of the intervention periods. A similar food frequency questionnaire has been found to be a suitable method in ranking energy and nutrient intake (Araujo et al. 2010). In addition, we measured body weight after both dietary periods to ensure that there was no difference in energy balance.

6.6.6 Assessment of erectile dysfunction

The IIEF questionnaire is a reliable method to assess the presence of ED (Cappelleri et al. 1999). However, men with erectile problems may avoid sexual intercourse and thus, are unable to answer the questions concerning erectile function during intercourse. In the present study, we used a single-question assessment of ED (IIEF question 15) among sexually inactive men. Previously, a single-question assessment of erectile function was found to appropriately identify men with ED (O'Donnell et al. 2005). Since we used the single-question IIEF to assess

the presence rather than the severity of ED, we presumably identified those with true, clinical ED as well.

The IIEF questionnaire has also been reported to properly classify the severity of ED (Cappelleri et al. 1999). Because of the relatively small number of subjects, we could not study the differences in risk markers between subjects with different severity classes of ED. However, it is not considered as a limitation since the presence, not the severity of ED, has previously been reported to be associated with the level of subclinical atherosclerosis (Vlachopoulos et al. 2008).

In the present study the IIEF response rate was markedly higher among PhA than MetS subjects. Men with erectile problems may be ashamed of reporting it even when told that the answers will be analyzed anonymously. Whether this affected the response rate and thus, the number of ED subjects is not known. In Study V, dealing with both PhA and MetS subjects, we wanted to be sure that subjects participating in the study truly had either completely normal erectile function or ED. Therefore, we accepted only men with either maximal or clearly decreased IIEF score to participate in the study.

Hypogonadism, associated with visceral obesity, is one mechanism linking MetS to ED (Corona et al. 2009). Physical activity may produce some benefits by inducing an increase in testosterone levels (Revnic et al. 2007). We did not assess testosterone levels in association with ED, which was a limitation of the study.

6.7 FUTURE RESEARCH PROSPECTIVES

According to guidelines, aggressive primary prevention should be targeted to patients at high calculated CVD risk. Thus, among these patients assessment of an intermediate CVD endpoint, such as impaired arterial elasticity, would not impact the treatment.

MetS, ED and high estimated CVD risk are associated with future CVD events. This thesis provides additional insight into the association of these high risk conditions with markers of subclinical atherosclerosis. Whether assessment of these markers among patients with medium or even low estimated CVD risk would reveal patients at high actual risk, or increase the risk known to be associated with MetS and ED is essential to define in prospective studies.

In addition, turnip rapeseed oil supplementation resulted in a decrease of oxLDL in comparison with butter in the present study. Whether a similar beneficial effect would occur in comparison with placebo, and whether alterations in arterial elasticity would emerge after a long-term intervention, are also interesting and important topics for future research.

7 Conclusions

The purpose of this thesis was to study whether arterial elasticity, circulating oxLDL, fibrinogen and RHR are associated with the presence of MetS, and high estimated 10-year cardiovascular risk, increasing number of MetS variables and ED among MetS subjects. In addition, the effect of dietary intake of CPTRO on these markers of subclinical atherosclerosis was assessed among subjects with MetS.

The main results of the study were:

1. MetS subjects had impaired C1, increased oxLDL and fibrinogen and elevated RHR compared with PhA subjects **(I, V)**.

2. MetS subjects at high estimated 10-year CVD risk according to FINRISK at the extrapolated age of 60 had impaired C1 and C2, increased oxLDL and fibrinogen and elevated RHR compared with medium-risk subjects **(II)**.

3. The number of MetS variables was not associated with markers of subclinical atherosclerosis **(II).**

4. OxLDL level was lower after the intervention period with CPTRO than after the butter period. There was no difference in arterial elasticity, fibrinogen, or RHR **(III)**.

5. MetS subjects with ED had impaired C1 **(IV)**, increased fibrinogen **(V)** and elevated RHR **(IV)** compared with those without ED. There was no difference in C2 or oxLDL. The difference in C1 between ED and non-ED MetS subjects was evident even among those with SCORE risk score < 5% **(IV)**.

6. Impaired C1 **(IV)** and increased fibrinogen **(V)** were associated with the presence of ED among MetS subjects, independently of traditional CVD risk factors. Physical activity was associated independently with normal erectile function among MetS subjects.

Impaired arterial elasticity, increased oxLDL and fibrinogen and elevated RHR may contribute to the increased CVD risk connected to MetS. The 10-year CVD risk calculation seems to differentiate MetS subjects with respect to these markers of subclinical atherosclerosis. However, in the presence of suspected vasculogenic ED, aggressive primary prevention should be considered for MetS patients, irrespectively of the estimated 10-year CVD risk. Dietary intake of CPTRO may delay the atherosclerotic process among MetS. Physical exercise may protect against ED in MetS.

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Arterial Elasticity, Oxidized LDL, Fibrinogen and Resting Heart Rate

Patients with metabolic syndrome presented with markers of subclinical atherosclerosis –especially in the presence of high estimated cardiovascular risk and erectile dysfunction. Turnip rapeseed oil supplementation resulted in a decrease of oxidized LDL. Physical activity was independently associated with normal erectile function.

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