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OXIDIZED LDL LIPIDS AS A RISK FACTOR FOR ATHEROSCLEROSIS

Meri Linna

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University of Turku

Faculty of Medicine

Department of Clinical Medicine
Department of Physical Activity and Health
Doctoral Programme of Clinical Investigation
Paavo Nurmi Centre

Supervised by

Tommi Vasankari, M.D., Ph.D., Professor
The UKK Institute for Health Promotion Research
Tampere, Finland

Markku Ahotupa, Ph.D., Docent
MCA Research Laboratory
Department of Physiology
University of Turku,
Turku, Finland

Reviewed by

Jukka Marniemi, Ph.D., Docent
The National Institute for Health and Welfare (THL)
Turku, Finland

Hannu Vanhanen, M.D., Ph.D., Docent
The Social Insurance Institution
Helsinki, Finland

Opponent

Leo Niskanen, M.D., Ph.D., Adjunct Professor
Specialist in Internal Medicine, Endocrinology
and Geriatrics
Unit of Pharmacovigilance and Drug Information
Finnish Medicines Agency Fimea
Helsinki, Finland

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ABSTRACT

Meri Linna

OXIDIZED LDL LIPIDS AS A RISK FACTOR FOR ATHEROSCLEROSIS – with special perspective to the adult lifespan of men

University of Turku, Faculty of Medicine, Department of Clinical Medicine, Department of Physical Activity and Health, Paavo Nurmi Centre, Doctoral Programme of Clinical Investigation, University of Turku, Turku, Finland. Annales Universitatis Turkuensis. Medica – Odontologica, Turku, Finland, 2014.

Background: Atherosclerosis progression spans an entire lifetime and has a wide pool of risk factors. Oxidized LDL (oxLDL) is a crucial element in the progression of atherosclerosis. As a rather new member in the atherosclerosis risk factor family, its interaction with the traditional pro-atherogenic contributors that occur at different ages is poorly known.

Aims: The aim of this study was to investigate oxLDL and its relation to major contributing risk factors in estimating atherosclerosis risk in data consisting mostly of adult men. The study subjects of this study consisted of four different sets of data, one of which contained also women. The age range of participants was 18-100 years and totaled 2337 participants (of whom 69% were men). Data on anthropometric and hormonal parameters, laboratory measures and medical records were assessed during 1998-2009.

Results: Obesity was paralleled with high concentrations of oxLDL, which consequentially was reduced by weight reduction. Importantly, successful weight maintenance preserveed this benefit. A shift from insulin sensitivity to insulin resistance increased oxLDL. Smokers had more oxLDL than non-smokers. A combination of obesity and smoking, or smoking and low serum total testosterone, resulted in even higher levels of oxLDL than any of the three conditions alone. Proportioning oxLDL to HDL-c or apoA1 stood out as a risk factor of all-cause mortality in the elderly.

Conclusions: OxLDL was associated with aging, androgens, smoking, obesity, insulin metabolism, weight balance and other circulating lipid classes. Through this variety of metabolic environments containing both constant conditions (aging and gender) as well as lifestyle issues, these findings supported an essential and multidimensional role that oxLDL plays in atherosclerosis pathogenesis.

Keywords: Oxidized LDL, atherosclerosis, risk factors, men

TIIVISTELMÄ

Meri Linna

HAPETTUNUT LDL VALTIMONKOVETTUMATAUDIN RISKITEKIJÄNÄ

Paavo Nurmi -keskus ja Terveysliikunnan oppiaine, Turun yliopisto, Turku. Annales Universitatis Turkuensis. Medica – Odontologica, Turku, 2014.

Tausta: Valtimoiden kovettuminen on tapahtuma, joka kehittyy tunnetusti koko eliniän ajan. Valtimokovettumataudille on tyypillistä myös se, että sillä on runsaasti riskitekijöitä. Hapettunut LDL on yksi keskeinen elementti valtimoiden kovettumisessa. Verraten uutena riskitekijänä sen rooli muiden valtimonkovettumatautia edistävien riskitekijöiden joukossa on merkittävältä osin vielä epäselvää.

Tavoitteet: Väitöskirjatyön tavoitteena on ollut tutkia hapettuneen LDL:n yhteyttä muihin merkittäviin valtimonkovettumatautia kuvaaviin riskitekijöihin aikuisiällä. Tutkimuksen koehenkilöt muodostuivat neljän eri aineiston pohjalta, joista yhdessä mukana on myös naisia. Yhteensä 2337 koehenkilön (joista 69% miehiä) ikähaitari oli 18-100 vuotta. Kehon koostumukseen, elintapoihin, verestä mitattaviin laboratoriomittauksiin sekä sairauskertomustietoihin liittyvät tarvittavat muuttujat kerättiin vuosina 1998-2009.

Tulokset: Liikapainoon liittyy suurentunut oxLDL –pitoisuus. Laihduttaminen pienentää oxLDL –pitoisuuksia merkittävästi, ja onnistunut painonhallinta myös ylläpitää tämän alentuneen tason. Insuliiniresistenssi on yhteydessä oxLDL –pitoisuuksien kohoamiseen. Tupakoivien oxLDL –pitoisuudet ovat korkeammat kuin tupakoimattomien. Liikapainon ja tupakoinnin tai tupakoinnin ja matalan seerumin kokonaistestosteronipitoisuuden yhdistelmä johtaa korkeampiin oxLDL –pitoisuuksiin kuin mikään näistä kolmesta tekijästä yksinään. Mitä suurempi vyötärön ympärysmitta, sitä korkeammat pitoisuudet oxLDL:ää. OxLDL:n ja HDL –kolesterolin välinen suhdeluku ennustaa osaltaan merkittävästi kokonaiskuolleisuutta iäkkäillä.

Johtopäätökset: Ikääntyminen, androgeenistatus, tupakointi, liikapaino, insuliiniaineenvaihdunta, painon hallinta ja seerumin muut lipidit näyttävät vuorovaikuttavan LDL-partikkelin sisältämien lipidien hapettumisen kanssa. Näiden useiden eri aineenvaihduntareittien sisältäessä sekä vakiona pysyviä tekijöitä (ikääntyminen, sukupuoli) että muuttuvia (elintavat), tässä väitöskirjassa esitetyt tulokset tukevat oxLDL:n moniulotteista ja keskeistä roolia valtimoiden kovettumisessa.

Avainsanat: Hapettunut LDL, valtimonkovettumatauti, riskitekijät, miessukupuoli

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications, which are referred to in the text by Roman numerals (I-VI). Additional unpublished data is also presented.

I Linna MS, Borg P, Kukkonen-Harjula K, Fogelholm M, Nenonen A, Ahotupa M, Vasankari T. Successful weight maintenance preserves lower levels of oxidized LDL achieved by weight reduction in obese men. *International Journal of Obesity* 2007;31:245-253.

II Linna MS, Kukkonen-Harjula K, Fogelholm M, Ahotupa M, Vasankari T. Co-existence of insulin resistance and high circulating concentrations of oxidized LDL lipids. Submitted.

III Linna MS, Ahotupa M, Irjala K, Pöllänen P, Huhtaniemi I, Mäkinen J, Perheentupa A, Vasankari TJ. Smoking and low serum testosterone associates with high concentration of oxidised LDL. *Annals of Medicine* 2008;40:634-640.

IV Linna MS, Ahotupa M, Löppönen M, Irjala K, Vasankari T. Circulating oxidised LDL lipids, when proportioned to HDL-c, emerged as a risk factor of all-cause mortality in a population-based survival study. *Age and Ageing* 2013;42:110-113.

V Linna MS, Ahotupa M, Kyröläinen H, Santtila M, Atalay M, Vasankari T. Atherogenicity of increasing abdominal obesity and smoking in young adult men measured by circulating oxLDL/HDL-c, IL-6 and IGF-1. Submitted.

ABBREVIATIONS

ApoA1= apolipoprotein A1

ApoB = apolipoprotein B

BMI = body mass index

BP = blood pressure

CHD = coronary heart disease

CV = coefficient of variation

CVD = cardiovascular disease

DBP = diastolic blood pressure

FM= fat mass

FT = free testosterone

HDL-c = high-density lipoprotein cholesterol

HOMA = homeostasis model assessment (for insulin resistance)

IL-6 = interleukin 6

LH = luteinizing hormone

LDL-c = low-density lipoprotein cholesterol

OxLDL = oxidized low-density lipoprotein

SBP = systolic blood pressure

SD = standard deviation

SHBG = steroid hormone binding globulin

SPSS = Statistical Package for Social Sciences

TC = total cholesterol

TG = triglycerides

WC = waist circumference

WHR = waist-hip-ratio

WRP = weight reduction period

WMP = weight maintenance period

1. INTRODUCTION

Circulation of blood is characteristic of any vital human organ. Consequently, the cardiovascular system has a profoundly important role in human health. As the circulatory system is fundamental, as logical it is for scientific curiosity to aim at increasing our understanding of its function. Currently, for cardiovascular research, there is some imbalance in the amount of different types of data: there are more tools than there are manuals. In other words, the accumulated number of different risk factors outweighs the data on the precise methods of action for each factor. Therefore, more investigation on the events at the physiological level is needed. This thesis was created to demonstrate the interconnection between conventional accelerators of atherosclerosis and a relatively novel risk factor.

Another issue of relevance is the considerable magnitude, at which atherosclerotic cardiovascular diseases debilitate nations across the globe. Currently, in Finland, non-communicable diseases, or lifestyle-related conditions, account for up to 89 percent of all causes of death. Of this, the cardiovascular disease pool constitutes 41 percentage-units (46%) (WHO statistics, 2011). Consequentially, an investigation promoting new knowledge and more accuracy to the disease mechanisms for preventive or therapeutic targeting, clearly supply for an existing demand.

Usually the key question encircling around risk factors is in fact rather a matter of choice than of complete avoidance: which of all the unavoidable risks is least harmful over the long-term? As an example, substituting groceries with high amounts of salt with increased consumption of soft energy drinks may, as such, improve blood pressure over the short-term, but at the possible expense of simultaneously accelerating the development of non-alcoholic fatty liver disease (Bray and Popkin, 2013). Unsustainable risk reduction is not risk reduction at all.

A further scenario highlighting and characterizing the importance of research around risk factors is a phenomenon called ‘reverse epidemiology’. It is defined as a risk-increasing effect which at another instance may have protective properties (Kalantar-Zadeh et al. 2004; Strandberg et al. 2014). For example, hypercholesterolaemia may – unconventionally – indicate better health in patients with chronic heart failure (Kalantar-Zadeh et al. 2004). Also, the role of BMI is a risk factor is coherent until the age 70, but thereafter its interpretation may be influenced by underlying diseases or smoking status (Patel et al. 2014). A J-curve shaped, non-linear association is characteristic of BMI and mortality (Manson et al. 1995; Song et al. 2014). Orchestration of the big picture should, therefore, be the ultimate companion in all scientific work. This highlights the importance of keeping in mind elements such as time-dependence discerning short- and long-term scale, relevance to general health, the variety of demands between different physiologic/pathologic conditions and the underlying interconnectedness behind all these. Medical research, mostly characterized and established around demonstrating statistical significances in scientific papers,

should never be oblivious to biologic importance. In front of a cause-effect- finding, or an association, there is a considerable human temptation to oversimplify, which we should be conscientious of. Additionally, an increasing global burden of a condition – such as obesity – calls for immediate effective solutions (Morrow, 2003) further endorsing the importance of asking the right questions.

Briefly defined, atherosclerosis is a condition or a process where the initiating event is a fatty streak formation in the wall structure. Later on, arteries eventually may become stiff and gradually occluded. Evaluating atherosclerosis risk through oxidized LDL lipids (oxLDL) levels was a consistent aim of this thesis. The interrelationships between oxLDL: i) with other established atherosclerosis risk factors such as smoking and low serum testosterone, ii) in weight maintenance and obesity, and finally, iii) its predictive potential, has been investigated, essentially, in the range of adult male life span. Expectantly, this work was intended to add new, interesting and applicable evidence to the accuracy of atherosclerotic disease management.

In laymen's terms, lipid (fat) metabolism is a complex entity of essential nature, focused on transportation, utility and deposition of fats. The metabolism functions to execute the necessary handling of lipid-containing compounds properly in a water-soluble environment, i.e. systemic (blood) circulation (Marcovina et al. 2006). To meet the demands of energy metabolism and tissue function, lipid compounds obtained from, for example, nutrition have to be packed, delivered and stored appropriately. Disturbances in any of these pathways may have disease promoting effects, particularly so considering diseases of the main elements of circulation, for instance, the heart and vasculature (hence the term 'cardiovascular diseases' or CVD).

Impairments in lipid quality or in volume, and incapacitated fat storage function may all have contributing effect to the development of atherosclerosis. Our current Western lifestyle tends to promote a significant and excessive burden to the fat deposition mechanisms (Oddy et al. 2013). This is often highlighted and reflected, for example, as centrally located obesity and serum lipoprotein abnormalities. Whereas the Mediterranean diet, intriguingly enough, has considerable advantages over its Western dietary colleague (Zazpe et al. 2014). A similar set of laws of nature exists for carbohydrate metabolism as well: an imbalance between insulin action and blood sugar level promotes diabetic conditions – the harmful consequences of which have been increasingly documented and elucidated during the past few decades. The big picture that depicts lipid-related cardiovascular pathways becomes further complicated by the observations that diabetic conditions often, in fact, stand out as disorders of lipid metabolism as well (Ueba et al. 2009).

The challenges enforced on lipid metabolism through living in a Western culture become further advanced by the phenomenon of aging. The average lifespan of mankind has never been as lengthy as it is at present. This extends the window to explore different metabolic environments and mechanisms. However, it also provides a milieu for a variety of diseases to become increasingly severe and chronic, posing more burden on both individual and societal levels. Currently, people have an unprecedented amount of time to suffer from diseases or, more preferably, implement preventive

measures to sustain wellbeing. Interestingly, characteristics of a metabolically burdened condition (metabolic syndrome), for example, predict the onset of diabetes even in the elderly (Salminen et al. 2013). Risk factors with predictive potential, therefore, emerge as tools antagonizing the agonistic effect of disease development, and propose the importance of prevention (Reis et al. 2013). From a medical perspective, all these characteristics of our modern time emphasize the quintessential question, how early and how efficiently could we intervene?

Recently, a comparison between two different mortality risk reducing tools was performed (Briggs et al. 2013). Statins – a widely used lipid-lowering drug group in the management of atherosclerosis risk – were compared to apples, and found to have comparable risk-reducing effects. Interestingly, this provoked critical suggestions, that to justify the comparability, the latter should be similarly investigated in placebo-controlled trials (Strandberg, 2014). However, in health the demand for nutrition is irreplaceable and consistent. This is not the case for a pharmacologic agent. Also, criticizing apples for the potential risks that are universally characteristic of all eating, is unreasonable. For drug molecules, on the contrary, their undesirable side-effects are often drug-specific. Therefore, the standards for a wide-scale recommendation cannot be similar for a food item and pharmacotherapy. Convenience is, naturally, a practical issue, a current cultural value and potentially deal-breaking in choosing the appropriate risk reducing tool. However, it is a highly subjective one as well. Fundamentally, from the context of general recommendation and preference rank, disease risk reduction is a question of objectivity, irreplaceability and safety of alternatives. Obviously, more discussions – including cross-disciplinary collaborations – are needed about the comparability and eligibility of different methods.

These introductory, preliminary notions hopefully at least partly explain the entity this thesis has eventually shaped into. It is imperative to consider the background emphasis or a special reference that should be discernible throughout the original publications: this thesis deals with atherosclerosis risk factors that are either directly or indirectly related to, or modifiable by, lifestyle factors. Basically, introducing a new agent, pharmacotherapy, into a finite but interactive system tends to be less physiological than rearrangement of the pre-existing metabolic condition habitually (for instance, *via* exercise, healthy nutrition and smoking cessation). Therapeutic position statements remain, however, outside the scope of this thesis. They are only briefly discussed in a lifestyle-oriented emphasis in order to sketch the aforementioned big picture for the reader. The connectivity of the findings presented here, to other works on the same field of research, indicates their utility and applicability potentials in a larger scale.

Without proper introduction of the context, a mere novelty is of no sustainable value. Metaphorically, there is a pivotal difference between a breath of wind and a breath of wind bending a tree.

2. REVIEW OF THE LITERATURE

2.1. ATHEROSCLEROSIS

2.1.1. Epidemiology and pathogenesis of atherosclerosis

Currently, European populations exhibit some variance in cardiovascular disease statistics. There is a contrast between East and West, where Eastern parts have higher mortality rates (Kromhout, 2001). Originating from the 1960's, Finland, in particular, has had a reputation of having the highest cardiovascular morbidity rates (Keys, 1970 and 1986). Interestingly, the Mediterranean regions seem to be particularly protected, as they, for example, demonstrate the lowest prevalence of obesity in Europe (Perez-Lopez, 2009; Gallus et al. 2014). Further studies are, nonetheless, required for corroboration while the exact mechanisms, especially for their diets role, remain partly undiscovered. (Grosso et al. 2014). Additionally, at present, the Mediterranean diet seems to be threatened by Western-based influences (Lairon, 2007).

By nature, atherosclerotic cardiovascular disease progression may develop already from the early years. Some have estimated the crucial onset-age around three to nine years of age (Jaquith et al. 2013; Juonala et al. 2013) with health risk ramifications potentially reaching adulthood (Raitakari et al. 2003). Considering the prospects of cardiovascular health from the early years on, nutrition and physical activity may be one of the key determinants (Juonala et al, 2013; Kaikkonen et al., 2013).

The term 'atherosclerosis' describes arteries that gradually lose their elasticity and have a decreased blood flow diameter. In greater detail, what is established is a system wherein a plaque develops *via* modification of LDL particles, involvement of macrophages, fatty streak formation, calcification and low-grade inflammation. Ultimately, a risk of an acute plaque eruption event with potentially fatal outcomes is generated (Glass and Witztum, 2001; Otsuka et al. 2014).

2.1.2. Clinical significance of atherosclerotic cardiovascular diseases

The clinical significance of atherosclerosis is easily comprehended when considering the elemental necessity of blood circulation to the physiology of any tissue. A process compromising vascular health, therefore, easily translates into a functional disturbance of the organ as well. Hardening of the arteries means less adjustability for different metabolic demands, such as alterations in blood pressure or perfusion. At an individual level, the development of atherosclerosis risk is initiated more robustly for men than for women. However, after approximately five years has elapsed from menopause, men and women share the same progression pace (Kiechl and Willeit, 1999).

The health costs of cardiovascular diseases – either financial or related to quality of life, and with or without diabetes – are significant. It is a major mortality factor among

diabetic patients (Stephens et al. 2009), although the overall lifetime risk scene of these diseases is also substantial (Kiechl and Willeit, 1999; Wilkins et al. 2012). As diabetes has a key role in contributing to the pool of cardiovascular morbidity, a great concern is justified. In developed countries, the prevalence of diabetic individuals has been estimated to increase by a fifth until the year 2030. For developing countries, the future seems even bleaker (Shaw et al. 2010).

2.2. RISK FACTORS FOR ATHEROSCLEROSIS

2.2.1. Age and sex

Aging and gender provide the general framework from which general health behaviour roots from. Accumulation of age is momentum acting within the boundaries of a limited-timed lifespan, whereas gender provides the appropriate 'origin' for a variety of hormonal milieus and predispositions. As a result, these factors attribute to the overall human condition from cellular to behavioural interaction with stimuli from the outside world. Tissues and metabolic pathways undoubtedly wear off by aging. The speed and magnitude of this decadence is, however, a much more complicated matter, and both directly and indirectly dependent on lifestyle choices.

Aging and gender have acknowledged contributing effects on atherosclerosis risk (Janghorbani et al. 1993; Willeit and Kiechl, 1993). Even though they are, *per se*, atherosclerosis risk factors with no room for improvement, they indeed merit attention. Also, race and ethnicity may in fact have a role (Jeng et al. 2002). Furthermore, the setting between atherosclerosis and its risk factors may change from middle-age on: a considerable risk factor in the first decades of life might be of uncertain relevance in the elderly (Fabris et al 1994; Krumholz et al. 1994). However, some risk factors may indeed maintain their prognostic value throughout lifespan (Howard et al. 1997). For example, elderly subjects with the highest oxLDL concentrations exhibit a high coronary heart disease (CHD) risk with an odds ratio of 2.8 (Holvoet et al. 2003).

The crucial status of ox-LDL in development of and reliability in atherosclerosis risk is verified by several investigations (Witztum and Steinberg, 1991; Ahotupa and Vasankari. 1999, Meisinger et al. 2005). The predictive value may even exceed that of the conventional risk factors. Particularly in the case of total cholesterol, this traditional measurement is a very gross one without the benefit of specifying the quality of cholesterol subclasses, the properties of which range from pro-atherogenic (LDL) to anti-atherogenic (HDL-c) (Meisinger et al. 2005; Johnston et al. 2006). There is also evidence, that in an elderly population, apolipoproteins may be more indicative or predictive of cardiovascular disease risk or mortality than conventional lipids (Bruno et al. 2006; Florvall et al. 2006).

In atherosclerosis risk, there are apparent differences between men and women: up until the end of middle-age, men are acknowledged to have a worse risk factor status than women. This, at least in part, is thought to be the result of antiatherogenic effects

of estrogen hormones in the premenopausal episode (Kalin and Zumoff, 1990). Middle-aged women tend to have higher concentration of HDL-c than men, and this partly explains some of the reduced atherosclerosis risk in women of that age (Kotchen et al. 1993). It has been proposed that menopause may, however, have hormonal repercussions that could contribute to an uprising in atherosclerosis risk among women. Therefore, with aging, the differences in risk factor profiles between men and women taper off (Jousilahti et al. 1999), thus changing the setting and possibly rendering women eventually at higher risk (LaRosa et al. 1994). In men, avoidance of factors that are disadvantageous for serum testosterone metabolism, may have an elemental value in the prevention of some chronic cardiovascular diseases, such as type 2 diabetes (Ohlsson et al. 2011; Salminen et al. 2014).

When considering a risk factor for a disease in the case of elderly – and particularly, when reviewing the scientific literature thereof – it cannot be omitted that the metabolism of the subjects is that of a more or less burdened system approaching its dawn (Corti et al. 1997). Metaphorically speaking, the engine has gathered some soot. Presently, the life expectancy in Finland is 78 years for men and 84 years for women (WHO statistics, 2011). Exclusion of factors that could easily be considered as confounding among younger individuals, would be a downright breach of scientific thinking in the elderly. By increasing study population age, there are less and less healthy individuals, or individuals with only one or two diagnosis – preferably of irrelevant nature to atherosclerosis. In the case of current life expectancy, being higher than ever in human history, seeking or highlighting disease-free subjects would equal to research focused on the rare few rather than on the actual laws of nature; i.e., course or characteristics of a disease.

2.2.2. Lipid metabolism

For decades now, in clinical practice and research settings related to cardiovascular disease risk, lipid metabolism has been examined by several parameters. These markers have mainly included total cholesterol, triglycerides and different classes of lipoprotein cholesterols, such as low- and high-density lipoproteins (LDL, HDL-c) (Grundy, 1995). These are nominated as conventional lipoprotein risk factors.

Increasing scientific evidence has established more precision within these conventional lipoprotein classes. Subclasses for HDL's (for example, HDL_2 and HDL_3) and LDL's (for example, oxLDL; small, dense LDL or sdLDL; large, buoyant LDL) have been characterized. As a rule, the less LDL the better, particularly so for oxLDL and sdLDL (Carmena et al. 2004). For HDL-c, the more the better – without additional clinical significance in the specific distribution of HDL subclasses (Superko et al. 2012). Dyslipidemic conditions, however, induce distinguishable manifestations in HDL subclasses indicating functional impairment in HDL's antiatherogenic capacity (Kontush and Chapman, 2006). Due to some confusion in the scientific arena regarding HDL-targeted therapeutics, currently, increasing HDL concentrations is not the primary focus of therapeutic lipoprotein guidelines. It appears that according to data thus far, measurements of HDL cholesterol do not represent HDL functionality

precisely enough, but rather warrant further investigation (Rosenson et al. 2013). Hence, lowering of non-HDL lipoprotein fraction in serum lipids remains as the top priority of clinical recommendations, emphasizing the role of LDL metabolism (Toth et al. 2013).

Alongside all the lipoprotein classes mentioned above, apolipoprotein A1 and B (apoA1 and apoB) have a pronounced role in CVD risk prediction, as for example, increased concentrations in LDL tend to promote apoB levels (Marcovina et al. 2006). Apolipoproteins are structural steering protein classes in HDL- and LDL particles responsible for guiding and executing the molecular assembly of these lipoprotein particles. Depending on scientific literary reference, their role varies from an auxiliary risk assessment tool to importance higher than that of older traditional lipid markers. Some suggest that apolipoproteins or the ratio of apoB/apoA1 may surpass the risk factor value of conventional markers or even some manual technical tools, such as ultrasonography (Walldius and Jungner 2004; Florvall et al. 2006; Walldius and Jungner, 2006). There are, however, voices of dissidence as well, questioning whether in fact conventional lipoproteins keep on pulling a significantly higher rank over apolipoproteins in risk reflection (Ingelsson et al. 2007).

By accumulation of age, the risk factor value of some lipid-related biomarkers may be altered (Willeit and Kiechl, 1993). For example, the risk factor status of total cholesterol and LDL seems to be compromised (Anum et al. 2004, Tikhoff et al. 2005). Similar findings have been observed for triglycerides and HDL-c as well (Baggio et al. 1998), but for the latter contradicting findings also exist (Packard et al. 2005; Landi et al. 2007). The use of oxLDL and HDL-c has been proposed as pragmatic and accurate lipid-related tools to risk assessment of cardiovascular diseases (Johnston et al. 2006). Oxidation of LDL has been characterized as a key event in atherosclerosis development (Witztum and Steinberg, 1991), and conversely, HDL-c as an essential counter-agent with outstanding preventive potential (Superko et al. 2012). As mentioned, at present, there yet remains some cautiousness as to exactly how should HDL-c be considered as a therapeutic target (Rosenson et al. 2013).

Currently, the CVD-related clinical significance of total cholesterol can be considered as too general and hence easily surpassed by the metabolically more precise lipoprotein biomarkers. The same seems to apply to LDL as well, as the mere absolute concentration of LDL oversimplifies the actual atherogenic mechanisms at cellular level, i.e. small and dense composition (Griffin BA, 1999) or modification of LDL lipids (Ahotupa et al. 2010). The simultaneous consideration of triglycerides, and contributors to its concentration, should not be forgotten either, as the triglyceride-rich VLDL in the post-prandial state appear to be a significant factor of modifying LDL particles (Griffin, 1999; Ahotupa et al. 2010).

As mentioned earlier, the joint use of oxLDL and HDL-c, or an apoB/apoA1 –ratio, might be one of the most pragmatic and accurate lipid-related tools in risk assessment of atherosclerotic cardiovascular diseases (Johnston et al. 2006, Walldius and Jungner, 2006). The anti-atherogenic properties of HDL exert a balancing effect on the pro-atherogenic nature of modified LDL particles, of which oxLDL seems to be the most

extensively studied. However, methodological discrepancies in oxLDL analysis render comparison between studies a challenge.

2.2.3. Obesity

The incidence of obesity has been on a consistent rise for the past three decades. In accordance, efforts to lose or maintain weight have been similarly common (Serdula et al. 1999, Mitchell et al. 2011). Today, due to the increasing trend, the original challenge still remains. A Western sedentary lifestyle, the prototype of which North America is often considered as, has in fact spread across the globe (Kong et al. 2013). Accordingly, since then it has been customary to denote obesity as something of an epidemic or pandemic nature (Swinburn et al. 2011). In Finland, the current overall prevalence of overweight ($25 \leq BMI < 30$ kg/m^2) and obesity ($BMI \geq 30$ kg/m^2) are 36 and 19 percent (Gallus et al. 2014). Over the past few decades, the occurrence of obesity in Finland has demonstrated a dramatic increase (Lahti-Koski et al. 2010), estimated to be as high as 89 percent. As a comparison, the corresponding increase in Sweden was 51%, as, currently, 10 percent of their population are obese (Gallus et al. 2014).

After a sluggish and reluctant start, the WHO has finally recognized the severity of obesity-related health threats in its action plans. In fact, inside Europe, Scandinavia was among the first to become targeted by WHO's initiatives to defeat obesity. The vigorousness to attack global health issues demands co-operation between several contributors. The motives or intentions of Health, Trade and Agriculture ministries should align globally as the obesity epidemic prevails. However, health promotion issues may not either share or serve the same purposes with the trading/financial sector – all nonetheless having an equally fundamental role in society, and thereby, at the individual decision-making level as well (James, 2008).

Although the context of this thesis clearly serves the individual level rather than the societal, the latter is an important aspect to briefly sweep over. The most vital dilemma in obesity is very simple: it is defined by excessive intake of energy. Naturally, we may – and we should – debate about different types of obesity, but the underlying math of caloric surplus yet remains. Secondly, the causal sequence leading to or accelerating the development of obesity involves several contributors (Kong et al. 2013). Availability or cost of healthy daily choices – and opportunities to avoid the unhealthy ones such as prolonged sitting or soft drinks – is one of the most important of these factors (Lobstein, 2002; Wells, 2012). It remains to be seen, how this modern obesitogenic predisposition of unprecedented nature eventually discloses, as presently, the tendency to develop obesity applies to children as well. Therefore, the offspring of current generations will enter adulthood with a history and commonness of overweight never seen before (Unger, 2003). What may additionally shape our knowledge on obesity in the future are findings, that adipose tissue is in fact by far nothing of a passive storage for fat, but a metabolically active, multifactorial environment (Galic et al. 2010).

From the perspective of cardiovascular relevance in a clinical setting, currently, waist circumference seems to be the most supported of the obesity-measuring tools (Menke et al. 2007; Tchernof and Després, 2013). BMI has been observed to be surpassed by waist-hip-ratio (WHR) (Yusuf et al. 2005). WHR, on the other hand, seems to be defeated by waist circumference (Wang et al. 2005; Tchernof and Després, 2013). One essential problem with waist-hip-ratio is that gaining or losing weight may change both the visceral (waist) and more peripheral (hip) measures resulting in no alteration in the ratio. For waist circumference, one essential characteristic that should not be forgotten – particularly considering the exact metabolic ramifications of visceral adiposity – is that it cannot discriminate between subcutaneous or visceral fat (Lemieux et al. 2000). The recommendation for waist circumference is less than 102 cm for men and less than 89 cm for women (Huxley et al. 2010; Azagury and Lautz, 2011), but some investigators have raised doubts whether in fact the limits should be tighter (Wang et al. 2005).

The complications derived from obesity are numerous. From the perspective of mortality and morbidity, the cardiovascular repercussions of obesity – of all the obesitogenic consequences – are the most significant (Grundy et al. 2005; Tchernhof and Després, 2013). In Finland, 41 percent of all deaths stem from cardiovascular conditions – and altogether 89 percent of all-cause deaths are presently connected to our lifestyle choices (WHO statistics, 2011). The long-term futuristic big picture around obesity appears, therefore, undisputedly adverse (Dyer et al. 2004).

The most physiological of the tools to overcome overweight-related health challenges are focusing on dietary factors and sufficient dosage of physical activity. Several studies show encouraging signals as it seems, that lifestyle interventions may, for example, lead to decrease in abdominal fat, lowered stroke risk, or decease the concentration of oxLDL (Rector et al, 2007; Middleton et al. 2013; Tchernof and Després, 2013). The amount of data considering the benefits of lifestyle interventions is consistently increasing. High waist circumference, for example, has been independently associated with increased concentrations of oxLDL (Weinbrenner et al. 2006). There is evidence suggesting, that not just oxidative stress but also inflammatory reactions may in fact be involved in obesity in a way we are only beginning to understand (Njaou et al. 2009; Bondia-Pons et al. 2012).

Furthermore, of particular concern related to obesity, are increasing observations related to fatty livers, meaning a considerable possibility of severe long-term liver complications (Zelber-Sagi et al. 2011; Rahimi and Landaverde, 2013). The challenges and risks related to visceral accumulation of fat are being more extensively recognized. Interestingly, and as a finding of novelty, it seems that visceral adiposity is also a reliable indicator of fat deposition in epi- or pericardial locations (Granèr et al. 2013). It has been proposed that fat deposition around the myocardium caused by weight gain may be directly cardiotoxic, potentially predisposing to systolic dysfunction (van Gaal et al. 2006).

2.2.4. Endocrine function: insulin action, IGF-1, testosterone

Insulin action

Insulin is a peptide hormone with anabolic characteristics and is secreted by the pancreatic β-cells (Dimitriadis et al. 2011). It is responsive of blood glucose concentrations. The main physiological targets for insulin action are adipose tissue, skeletal muscle and the liver (Borai et al. 2007). However, interestingly, insulin action is also implicated in apparently distant metabolic axes, such as osteocalcin/bone (Klein, 2014) and gut microbiota (Shen et al. 2013). Additionally, and importantly, disturbed testosterone metabolism may have ramifications extending to insulin function, with the potential of being an early manifestation of glycemic pathogenesis (Laaksonen et al. 2004). Insulin resistance, or low insulin sensitivity, is defined as a condition, where insulin's capacity to exert its biological effects is diminished. Accumulation of adipose tissue and inflammatory processes are important examples of conditions that may impair insulin action (Sesti, 2006). Furthermore, oxLDL disturbs adipocytes responsiveness to insulin (Scazzocchio et al. 2009).

Considering the substantial input of lifestyle factors in current overall mortality, it is no surprise that environmental conditions may also promote the onset of diabetic conditions (Fujimoto et al. 2000; Zelberg-Sagi et al. 2011). Obesity is a considerable, and – although not solely responsible – a highly liable element in the development of diabetic conditions (mainly insulin resistance, type 2 diabetes and metabolic syndrome) (Kong et al. 2013). As the rise in the prevalence of obesity (see chapter 2.2.3) has been both consistent and international, the simultaneous trend in disorders of the glucose metabolism indicate an apparent co-existence (Kissebah and Krankower, 1994). Approximations of the global burden of diabetes in the future are discouraging throughout, with developed countries having an estimated 20 percent increase in prevalence during 2010 and 2030 (Shaw et al. 2010). Additional visceral adiposity in particular may be elemental in the prediction and development of diabetic disorders (Boyko et al. 2000; Balkau et al. 2007).

Obesity, particularly centrally located, usually coexists with dyslipidemia - a disturbance of serum lipids. Abdominal obesity is suggested as a reliable tool to differentiate individuals with a significant cardiometabolic risk (Després et al. 2008). In type 2 diabetes, characterized as maturity- or environmentally-onset imbalance between serum glucose and insulin response, the characteristic dyslipidemic state may involve hypertriglyceridemia and/or impaired HDL antiatherogenic action (Gowri et al. 1999). Furthermore, some findings indicate that individuals with cardiometabolic disorders exhibit elevated concentrations of oxidatively modified LDL (Pohjantähti-Maaroos et al. 2010). This is suggestive of the possibly crucial role of oxidative events in accounting for the increased cardiovascular morbidity related to diabetic conditions (Hopps et al. 2010). Further considering the link between oxidative stress, disturbed glucose homeostasis and obesity, insulin resistance may in fact emerge as a superior factor over an actual diabetic state (D'Archivio et al. 2012).

An undiagnosed diabetic condition conveys an additional morbidity burden to an existing cardiovascular disease – emphasizing the overall health-promoting effect of treatment initiation and follow-up (Tenenbaum et al. 2000). Presently, lifestyle choices bear a great significance in overall morbidity and mortality across continents. Interventions, tools and projects aiming at reducing obesity – and thereby curbing the incidence of diabetic conditions as well – are, therefore, of outstanding significance. As insulin secretion is a postprandial event, healthy nutrition is a fundamental issue. Therefore, studies showing the beneficial effects of, for example, berry consumption in reducing glycemia/insulin burden after meals, translate into biologic importance and promising investments on health (Basu et al. 2010; Törrönen et al. 2012 and 2013).

Initiatives carried out either as individual contact or at workplace, seem promising (Allen et al. 2012; Salinardi et al. 2013). A preventive effect can be observed already with five percent weight loss (Penn et al. 2013). However, the achieved risk reduction or health benefit, to a considerable extent, suffers from remaining only short-lived. Some have investigated different patterns of weight loss and found that physical activity, for instance, and some psychosocial factors during weight reduction have a contributing role in the weight loss success (Yank et al. 2014). In a broader sense, designing protocols to accomplish clinically significant reductions in risks related to diabetic conditions, still maintain long-term efficacy issues between resources and outcomes (Penn et al. 2005 and 2013).

IGF-1

Insulin-like growth factor I is a widely acting, closely regulated hormone in the human body found in many tissues. One of its functions is to modulate systemic growth hormone action – one important of which occurs in hepatocytes as an endocrinological response. However, a number of other types of activities have been observed (e.g. anabolic, antioxidant and anti-inflammatory) (Puche and Castilla-Cortázar, 2012). Despite the homologous structural similarity to insulin, IGF-1 is responsive of growth hormone and paracrine IGF-1 (Klein, 2014). Age, sex, smoking and aerobic fitness may also have a determining role in the level of IGF-1 (Kaklamani et al, 1999; Nindl et al. 2011).

A considerable body of evidence points out that abdominal adiposity causes suppression on the excretion of growth hormone secretion from the pituitary. Additionally, administration of growth hormone has yielded improvements in losing visceral adipose tissue. However – as was the case in testosterone administration – some findings have been suggestive of possible impairments on glucose metabolism (Berryman et al. 2013). Nonetheless, while discussing abdominal obesity and atherosclerosis risk, as is the case in this thesis, IGF-1 may be an interesting element, providing a view to the endocrinological aspect of carrying excess visceral fat.

Besides abdominal obesity, some findings have implicated IGF-1 in insulin resistance/type 2 diabetes (Heald et al. 2003) and cardiovascular disorders (Colao et al. 2005). It seems that the metabolic disturbances typical of these conditions provoke the possibility of a reduced IGF-1 availability, thereby explaining for the association

(Abbas et al. 2008). As for the therapeutic potential, some consider the prospects quite positively (Berryman et al. 2013) while others emphasize that therapies targeting growth hormone/IGF-1 –axis should be limited only to clinical diseases and/or to compensate for a possible deficiency (Puche and Castilla-Cortázar, 2012).

Testosterone

Until the age of 55 years, adult male serum total testosterone concentrations remain relatively constant (Vermeulen et al. 1996). Thereafter, the rate of decline has been suggested to be an entity of several different potential contributors, such as BMI (Vermeulen et al. 1996). A considerable volume of evidence indicates that cardiovascular morbidity and low levels of serum testosterone are associated. (Mäkinen et al. 2005; Jones and Saad, 2009; Hu et al. 2011; Empen et al. 2012). In fact, a similar kind of association seems to extend to all-cause mortality as well (Lehtonen et al. 2008; Haring et al. 2010). Mechanistically, one scenario explaining this finding is that low serum testosterone may impair endothelial function (Empen et al. 2012). Additionally, low free testosterone may be associated with higher intima-media thickness (Mäkinen et al. 2005; Tsujimura et al. 2012). There seems to be an interaction between testosterone and lipid metabolism as well, as decreased concentrations of serum testosterone may co-exist with low HDL-, high LDL levels or hypertriglyceridemia (Mäkinen et al. 2008; Monroe and Dobs, 2013). Accordingly, high concentrations of testosterone in young and middle-aged men are accompanied by beneficial impacts on insulin and lipid profile, indicating benefits in cardiovascular health (Firtser et al. 2012). There has been, however, some inconsistencies in the actual role of steroid hormone metabolism, potential atheroprotection and serum lipids (Gyllenborg et al. 2001; Kelly and Jones, 2013).

What has remained less explored is the possible connection between serum testosterone and oxidative stress, both independently recognized as atherosclerosis risk factors. The existing few studies suggest that low serum testosterone seems to co-exist with elevated concentration of oxLDL (Barud et al. 2002; Kosola et al. 2013b). Furthermore, the exact details of the potential benefit of testosterone administration in atherosclerosis risk reduction among middle-aged or aging men, sustain some unanswered questions (Monroe and Dobs, 2013). For example, as data on the beneficial effects of testosterone supplementation on body composition seems consistent, the issue of administering testosterone and insulin resistance, on the contrary, warrants further studies (Isidori et al. 2005; Emmelot-Vonk et al. 2008). Therefore, although the supplementation of certain hormones (growth hormone, testosterone) might mitigate the frailty-related consequences of aging, improvements in body composition may occur at the expense of possibly compromising insulin and glucose metabolism (Blackman et al. 2002).

Although a substantial decline in fertility factors typically characterizes female middle-age, it has been known for decades, that indices of male fertility are on an obvious decline as well (Vermeulen, 1993). Naturally, what is highly interesting in this phase of life are the possible alternatives to mitigate the process. As noted earlier, low levels of testosterone may be associated with some health risks. Certain lifestyle factors have

achieved significant success from the andropause perspective (Kratzik et al. 2009), such as losing excess weight (Niskanen et al. 2004) while some less confirming findigs also exist (Ponholzer et al. 2005). Furthermore, some data indicate, that aging is the strongest determinant of androgen bioavailability in men aged 70 or older (Allen et al. 2002). It is, therefore, obvious that the connection between lifestyle factors and androgen metabolism very likely would benefit from further scientific exploration and scrutiny.

2.2.5. Smoking

Of the entirety of cardiovascular disease risk factors, smoking is among the trickiest to understand because it is preventable, but yet even today, it kills six million people per year (Messner and Bernhard, 2014). An increased risk to develop a variety of compromising cardiovascular conditions applies to all forms of tobacco: cigarettes, chewing tobacco/snuff and smoking cannabis (Katsiki et al. 2013). Considering the intensity of global efforts to cut down smoking, its current prevalence figures are staggering (Yanbaeva et al. 2007). Therefore, urgency still describes the actions needed to overcome the problem (Asia Pacific Cohort Studies Collaboration, 2005). Particularly compelling are findings that the extent to which second-hand smoke burdens the cardiovascular system, is 80 to 90 percent of the effects of first-hand smoke (Barnoya and Glantz, 2005). The contagiousness of the adverse effects of second-hand smoke worldwide has reached proportions that cannot be ignored. In 2004, 40 percent of children, 33 percent of male and 35 percent of female non-smokers were exposed (i.e., had a smoking parent or spouse or had exposure at workplace) (Öberg et al. 2011). Of these, the single largest disease burden was lower respiratory tract infections (5,9 million cases per year) among children less than five years of age. The burden from ischaemic heart diseases in adults was the second largest (Öberg et al. 2011).

A single puff of cigarette smoke (gas-phase) contains approximately 10^{15} radical molecules (Pryor and Stone, 1993). They induce a variety of chemical reactions that induce a complex load for the metabolic capacity to overcome oxidative burden. A considerable body of evidence has shown that oxidative factors, to a critical extent, may explain the undisputed cardiovascular dysfunction associated with smoking (Ambrose and Barua, 2004; Yamaguchi et al. 2005). One form of oxidative burden is the peroxidation of lipids (Ambrose and Barua, 2004). In fact, peroxidation of lipids *via* smoking has been proposed as a potential major culprit in explaining the overall acceleration in atherosclerosis development connected to smoking (Santanam et al. 1997). A formation of dysfunctional connection between actions of HDL-c and LDL by introduction of cigarette smoke has also been proposed (Nishio and Watanabe, 1997). An additional adversity of smoking, from the dyslipidemic perspective, is the finding that smoking tends to lower HDL cholesterol levels. Logically, the improvement in cardiovascular disease risk profile by cessation of smoking (Jiang et al. 2010) could be, in part, accounted by an increase in HDL-c (Fortmann et al. 1986).

In a physiological sense, aging provides a continuum of events whereby different organs and metabolic pathways incessantly aim at reaching a new balance. In the

framework of reproductive hormones, its definitive course of action may be put forth as early as the fetal stage (Juul et al. 2014). Men who are exposed to smoking while *in utero*, after controlling for man's own smoking habits, tend to have smaller testis size and have 20 to 25 percent reductions in sperm volume and count (Jensen et al. 2004). In this respect, the role of maternal smoking in paving the way for the reproductive development of the male offspring further in life may outweigh man's own choices. The range of repercussions related to smoking are, therefore, potentially very long-term and diverse (Jensen et al. 2004).

2.2.6. Interleukin-6

The evolution of an atheromatous plaque involves inflammatory properties and processes, initiating from the involvement of monocytes and macrophages in foam cell formation. Interleukin-6 (IL-6) is involved in a variety of cascades relevant in the combination of inflammation and atherosclerosis development (Yudkin et al. 2000). It has a particularly pivotal role in coordinating the entrance of inflammatory cells to the atherosclerotic lesion (Hartman and Frishman, 2014). Furthermore, interestingly, both visceral and subcutaneous adipose tissues produce IL-6, the magnitude of production being some three-times greater for visceral (Mohamed-Ali et al. 1997; Fried et al. 1998). Favourable lifestyle changes may have a reducing effect on the level of IL-6 (Urpi-Sarda et al. 2012; Richard et al. 2013).

2.3. OXIDIZED LDL LIPIDS IN ATHEROSCLEROSIS

2.3.1. Definition of oxidized LDL

Approximately 75-78 percent of LDL is lipids, including cholesterol esters, phospholipids, unesterified cholesterol and triglycerides (Scanu and Wisdom, 1972). Some of LDL particles undergo oxidative modification, and in this process diene conjugation – reorganization of carbon bonds – occurs in the lipid moieties of LDL particles. Under this chemical reaction of oxidative load or stress, the structural configuration of an LDL molecule is altered, and is termed oxidized LDL (oxLDL). Apoprotein B – a protein that mediates LDL particles uptake from the circulation by the liver – is also affected in this procedure (Rice-Evans et al. 1992; Mackness et al. 2000). These structural modifications are considered as damage, potentially transforming native LDL into oxLDL. After oxidation has reached a certain threshold of severity, it makes macrophages in the arterial wall to recognize only the oxidized form with their scavenger receptors. These events are a prelude in a cascade that concludes into an atheromatous plaque occluding the arterial wall. In addition to the scavenger receptors in macrophages, the vascular endothelial cells also exhibit receptors (LOX-1) for oxLDL (Sawamura 1997; Hoshikawa 1998). The accumulation of oxLDL into the arterial wall, together with cholesterol esters, calcium and immigration of smooth muscle cells, by these mechanisms stiffens the arteries, justifying the name atherosclerosis.

Because severe oxidation of LDL in circulation is prevented by means of antioxidants, it is logical to assume that the circulating oxLDL represents mostly LDL that is oxidized but only minimally in extent, so that the permanent lodging into the vascular wall has not yet been possible. Simultaneously, the extensively oxidized LDL particles may be so rapidly cleared from the circulation *via* the reticuloendothelial system, that their detection *via in vivo* sampling is virtually impossible (Levitan et al. 2010). It should be noted, that these minimally oxidized LDL particles may still be recognized by the LDL receptor which recognizes native LDL as well. As these mildly oxidized LDL particles undergo further oxidation, ending up being maximally, fully or extensively oxidized, they ultimately get taken up by the scavenger receptors in macrophages and smooth muscle cells, and become lodged into the endothelium (Juul et al. 1996; Glass and Witztum, 2001, Levitan et al. 2010). However, due to the heterogeneous nature, no definition exists for the actual molecular/chemical properties for either minimally or for extensively oxidized LDL particles. The pathways, by which LDL becomes modified, consist of both enzymatic and non-enzymatic reactions. Lipid peroxidation is the foremost reaction in a lipoxygenase-enzyme and free radical – delivered pathways (Levitan et al. 2010).

2.3.2. Methods of measuring oxidized LDL

There are several methods available for estimating or measuring the level of oxLDL in the circulation. One of these is an immunological method, where oxLDL is considered as an antigen – a stimulator in the system for antibodies to attack against. OxLDL, while taken up by scavenger receptors in macrophages, thus expresses immunological properties. The lack of precise definition for oxLDL makes it possible to generate oxLDL *in vitro via* a variety of reagents and regard it as an antigen amidst the immunological system to which antibodies can be manufactured. However, autoantibodies against oxLDL do not seem to be a suitable parameter for detecting rapid changes in oxidative stress, thus weakening the basis from interventional usage. After an antigen has been introduced and antibody production initiated, neither the process rate nor the lifespan of antibodies are known. Lack of such experience is a serious defect considering scientific experimentation. Furthermore, autoantibodies exist in many diseases other than atherosclerosis (Steinerova et al, 2001). There is also evidence to suggest that antibodies against oxLDL are not a reliable indicator of oxidative damage (Miller et al. 2005). Perhaps most importantly, the immunological method in oxLDL analysis offers no structural or compositional information of the oxLDL particles *in vivo* (Levitan et al. 2010). Taken together, due to these inadequacies, antibodies against oxLDL as a method can been deemed as insufficient to meet the demands of our study principles and objectives.

Another tool to get insight into oxidative stress milieu is the measurement of F2-isoprostanes. F2-isoprostanes can be measured from either plasma or urine and are generated in peroxidation of arachinoid acid – a fatty acid being a precursor of eicosanoids and found in cell membranes. Even though F2-isoprostane levels are elevated in hypercholesterolemia (Reilly et al. 1998), and seem to stand out as a risk

factor for CHD (Davies et al. 2011) the method suffers from lack of specificity. Although mechanistically similar, systemic lipid peroxidation should not be confused with peroxidation taking place in a confined locus, such as lipid compartment of a certain lipoprotein particle. Furthermore, urinary measurement of F2-isoprostanes has the vulnerability and dependency of urinary or kidney function to be considered. This may compromise the method's applicability in circumstances with impaired kidney function, which is, for example, remarkably common in diabetic patients (Metsärinne et al. 2014). Of all the lipid peroxidation in fatty acids at different metabolic environments, the lipids especially in LDL particles are of supreme importance in atherosclerosis development due to the key role of LDL in atheromatous plaque evolution. However, collection of data by Davies et al. (2011) elucidates the role of lipid peroxidation in CHD risk. Although not connected to modification of LDL particles, lipid peroxidation is an important encouragement for the overall importance of lipid metabolism in health and disease. All in all, to consider F2-isoprostanes as a relevant window to oxidative events in LDL particles - and thereby to lipoprotein-related atherosclerosis risk - would not be within the limits of reason.

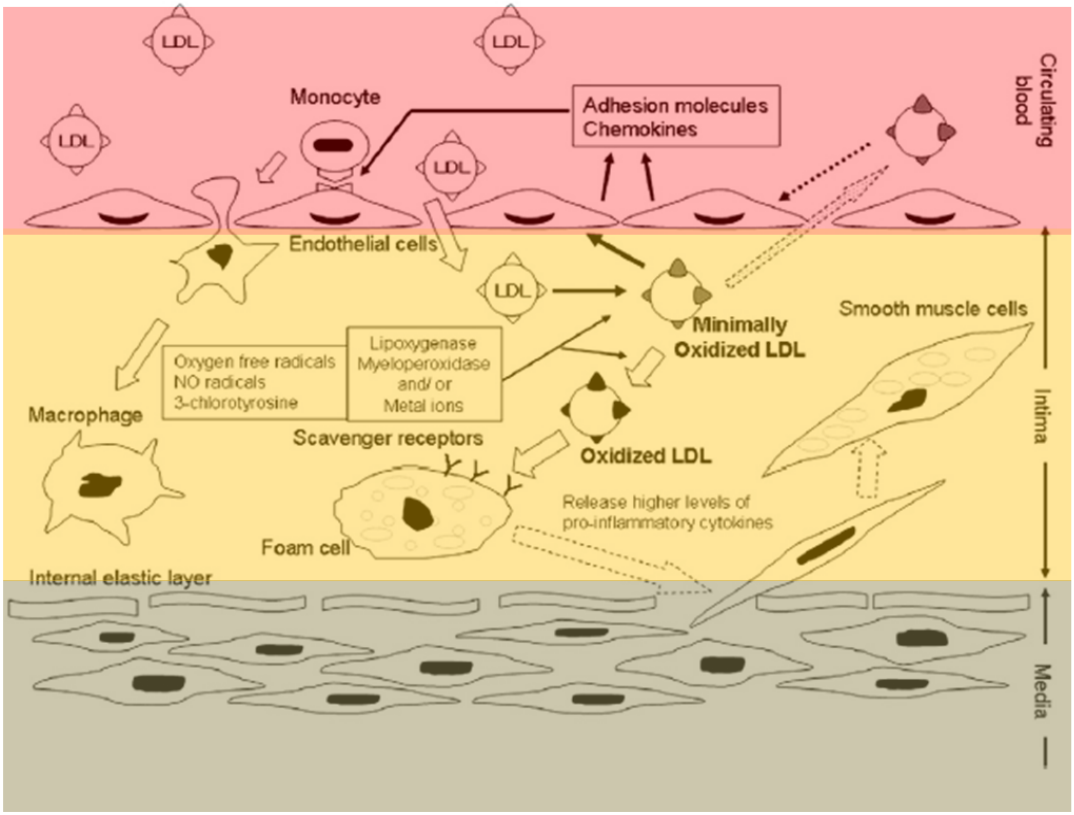
In contrast to these, measurement of oxidized LDL lipids is based on quantifying diene conjugates – an outcome of oxidative modification in certain fatty acids in LDL described earlier – and this measurement lacks the shortcomings of the above methods. Additionally, studies on the storage conditions of serum samples have shown that, when kept at -80 C, the levels of oxidized lipoprotein lipids are not changed during prolonged (up to 8 years) follow-up (Kresanov et al. 2013). First, it is a method that measures oxidatively modified lipids in LDL particles *in vivo*, thus being a specific method. Second, oxidation particularly in the lipid compartment of LDL has a substantial role in its being recognized by the scavenger receptor (Nicholson et al. 1995, Hörkkö et al. 2000). Third, this method is simple to perform with the general facilities of a standard laboratory (Ahotupa and Vasankari, 1999). Fourth, it is suitable for a variety type of study protocols, including interventions. Fifth, the data thus far has confirmed its feasibility as a parameter reliably reflecting atherosclerosis risk (Toikka et al. 1999, Vasankari et al. 2001, Ahotupa et al. 2010).

2.3.3. Oxidized LDL lipids as a risk factor of atherosclerosis

Often, studies show that oxLDL is a crucial parameter in evaluating atherosclerotic disease (Toshima et al. 2000, Faviou et al. 2005, Meisinger et al. 2005). CD36, a type of receptor found in many cell lines in humans, for example, monocytes and endothelial cells, plays a crucial role in the development of atherosclerosis. OxLDL has been observed to have the necessary characteristics of acting as a ligand for this receptor – a conception more than two decades old (Endemann et al. 1993). Furthermore, CD36 seems to act as the scavenging element in initiating foam cell formation (Silverstein and Ferraio, 2000). Of particular importance is a finding that the binding site in oxLDL, by which it actually contacts with the scavenger receptor in macrophages, seems to be located in the lipid compartment (Silverstein and Ferraio, 2000). These findings highlight the importance of not just the lipid compartment in

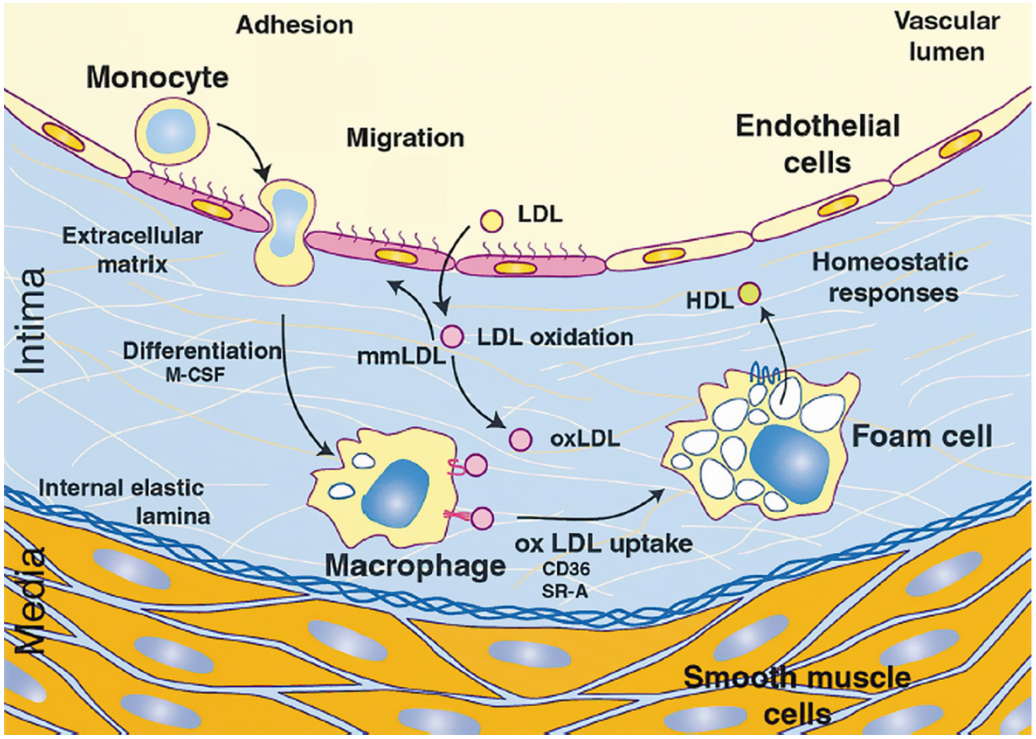
LDL particles, but also the role of modifications in these lipids, as native LDL – unlike oxLDL – has no necessary properties to launch its own unregulated uptake by the reticuloendothelial system.

Numerous studies have corroborated the suitability of measuring oxidized LDL lipids as an atherosclerotic cardiovascular risk factor (Toikka et al. 1999, Vasankari et al. 2001, Kosola et al, 2013a,b). Further highlighting the clinical relevance, after an onset of a sudden atherosclerotic event such as acute myocardial infarction, consistently high levels of ox-LDL have been predictive of a restenosis (Naruko et al. 2006). The caution needed in interpreting studies with oxLDL as main outcome measure, in addition to the existing methodological differences, concerns the influential effects of varying lifestyle habits in oxLDL metabolism (Verhoye et al. 2009). However, as lifestyle factors may contribute to the oxidation events implicating LDL, with potential confounding role needed to address in scientific papers, correspondingly, healthy choices may also serve as tools for improvement. Consumption of some berries, for example, decreases lipid peroxidation and LDL oxidation (Basu et al. 2010).



Adapted with the publisher's permission from Yoshida and Kisugi, 2010.

Figure 1. Schematic view of actions relevant to atherosclerotic lesion development involving LDL particle within the arterial wall structures.



Adapted with the publisher's permission from Glass and Witztum, 2001.

Figure 2. Interaction between oxLDL, macrophages and involvement of HDL as a mediator of antiatherogenic actions.

3. AIMS OF THE STUDY

At present, Western lifestyle propels serious challenges to maintaining health throughout life. An increasing prevalence of obesity, difficulties in reducing and maintaining a healthy weight, disturbances of insulin or testosterone metabolism, smoking and the longest life expectancy ever in history are examples of phenomena, by which health issues can be threatened and become increasingly chronic. Modern circumstances, such as sedentary lifestyle, inadequate cardiorespiratory fitness or poor nutrition quality must therefore be taken seriously, as they provide the possibility of a significant cumulative daily effect, which may emerge through a prominent waist line, insulin resistance or accelerated decline in serum testosterone with age. Amidst these conditions, risk factors either elucidating the disease risk mechanisms, or having prognostic value, might be of value. For cardiovascular diseases, considering the globally substantial prevalence and incidence, these factors are especially crucial. Lastly, research focusing on potential means to overcome these unhealthy consequences as sustainably as possible (for instance, weight management *via* exercise or diet) and further exploring the big (metabolic) picture to gain as full a view as possible, are needed.

Therefore, the main aim of this thesis was to investigate the interconnectivity of a relatively novel biomarker of atherosclerosis, oxLDL, to the milieu of traditional cardiovascular disease risk factors.

The specific aims were:

I. To investigate the trends in oxLDL in weight reduction and subsequent weight maintenance, with a particular interest for success in the latter

II. To study if a combination of smoking with low serum total testosterone – both known atherosclerosis risk factors – would be indicated in concentrations of oxLDL, and if there were differences to normotestosteronaemic and non-smoking men

III. To explore if oxLDL either alone or in relation to other lipid markers, stands out as having a prognostic value in an all-cause mortality setting in the elderly

IV. To examine the association between oxLDL and insulin resistance

V. To inspect the trends in oxLDL by increasing waist circumference

4. SUBJECTS AND METHODS

Subjects were derived from four different sets of data, which in total means 2337 participants. Men formed the majority of subjects (69%) with an age span of young adults to senior elderly (range 18-97 years). The women in the data were all elderly females from Lieto, aged 64-100 years.

4.1. SUBJECTS AND DATA COLLECTION

4.1.1. Obese middle-aged men in Tampere

This data is based on a group of obese, middle-aged men with the following inclusion criteria: age 35 to 50 years, BMI 30 to 40 kg/m^2 and waist circumference of greater than 100 cm (Borg et al. 2002). Subjects with regular medication, physical activity once per week or more, smoking habit or suspected binge eating disorder were excluded. 214 men answered the newspaper advertisement, and of these 90 met the study participation criteria. The study design was based on five measurements: baseline, after weight reduction (2 months from baseline), after weight maintenance period (eight months), after one-year of follow-up (20 months), after two-year of follow-up (31-32 months). All 90 subjects completed the weight reduction period of two months. Of these, 82 (91%) finished the weight maintenance phase. A total of 72 (80%) finished the first follow-up year and 68 (76%) the second follow-up year. Full data from those, of whom oxidized LDL and insulin concentration was available, was 67 (74%). The local Commission of Ethics approved of the study protocol. For further details, see original publication no. I and II.

In addition to exploring the study group as a whole, from the 67 participants, a subgroup analysis was performed: 20 subjects most successful in weight maintenance during the 2.5 years of follow-up after the weight reduction period constituted subgroup 1 (S1) and the remaining 47 constituted subgroup 2 (S2). During the 2.5 year-follow-up, the maximum increase in weight for S1 was +6.2 kg and +23.1 kg for S2. An illustration of study design is given in figure 3. For subjects' characteristics, see table 1.

Blood glucose was measured from fresh venous samples between 0800 and 0900 after a 12 h fast. A 2-h oral glucose tolerance test (OGTT) was performed with sampling at 0, 30, 60 and 120 min with a dose of 75 g glucose. Serum concentrations of cholesterol and triglycerides (TG) were analyzed by enzymatic methods (CHOL-PAP for cholesterol and GPO-PAP for TG, Boehringer Mannheim, Mannheim, Germany). High-density lipoprotein cholesterol (HDL-c) was measured by selective precipitation (dextran sulfate) (Nguyen and Warnick, 1989). LDL-c was estimated as presented by Friedewald et al. (1972) Body weight was measured using a high-precision scale (F150S-D2, Sartorius, Goettingen, Germany). The subjects wore light underwear while

being weighed. Waist circumference (WC) was measured in the horizontal plane halfway between the lowest rib and the suprailiac crest. Hip circumference was measured at the horizontal level of the tips of greater trochanters and the average of three measurements was used. Fat mass (FM) was measured by underwater weighing after full exhalation by the subject. Body composition was calculated by a two-component model (Siri et al. 1956). A random zero sphygmomanometer was used to measure blood pressure (Hawksley & Sons Ltd, Lancing, Sussex, England). The mean of two measurements was used.

Table 1. Means (SD) of study participants, baseline (obese middle-aged men).

	Subjects		
Variable	**All**	**Subgroup 1**	**Subgroup 2**
Age	42,7 (4,6)	43,1 (5,7)	42,5 (4,2)
BMI	32,8 (2,6)	32,7 (2,2)	32,8 (2,8)
Waist girth (cm)	112,6 (7,2)	111,7 (5,1)	112,9 (8,0)

(For further details, see original publication no. I)

4.1.2. Aging men in Turku

The original data, to which our substudy is based on, consisted of 600 men 40-70 year-old (100 men from each five-year age group) living in the Turku City area, who were randomly chosen from the Population Registry. To these men, a questionnaire (validated by Pöllänen et al. 2000) regarding medical history, sociobehavioral factors, education, lifestyle habits (amount of exercise, smoking, alcohol consumption) and life satisfaction was sent by post. A total of 336 men (56%) answered. Blood samples were collected from 214 men, who came to the laboratory. The data collection lasted from December 1998 to March 2000. The project's main focus was on the frequency, diagnosis and treatment of the sex-hormone related conditions of aging males. The study was approved by the local Commission of Ethics. For some further details, see original publication no. III.

Sufficient data of smoking habits and blood samples with measurements of lipids, sex hormones and oxidized LDL was available from 165 men. Our study design was a cross-sectional setting, in which the lipoproteins and sex hormones were compared between smokers and non-smokers. Additionally, the joint effect of smoking plus hypotestosteronaemia was investigated. An outline of the study design is given in fig. 4. For subjects' characteristics, refer to table 2.

Lipid analyses took place in the Turku University Central Hospital. All serum lipids were determined from fresh Samples with a Hitachi 717 chemistry analyser. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TG) were measured enzymatically with the use of Boehringer (triglyceride) and Bio Merieux (TC and HDL-c) reagents. Luteinizing hormone (LH) and sex hormone

binding globulin (SHBG) were analysed by timeresolved immunofluorometric assay (Wallac PerkinElmer, Turku, Finland). Testosterone and estradiol concentrations were analysed by Spectria radioimmunoassay (Orion Diagnostica, Helsinki, Finland). Free testosterone (fT) concentration was calculated using the Anderson equation (Anderson et al. 1975) by which the non-SHBG-bound fT was calculated as follows: proportion (%) of fT (fT%)_2.28_1.38_log(SHBG nmol/L/10), and serum fT (pmol/L)_fT%_T (nmol/L)_10. The inter-assay precision of the assays were: for testosterone: 6.9% (21.6 nmol/L), for estradiol: 5.3% (0.136 nmol/L), for LH: 4.5% (0.4 U/L), and for SHBG: 7.0% (10 nmol/L), respectively. Analysis of oxidized LDL lipids was based on determination of the base-line level of conjugated dienes in LDL lipids. Blood pressure was measured twice in the sitting position with a standard mercury sphygmomanometer (Omron HEM-705CP, Japan). The arithmetic mean of the two measurements of systolic and diastolic blood pressures (SBP, DBP) was used in the analyses. Weight was measured by a high-precision scale (Seca, Germany) in light underwear clothing.

Table 2. Means (SD) of study participants (aging men in Turku). No outliers excluded.

Variable	Smokers (n=34)	Non-smokers (n=132)
Age	53,6 (7,5)	58,8 (8,1)
BMI	27,7 (5,4)	26,9 (3,2)
Serum testosterone	16,6 (4,5)	14,8 (4,3)

(For further details, see original publication no. III)

4.1.3. Elderly men and women in Lieto

The data consists of inhabitants of Lieto, a semi-industrialized rural municipality in southwestern Finland. During March 30. 1998 – September 22. 1999, all Lieto residents 64 years of age or more (born 1933 or earlier) were invited. This group of elderly formed 12 % of the entire population in Lieto in January 1998. Of the 1596 elderly inhabitants, 1260 subjects (79%) participated and gave their informed consent, which consisted of 533 men (42%) and 727 women. Compared to the entire population of men and women in Lieto at the time, 80% of elderly men and 78% of elderly women attended the study. Five percent of the population consisted of individuals, who were institutionalized. There were 61 men and 34 women (12% of men and 5% of women, respectively) who reported themselves as current smokers. The medical histories (frequencies of different disease categories) of the participants at the time of data collection in 1998-1999 are outlined in table 3. For some further details, see original publication no. IV.

The mean age of the study population was 74 years, ranging from 64-100 years. For men and women separately, the mean age was 72.7 and 74.1 years, respectively. Laboratory analyses (early-morning fasting venous blood samples) were performed in

the Central Laboratory of Turku University Central Hospital. Oxidized LDL measurements took place in 2003.

The study data was collected by three to five visits to the local health care centre. The original research design was a community-based epidemiological survey. This cross-sectional data was, however, completed with a review of medical records in January 2009, enabling also a prospective view of approximately ten years' duration on the data. The study protocol is outlined in figure 5. The study protocol was approved by the Joint Ethics Committee of Turku University and Turku University Central Hospital.

To discriminate patients with cardiovascular disease (CVD) from subjects free of disease, we used ICD-10–codes (Rose et al. 1982). Of the 1260 participants, 380 had been diagnosed with either coronary heart disease (n=103, ICD-10 codes I20-23), cerebrovascular disease (n=93, ICD-codes I61, I63-64, I691, I693-4, I698) and/or they had undergone a major surgical procedure of the coronary arteries (n=38). Of these 380 patients, 187 subjects (49%) were diagnosed during the 10-year period.

Ten most prevalent underlying and immediate causes of death are listed in tables 4 and 5. Immediate causes of death were available from 377 subjects (175 men, 202 women). By January 2009, the mean age (SD) of subjects surviving the follow-up was 81.0 (4.8) years. The mean age of the deceased by the time of their death was 82.6 (7.2) years.

Table 3. Prevalence of major disease categories in Lieto elderly.

ICD-10 disease category	Men (n/% of men)	Women (n/% of women)	Total (n/% of all)
Malignant tumors (C00-C97)	50/9.3%	77/10,6%	127/10,1%
Blood and blood forming organs (D50-D89)	30/5.6%	38/5,2%	68/5,4%
Endocrine, nutritional and metabolic (E00-E90)	218/40,9%	360/49,5%	578/45,9%
Mental and behavioral (F00-F99)	131/24,6%	268/36,9%	399/31,7%
Nervous system (G00-G99)	108/20,3%	180/24,8%	288/22,9%
Eye and adnexa (H00-H59)	79/14,8%	181/24,9%	260/20,6%
Ear and mastoid process (H60-H95)	167/31,3%	146/20,1%	313/24,8%
Circulatory system (I00-I99)	328/61,5%	455/62,6%	783/62,1%
Respiratory system (J00-J99)	140/26,3%	123/16,9%	263/20,9%
Digestive system (K00-K93)	186/34,9%	279/38,4%	465/36,9%
Skin and subcutaneous tissue (L00-L99)	52/9,8%	69/9,5%	121/9,6%
Musculoskeletal system and connective tissue (M00-M99)	337/63,2%	518/71,3%	855/67,9%
Genitourinary system (N00-N99)	185/34,7%	162/22,3%	347/27,5%

Table 4. Ten most prevalent underlying causes of death (n=467; 213 men, 253 women).

ICD-10 code	Men (n/% of men)	Women (n/% of women)	Total (n/% of all)
Atherosclerotic heart disease (I25.1)	23/11,0%	18/7,1%	41/8,8%
Dementia, unspecified (F03)	10/4,7%	28/11,1%	38/8,1%
Cerebral infarction, unspecified (I63.9)	3/1,4%	15/5,9%	18/3,9%
Acute myocardial infarction, unspecified (I21.9)	9/4,2%	9/3,6%	18/3,9%
Alzheimer's disease with late onset (G30.1)	5/2,3%	7/2,8%	12/2,6%
Chronic ischaemic heart disease, unspecified (I25.9)	5/2,3%	7/2,8%	12/2,6%
Acute transmural myocardial infarction of anterior wall (I21.0)	8/3,7%	3/1,2%	11/2,4%
Malignant neoplasm of prostate (C61)	10/4,7%	-	10/2,1%
Hypertensive heart disease with (congestive) heart failure (I11.0)	1/0,5%	9/3,6%	10/2,1%
Old myocardial infarction (I25.2)	8/3,7%	2/0,8%	10/2,1%

Table 5. Ten most prevalent immediate causes of death (listed from 377 subjects; 175 men, 202 women)

ICD-10 code	Men (n/% of men)	Women (n/% of women)	Total (n/% of all)
Atherosclerotic heart disease (I25.1)	21/11,9%	16/8,0%	37/9,8%
Pneumonia, unspecified (J18.9)	17/9,7%	10/5,0%	27/7,2%
Dementia, unspecified (F03)	6/3,4%	19/9,5%	25/6,6%
Cerebral infarction, unspecified (I63.9)	3/1,7%	13/6,5%	16/4,2%
Chronic ischaemic heart disease, unspecified (I25.9)	5/2,8%	7/3,5%	12/3,2%
Old myocardial infarction (I25.2)	7/4,0%	3/1,5%	10/2,7%
Acute myocardial infarction, unspecified (I21.9)	5/2,8%	3/1,5%	8/2,1%
Heart failure, unspecified (I50.9)	2/1,1%	6/3,0%	8/2,1%
Bacteropneumonia, unspecified (J18.0)	5/2,8%	3/1,5%	8/2,1%
Other specified chronic obstructive pulmonary disease (J44.8)	6/3,4%	1/0,50%	7/1,9%

4.1.4. Young adult male reservists in the Finnish Defence Forces

This study population consisted of a cohort of reservists from the Finnish Defence Forces at 2008. The aim of the project was to investigate the reservists' health from perspectives such as cardiovascular and musculoskeletal health, physical activity, ability to function/work and quality of life. Originally, 1155 reservists were invited to attend the refresher courses together with the combination of this study protocol. The invited consisted of men from three different branches of military service: the infantry, anti-aircraft warfare and military engineers, and they were selected with the attempt to gain both nationally and socially as representative of a study group as possible. For some further details, see original publication no. V.

A total of 846 participants, aged 18-48 (mean age 25,1 years), volunteered for the study protocol from the 920 men actually attending the refresher courses. Hence, the total participation rate was 73 percent. The main reasons for absence included: impediment posed by an employer or other work status, studying or health. The data collection took place during the year 2008.

The study population was divided by smoking status using the data achieved through a health questionnaire. The smokers, 323 (38%) men, in this study are those who reported to smoke regularly at the time of the inquiry and the non-smokers, 521 men, are those who answered never to have smoked regularly. As a subanalysis, the smokers and non-smokers were categorised by their waist circumference into three classes: i) less than 90 cm, ii) 90 to 99,9 cm, iii) ≥ 100 cm. Approximately 100 cm is considered as a limit, above which the visceral condition in the abdominal area is considered to be most likely associated with atherogenic and metabolic disorders (Pouliot et al 1994; Wang et al. 2005). The proportion of participants with a waist circumference of 100 cm or more, was 13 percent of smokers and 10 percent of non-smokers. As a second subanalysis, smokers and non-smokers were divided into two groups by the concentrations of serum total testosterone. The study design with the subanalyses is illustrated in figure 6. Subjects' characteristics are described in table 6.

The blood samples were gathered in a fasting state from the antecubital vein by trained personnel using a method of Terumo VenoSafe (Terumo Europe, Leuven, Belgium). During the procedure, subjects were in supine position. At location, a low blood count was measured from blood specimen with EDTA (Sysmex Co., Kobe, Japan). Fasting glucose, total serum cholesterol, HDL, LDL (Friedewald) and triglycerides were analysed during the following day (Konelab 20 Xti, Thermo Electron Co, Vantaa, Finland). Non-SHBG-bound free testosterone (fT) was calculated as follows (Anderson et al. 1975): proportion (%) of fT (fT%) = 2.28 x [1.38 x log(SHBG nmol/L/10)], and serum fT (pmol/l) = [fT% x T (nmol/L) x 10].

Table 6. Descriptives of reservists from Finnish Defence Forces.

Variable	Mean (SD)
Age (years)	25,1 (4,6)
Height (m)	1,80 (0,6)
Weight (kg)	80,6 (13,4)
Waist circumference (cm)	86,3 (10,4)
BMI (kg/m^{-2})	24,8 (3,8)

*) expressed as median due to skewed distribution

For some further details, see original publication no. V.

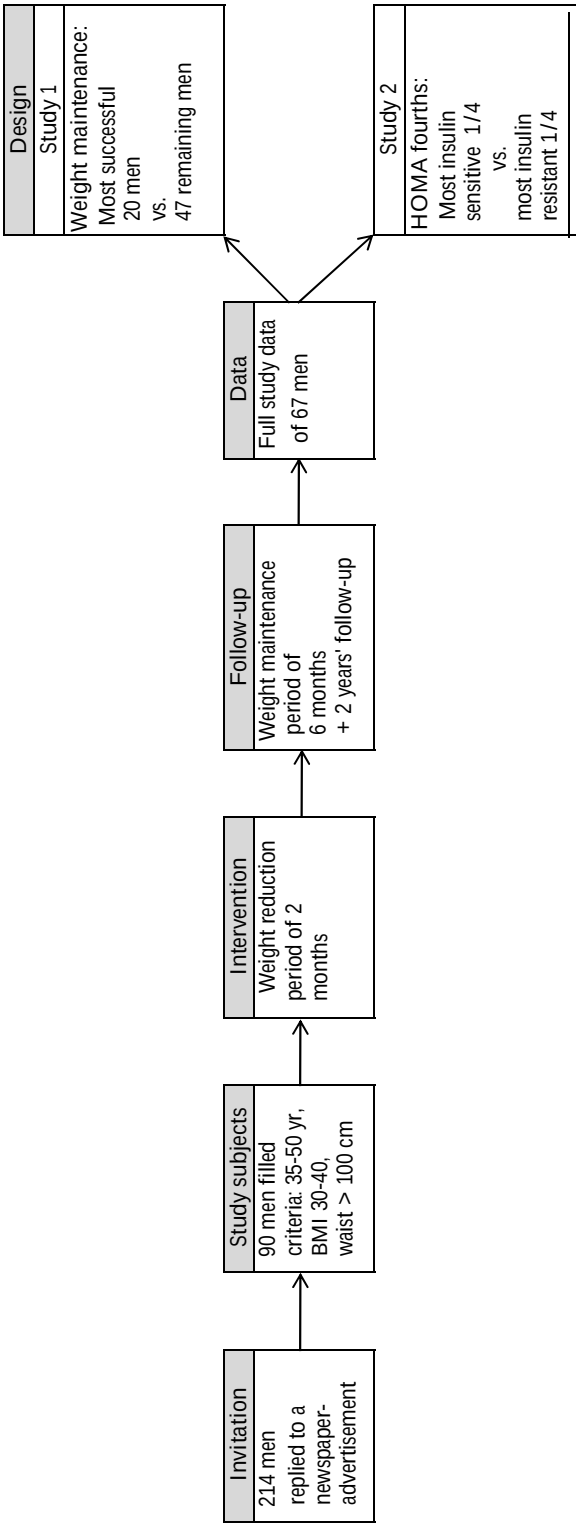


Figure 3. Study protocol of 'Obese middle-aged men in Tampere' (original publications no. I and II).

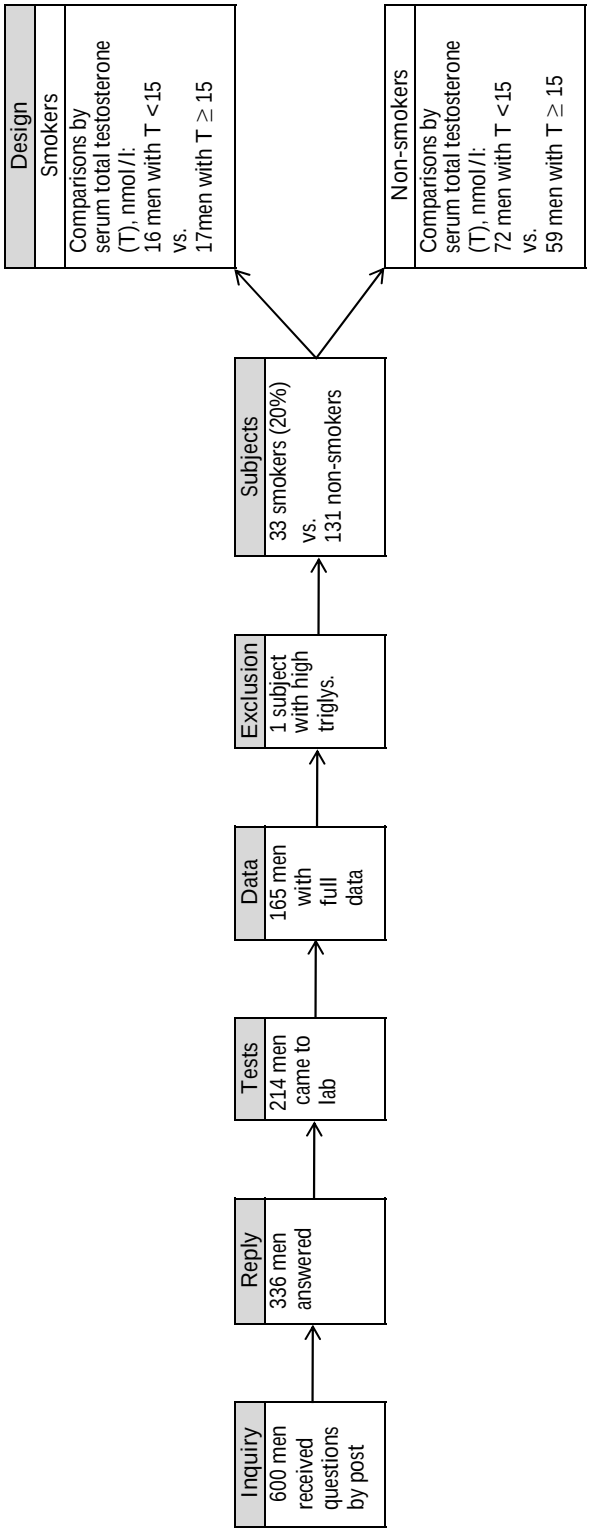


Figure 4. Study protocol of 'Aging men in Turku' (original publication no. III).

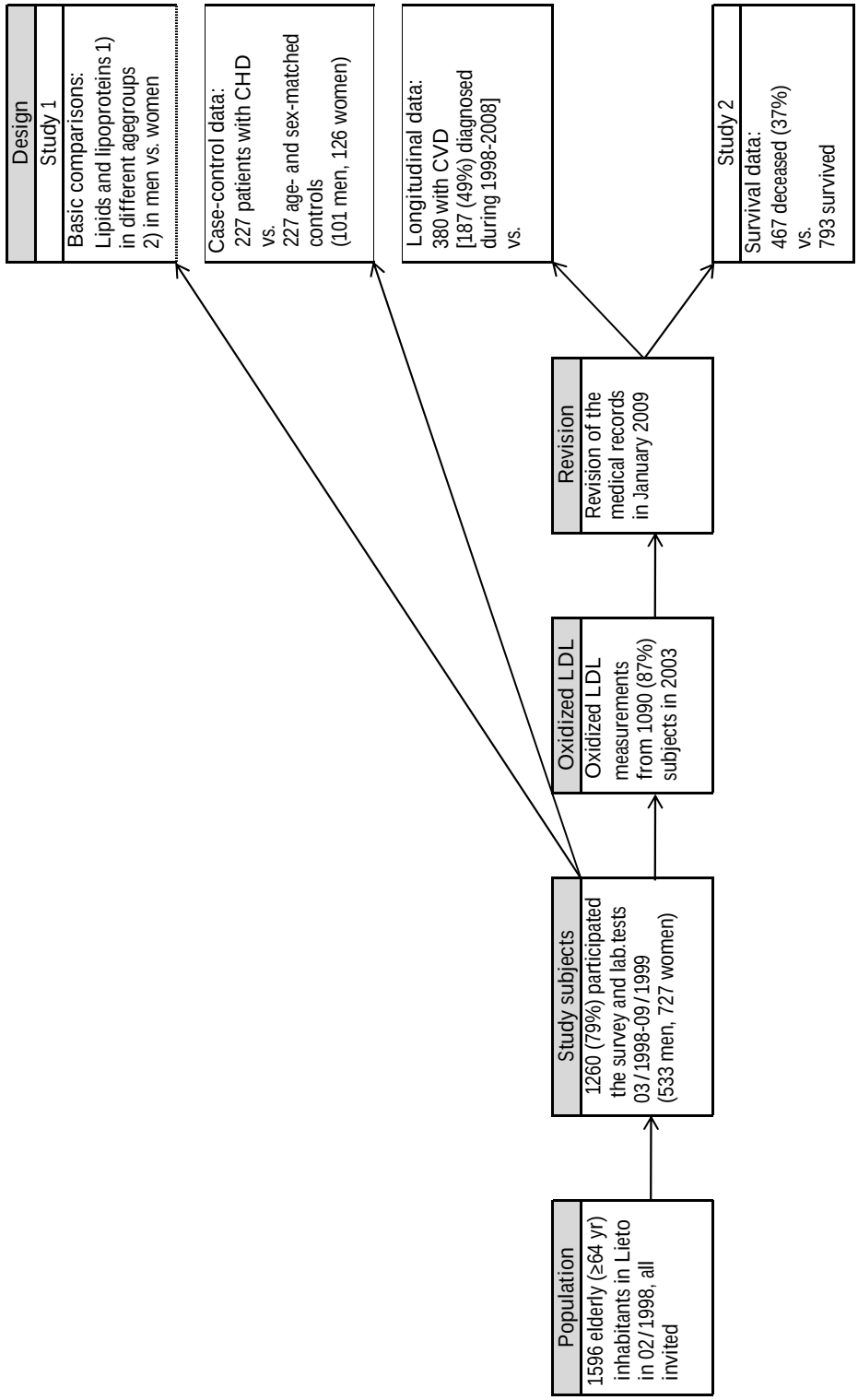


Figure 5. Study protocol of 'Elderly men and women in Lieto' (original publication no. IV).

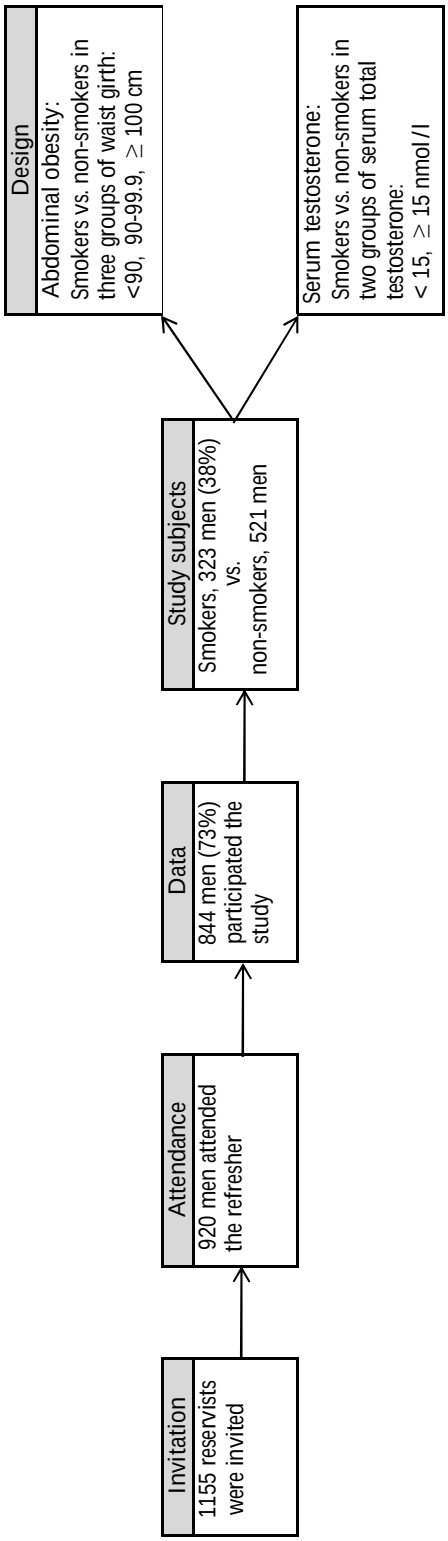


Figure 6. Study protocol of 'Young adult male reservists in the Finnish Defence Force' (original publication no. V).

4.2. METHODS

Analysis of oxidized LDL lipids

As described in chapter 2.3, a variety of methods exists for the purpose of evaluating the level of oxLDL. In addition to the methodological differences making studies quite difficult to compare to one another, the varying thoroughness in reporting the analysis method further complicates the big picture. The data and results described in this thesis are based solely on quantifying oxidized LDL lipids by measuring the amount of conjugated carbon double-bonds, or dienes, in LDL particles sequestered from a venous blood sample.

The main loci where oxidizing events in LDL take place are two polyunsaturated fatty acids called arachidonic acid and linoleic acid. The first metabolite of this oxidation is hydroperoxy derivative of a phospholipid, at which an instant diene formation also occurs. Further oxidation leads to the formation of carboxy or aldehyde derivatives (Levitan et al. 2010).

Consequentially, the analysis of oxidized LDL lipids in this thesis was based on determination of the baseline level of conjugated dienes in LDL lipids. Appearance of conjugated dienes is characteristic of peroxidation of all polyunsaturated fatty acids. In *in vitro* and *ex vivo* studies on LDL oxidation, diene conjugation has been commonly used as the index of LDL oxidation. Validation studies for the assay have ruled out interference by nonspecific substances, and shown that the assay is able to detect oxidative modifications in all LDL lipid classes. The assay method consists of precipitation of LDL, extraction of LDL lipids, and spectrophotometric analysis of conjugated dienes in LDL lipids. Briefly, serum LDL was isolated by precipitation with buffered heparin. Lipids were extracted from isolated LDL by chloroform-methanol (2:1), dried under nitrogen, and redissolved in cyclohexane. The amount of peroxidized lipids in LDL was assessed spectrophotometrically as the amount of diene conjugation (234 nm) (Ahotupa et al. 1998 and 1999).

Homeostasis Model Assessment for insulin resistance

Traditionally, tests measuring the balance between insulin secretion and blood glucose levels (clamp-techniques) have been laborious and time-consuming (Borai et al. 2007). Homeostasis model assessment for insulin resistance, or HOMA (occasionally alternatively abbreviated HOMA-IR), is a simple measure calculated from fasting concentrations of blood glucose and insulin as follows:

HOMA = [fasting glucose (mmol/l) x fasting insulin (mU/l)] / 22.5

This quantification tool for insulin resistance was first introduced in 1985. The fundamental idea is that in the fasting state, the level of hyperglycaemia is reflective of both β-cell dysfunction in the pancreas, and insulin resistance (Matthews et al. 1985). The method may be criticized over its relatively high coefficient of variation, but flattered for its inexpensiveness and strong associations with clamp-techniques. The biological representativeness of a single measurement is challenged, while repetition

may improve its reliability. However, considering the physiological circumstances, it must be remembered that HOMA falsely assumes peripheral and hepatic insulin sensitivity to be identical (Borai et al. 2007). In insulin sensitivity assessment, the authors therefore support log-transformed HOMA as more accurate (Levy et al. 1998; Fukushima et al. 2000).

5. RESULTS

5.1. LIFESTYLE INTERVENTION IN OBESE MEN

5.1.1. Weight reduction period

At baseline, the mean age of study participants was 43 years (range: 34 – 51 years). During the weight reduction period (WRP), the decrements in weight, waist circumference (WC) and fat mass (FM) were 14 % (14,5 ± 4,2 kg), 13 % and 28 % ($p<0.001$ for all), respectively. OxLDL decreased by 22 **%** ($p<0,001$). The reductions in serum lipid concentrations during WRP were 21 % for serum total cholesterol (TC), 23 **%** for LDL-c and 28 **%** for triglycerides ($p<0,001$ for all). HDL-c did not change. Systolic and diastolic blood pressure (SBP and DBP) decreased by 5 and 9 %, respectively ($p<0,001$ for both). 0-h and 2-h glucose decreased by 6 **%** and 9 **%** ($p<0,001$ for both). Further details are presented in the original publication no. I

5.1.2. Follow-up period

From the end of WRP to the end of the two-year follow-up, WC and FM increased by 9 % and 29 % ($p<0,01$ for both), respectively. The corresponding regain in weight was 11 % (9.6 ± 6.2 kg, $p<0,001$). OxLDL increased by 30 **%** ($p<0,001$). Increases in TC, LDL-c and TG concentrations were 27 **%**, 31 **%** and 41 **%** ($p<0,001$ for all). SBP and DBP increased by 8 and 14 %, respectively ($p<0,001$ for both). 0-h and 2-h glucose increased by 7 **%** ($p<0,001$) and 13 **%** ($p=0,021$). During the weight maintenance period (WMP), HDL-c increased by 15 % ($p<0,001$). During the two-year follow-up, HDL-c decreased by 7 **%** ($p<0,001$).

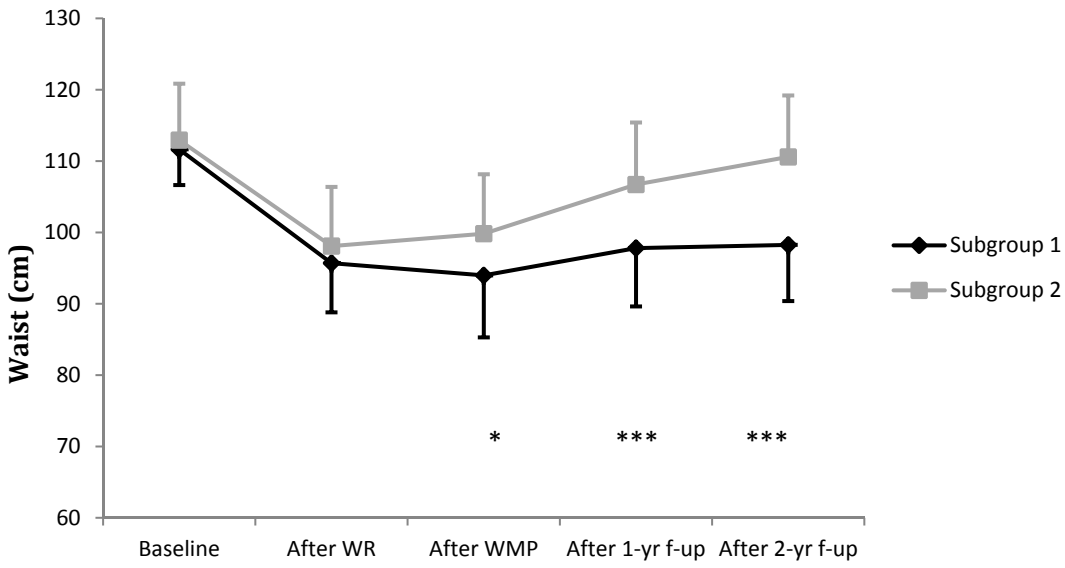
5.1.3. Most successful vs. least successful

During WRP, weight, WC and FM decreased similarly in both subgroups. From the end of WRP to the end of the two-year follow-up, there was a significant increase in weight, WC and FM by 3 **%** (2,2 kg ± 3,0 kg), 3% and 12 **%** in subgroup 1 ($p<0,01$ for all; for WC, see figure 7). In subgroup 2, weight, WC and FM increased by 14 % (12,8 ± 4,1 kg), 12% and 37 **%** ($p<0,001$ for all) (the difference of change between the subgroups for weight, WC and FM $p<0,001$ for all).

During WRP, oxLDL decreased by 17 **%** and 25 **%** ($p<0,01$ for both) in subgroups 1 and 2, respectively (subgroups 1 vs. subgroup 2, NS; see fig. 8). From the end of the WRP to the end of the two-year follow-up, oxLDL did not change in subgroup 1, whereas it increased by 39 **%** in subgroup 2 ($p<0,001$) (the difference between the subgroups: $p=0,008$). Ox-LDL/HDL-c decreased by

During WRP, TC, LDL-C and TG decreased similarly in both subgroups. From the end of the WRP to the end of two-year follow-up, TC and LDL-C both increased in subgroup 1, by 22**%** and 29 **%** ($p<0,001$ for both), whereas TG did not change. In subgroup 2, the respective increases were 30 **%**, 31 **%** and 52 **%** ($p<0,001$ for all) (the differences between the subgroups for TC $p=0,030$ and TG $p=0,005$).

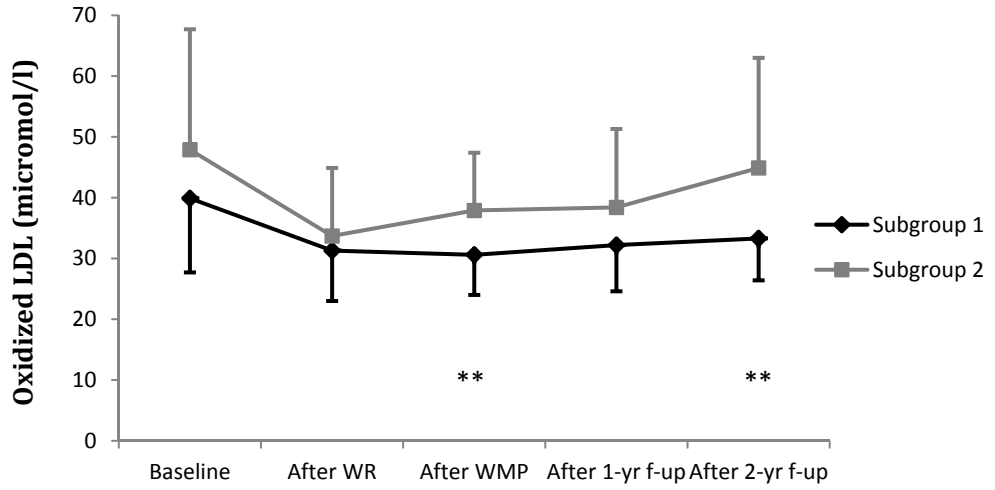
During WMP, HDL-c increased by 16 and 15 **%** ($p<0,001$ for both) in subgroups 1 and 2, respectively (see fig. 9 and 10). Figure 8 illustrates, how the positive effects of WMP on HDL-c indicates a beneficial shaping effect on a risk factor behaviour compared to measuring oxLDL alone. During the two-year follow-up, HDL-c did not change in subgroup 1, while in subgroup 2 it decreased by 9 **%** ($p<0,001$) (the difference between the subgroups $p=0,005$). During WRP and from there on the changes in SBP and DBP were similar between the subgroups. Exceptionally, results for oxLDL/HDL-c are not included in the original publication no. I.



* $p<0,05$

*** $p<0,001$

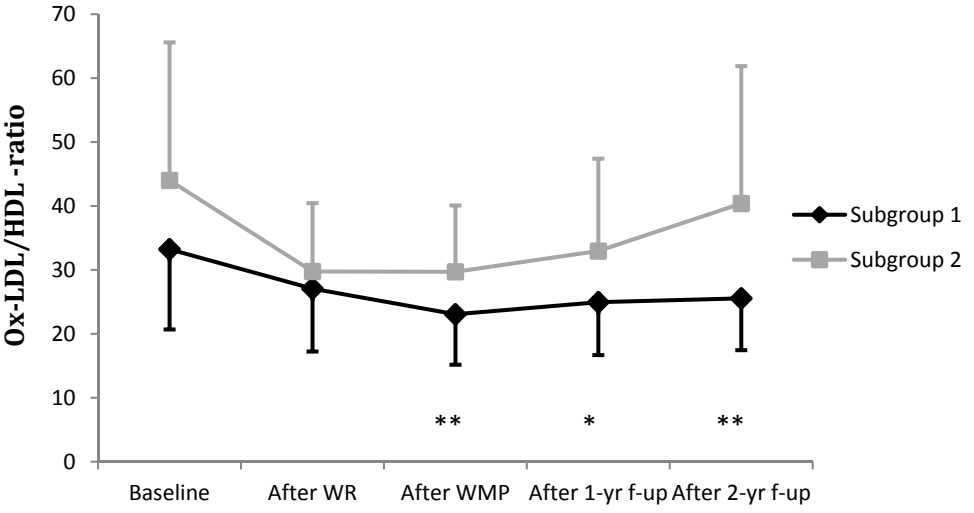
Figure 7. Concentrations in waist circumference during the whole study. Further details are presented in the original publication no. I.



* p<0,05

** p<0,01

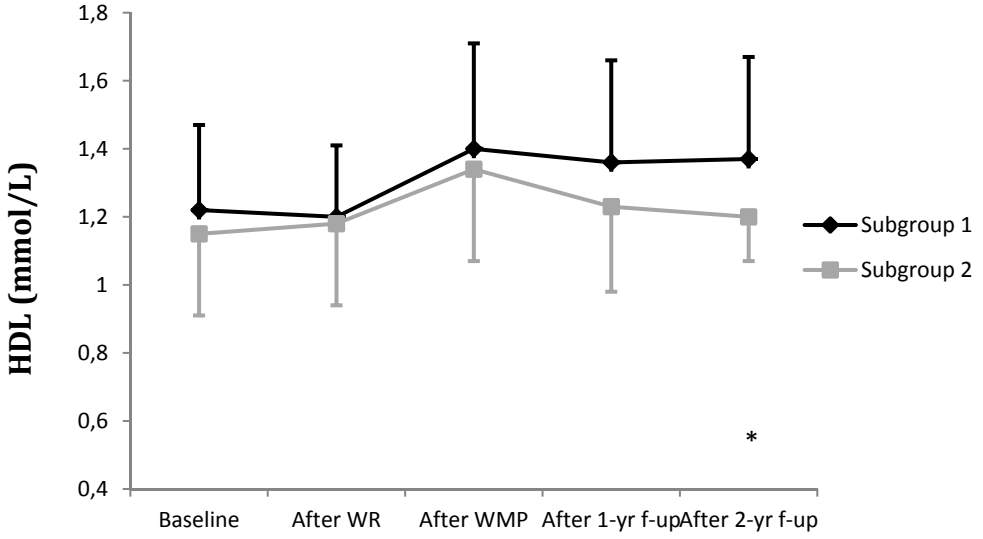
Figure 8. Concentrations of oxidized LDL during the whole study.



* p<0,05

** p<0,01

Figure 9. Concentrations in oxLDL/HDL-c during the whole study.



* p<0,05

Figure 10. Concentrations in HDL-c during the whole study.

5.1.4. Insulin metabolism and oxidized LDL

Throughout the study, concentrations of oxLDL and oxLDL/HDL-c were significantly higher among the highest HOMA quartile (see fig. 11 and table 7). When the participants were divided by quartiles of HOMA-IR –values in each measurement, during WRP, mean (SD) weight reductions in the first and fourth HOMA-fourths were 15.7 (3.1) and 12.6 (4.4) kg, respectively. Corresponding changes in waist circumference were 16.2(5.6) and 12.6 (4.2) cm, respectively. During follow-up, regains were 4.9 (5.5) and 14.0 (5.7) kg in weight, and 5.5 (6.1) and 11.4 (6.8) cm in waist (table). Throughout the study, the detected changes in HOMA-IR appeared strikingly similar to those in ox-LDL and ox-LDL/HDL-c seen in these two quartiles (Fig. 1a and 1b). In comparison to the first quartile, the fourth quartile had 23 to 37 % higher concentrations of ox-LDL throughout the study ($p<0.05$ for all; Fig.1a). Likewise, the ox-LDL/HDL-c ratio was 27 to 51 % higher in the fourth quartile at all measurement points ($p<0.05$ for all; Fig.1b). The table shows the values of ox-LDL, blood lipids, insulin, glucose and anthropometric measures in each of the four quartiles.

The correlation analyses showed that concentrations of both ox-LDL and ox-LDL/HDL-c associated significantly with both fasting insulin and HOMA-IR levels in each of the five sampling times ($\rho=0.304$ – 0.559, $p=0.012$ – 0.000 for ox-LDL; $\rho=0.274$-0.538, $p=0.025$ – 0.000 for ox-LDL/HDL-c). The changes in ox-LDL and ox-LDL/HDL-c during follow-up correlated with the corresponding change in HOMA-IR ($\rho=0.330$, $p=0.007$; $\rho=0.424$, $p<0.001$ respectively) and insulin ($\rho=0.349$, $p=0.004$; $\rho=0.455$, $p<0.001$, respectively), but not with fasting glucose. Even after controlling

for changes in BMI and waist during the 2.5 year follow-up, both ox-LDL and ox-LDL/HDL-c correlated with both insulin (0.289, p=0.021; ρ=0.382, p=0.002 respectively) and HOMA-IR (ρ=0.277, p=0.026; ρ=0.365, p=0.003 respectively) but not with fasting glucose.

An increase in waist circumference by 10 cm was paralleled by 11 to 55 percent increase in insulin concentration and 25 to 30 percent increase in oxLDL and oxLDL/HDL-c concentrations, indicating the metabolic impacts related to gains in abdominal girth. Further details are presented in the original publication no. II.

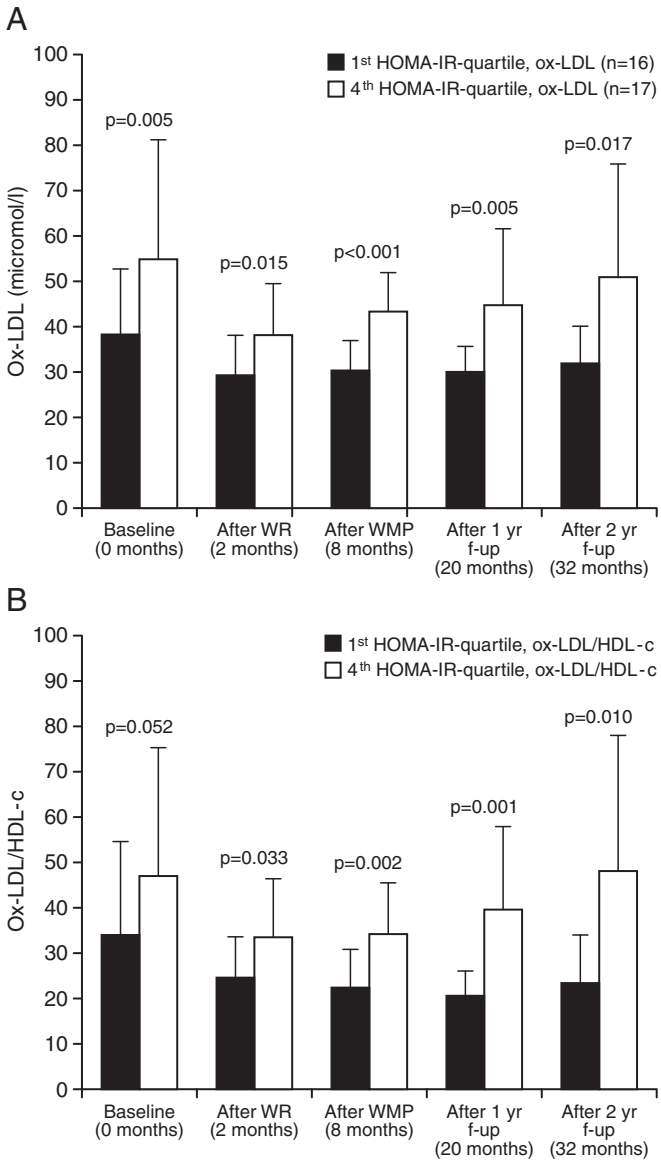


Figure 11. Oxidized LDL and oxidized LDL/HDL-c according to HOMA-quartiles. Further details are presented in the original publication no. II.

Table 7. Means (SD) of study variables according to first and fourth HOMA quartiles at each time point during the study.

Variable		Baseline	After WR	After WMP	After 1 yr f-up	After 2 yr f-up
Waist						
HOMA	1st q.	110.0 (5.7)	92.8 (4.7)	90.9 (4.9)	99.0 (9.2)	98.7 (7.0)
	4th q.	115.9 (9.2)	100.2 (6.9)	104.9 (6.1)	112.6 (6.1)	113.7 (9.6)
	P.	0.038	0.001	<0.001	<0.001	<0.001
HDL-c						
HOMA	1st q	1.26 (0.3)	1.23 (0.2)	1.45 (0.4)	1.51 (0.3)	1.45 (0.3)
	4th q.	1.23 (0.2)	1.21 (0.3)	1.35 (0.3)	1.19 (0.2)	1.19 (0.2)
	P.	NS.	NS.	NS.	0.001	0.014
Triglycerides						
HOMA	1st q.	1.64 (0.9)	0.99 (0.2)	1.03 (0.3)	1.00 (0.2)	1.12 (0.5)
	4th q.	2.53 (1.8)	1.80 (0.5)	2.05 (0.7)	2.29 (1.0)	2.95 (2.0)
	P.	NS.	<0.001	<0.001	<0.001	<0.001
Triglycerides/HDL-c						
HOMA	1st q.	1.48 (1.2)	0.82 (0.2)	0.78 (0.4)	0.69 (0.2)	0.84 (0.5)
	4th q.	2.24 (2.0)	1.62 (0.7)	1.65 (0.9)	2.0 (1.0)	2.8 (2.2)
	P.	0.114	<0.001	<0.001	<0.001	<0.001
Total cholesterol/HDL-c						
HOMA	1st q.	4.50 (1.3)	3.30 (0.6)	3.36 (1.0)	3.38 (0.7)	3.49 (0.9)
	4th q.	4.8 (1.1)	4.17 (0.9)	4.34 (0.9)	4.96 (1.2)	4.61 (1.3)
	P.	NS.	0.005	0.003	<0.001	<0.001
LDL						
HOMA	1st q.	3.46 (0.7)	2.43 (0.5)	2.72 (0.7)	2.85 (0.6)	2.79 (0.6)
	4th q.	3.61 (0.5)	3.14 (0.5)	3.22 (0.5)	3.31 (0.7)	3.60 (0.6)
	P.	0.434	<o.001	0.015	0.049	0.001
HOMA						
HOMA	1st q.	1.75 (0.2)	1.1 (0.1)	1.1 (0.2)	0.91 (0.1)	0.98 (0.2)
	4th q.	5.16(1.1)	3.12 (1.7)	2.75 (0.4)	3.49 (1.8)	4.33 (3.3)
	P.	<0.001	<0.001	<0.001	<0.001	<0.001

5.2. CROSS-SECTIONAL STUDY OF AGING MIDDLE-AGED MEN

5.2.1. Smokers vs. non-smokers

Smokers had 27 percent more oxLDL, 53 percent more TG, 13 percent more free testosterone (FT) and 18 percent greater oxLDL/LDL-c -ratio than non-smokers ($p \leq 0{,}01$ for all). HDL-c in smokers was 10 percent lower than in non-smokers ($p < 0{,}05$). In addition, the mean age of smokers was about fve years (9 %) lower than that of non-smokers ($p = 0{,}001$). The difference in oxLDL between smokers and non-

smokers remained statistically significant (p<0,001) even after controlling for possible confounding factors attained from the medical history records. Cardiovascular diseases, related symptoms and medication, age and alcohol consumption were used as covariates. Additionally, when HDL-c, TC, SHBG and free testosterone were also included to the list of covariates (those being variables differing significantly between smokers and non-smokers in previously conducted independent samples` t-tests) the difference in oxLDL between smokers and non-smokers still remained significant (p=0,007). Further details are presented in the original publication no. III.

5.2.2. High vs. low serum testosterone and smoking status

Smokers had 51 percent higher levels of oxLDL/HDL-c than non-smokers (see fig. 12) (For results in oxLDL alone, see original publication no. III) Smokers with serum testosterone concentration ≤ 15 nmol/l had 43 percent more oxLDL than smokers with serum testosterone > 15 nmol/l (p=0,006) and 58 percent higher oxLDL/HDL-c (p=0,015, see fig. 13 and 14). Among non-smokers, when divided into two subgroups in similar manner, the lower testosterone –group had higher levels of oxLDL and oxLDL/HDL-c (13 and 24%, respectively; p<0,05 for all, see fig. 15 and 16).

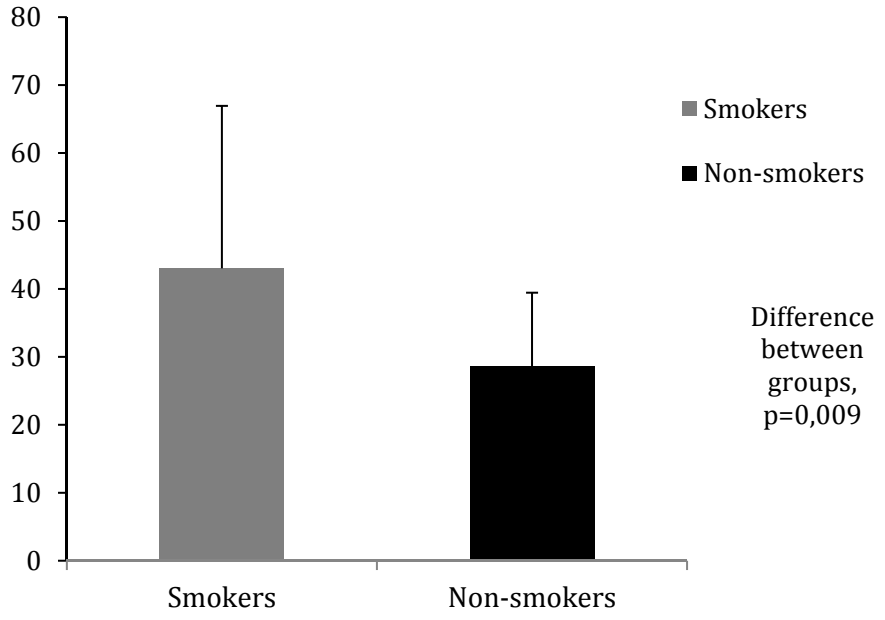


Figure 12. OxLDL/HDL-c in smokers and non-smokers.

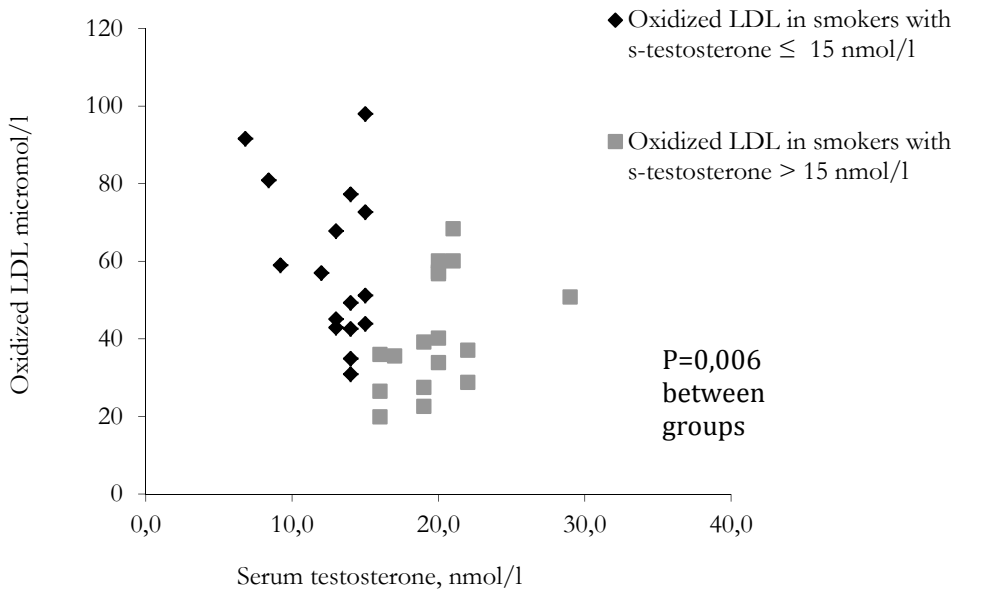


Figure 13. Ox-LDL in smokers by their serum testosterone concentration. Further details are presented in the original publication no. III.

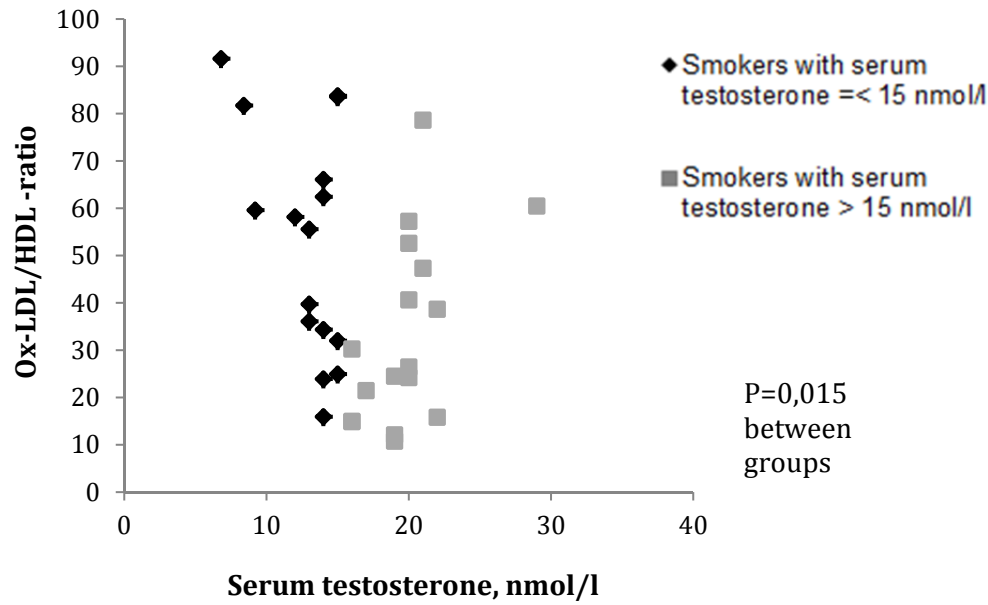


Figure 14. OxLDL/HDL-c in smokers by their serum testosterone concentration.

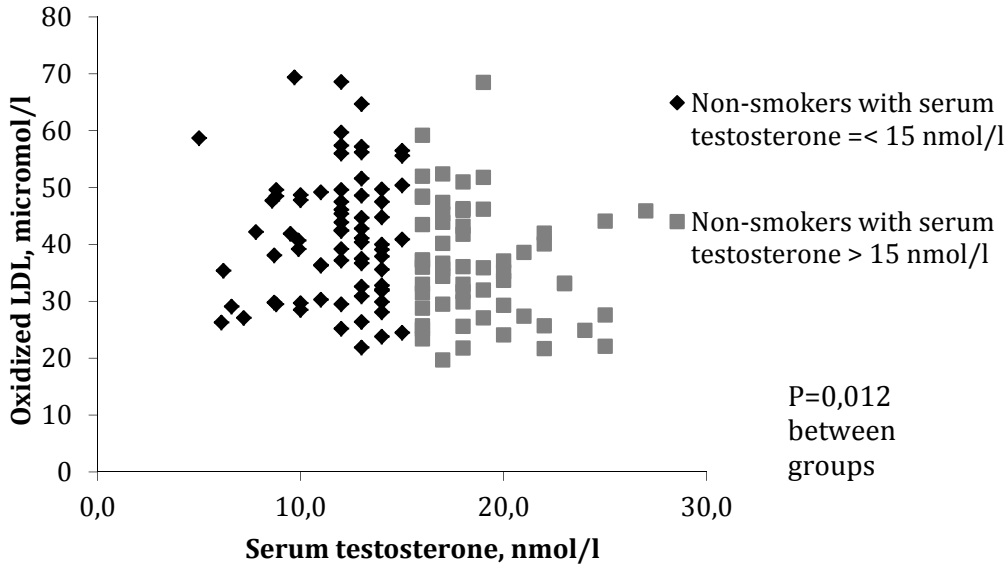


Figure 15. OxLDL in non-smokers by their serum testosterone concentration.

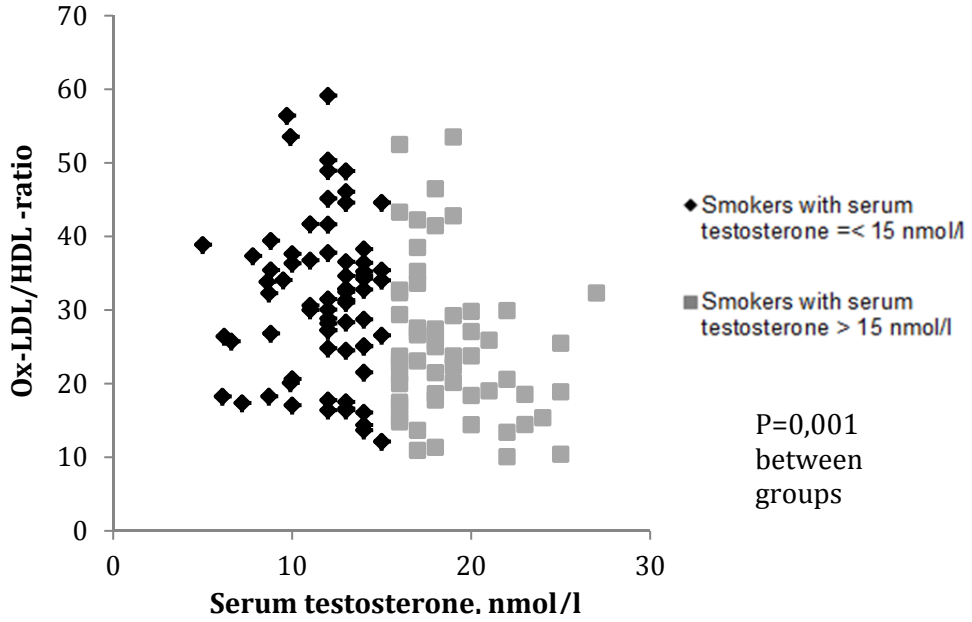


Figure 16. OxLDL/HDL-c in non-smokers by their serum testosterone concentration.

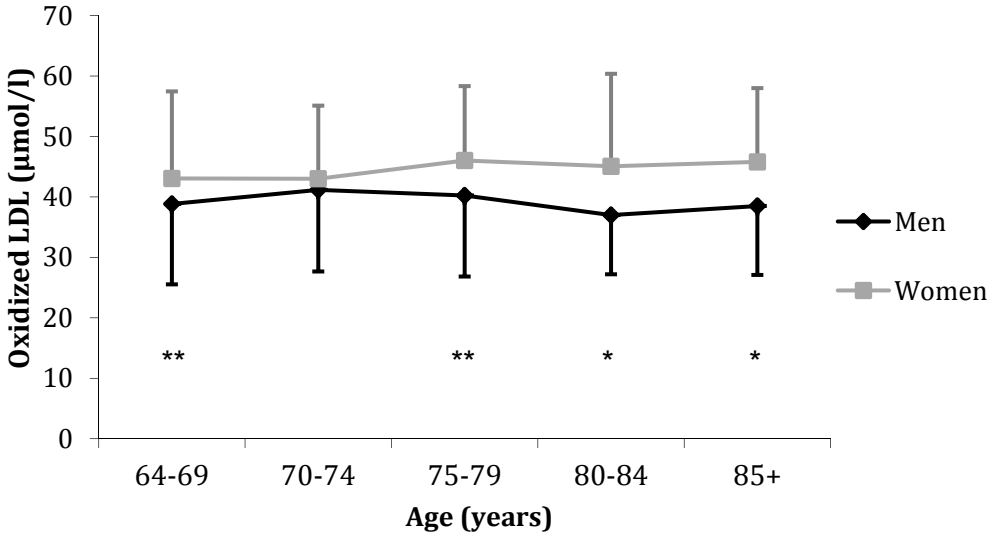
5.3. SURVIVAL STUDY OF ELDERLY MEN AND WOMEN

5.3.1. Effects of aging and differences between men and women

Mean ages ± SD for men and women were 73 ± 6 and 74 ± 7 years. With the exception of the age-group 70-74 years, oxLDL was significantly higher among women throughout age categories ($p<0,01$ for all, see fig. 17). The trends by age were significantly decreasing in TC, LDL-c, HDL-c, apoA1 and apoB in both men and women ($p<0,05$ for all). OxLDL/HDL-c was similar between men and women (see fig. 18). In women, the continuously increasing trend by age in oxLDL/HDL-c and oxLDL/LDL-c indicated 21% and 17% difference, respectively, between the eldest and youngest ($p<0,05$ for both). In men, oxLDL/LDL-c increased consistently with a difference of 13% between the eldest and youngest ($p<0,05$).

5.3.2. Oxidized LDL and all-cause mortality

The mean (SD) age of death for those who deceased during the 10-year follow-up after the original survey in 1998-1999, was 83 (7,2) years. Survivors by the end of 2008 were 81 (4,8) years old. Of the deceased participants, 36 % had died of atherosclerotic and/or ischemic cardiovascular diseases. The survived had 7-10% less oxLDL/LDL-c and oxLDL/HDL-c and 5 % more LDL-c and HDL-c than the deceased ($p<0,01$ for both). oxLDL/LDL-c, oxLDL/HDL-c and oxLDL/apoA1 were significant predictors of all-cause mortality, and these associations remained significant after controlling for age, sex, BMI, smoking, blood pressure and diabetes. HDL-c and apoA1 had a significant protective effect ($p<0,05$ for all). For further details, see table 8 below and tables in ch. 4.1.3. Further details are presented in the original publication no. IV.



* p<0,05

** p<0.01

Figure 17. OxLDL in men and women by age-group.

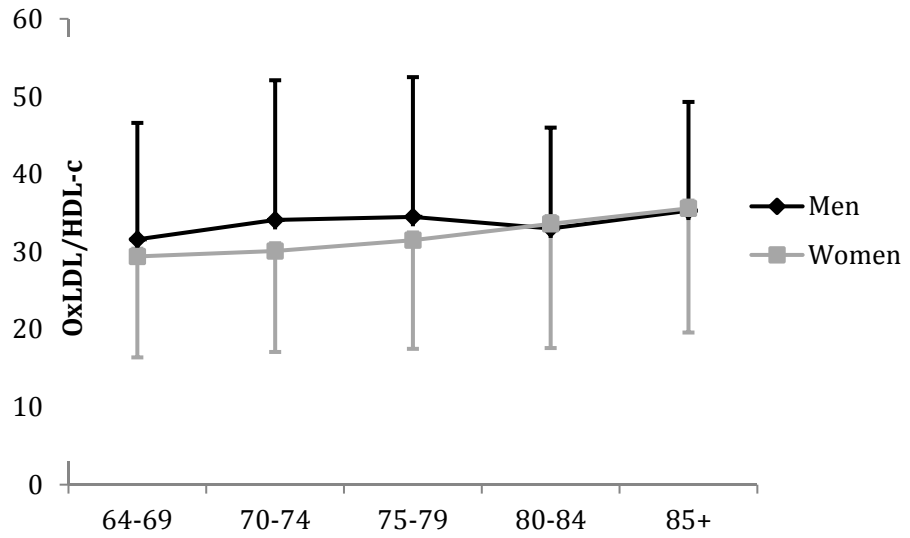


Figure 18. OxLDL/HDL-c between men and women in different age groups in Lieto elderly.

Table 8. Means (SD) and Cox hazard ratios adapted from the mortality data of 10 years after the survey in 1998-1999. Further details are presented in the original publication no. IV.

Variable	Mean (SD) Survived (n=793)	Deceased (n=467)	P-value controlled for sex
Ox-LDL * (µmol/l)	42,1 (13,2)	42,7 (13,7)	0,780, ns.
LDL-c † (mmol/l)	3,72 (0,9)	3,54 (0,8)	0,004
HDL-c ‡ (mmol/l)	1,47 (0.4)	1,39 (0,4)	0,027
Ox-LDL/LDL-c	11,5 (3,0)	12,3 (3,4)	<0,001
Ox-LDL/HDL-c	30,8 (13,8)	33,9 (16,5)	<0.002
Variable	**Hazard Ratio§**	**95% Hazard Ratio Confidence Limits**	**P-value**
Ox-LDL (µmol/l)	1,006	0,998 – 1,014	0,157; ns.
LDL-c (mmol/l)	0,956	0,845 – 1,081	0,476; ns.
HDL-c (mmol/l)	0,743	0.569 – 0,970	0,029
ApoB†† (g/l)	1,089	0,754 – 1,572	0,651; ns.
Ox-LDL/LDL-c	1,037	1,006 – 1,070	0,021
Ox-LDL/HDL-c	1,009	1,002 – 1,015	0,010
Ox-LDL/apoB	1,005	0,995 – 1,016	0,322; ns.
Ox-LDL/apoA1	1,013	1,003 – 1,022	0,008

* Ox-LDL, oxidized low-density lipoprotein

† LDL-c, low-density lipoprotein cholesterol

‡ HDL-c, high-density lipoprotein cholesterol

§ The Cox Hazard Ratios are controlled for age, sex, BMI, smoking, blood pressure and diabetes.

** apoA1, apolipoprotein A1

5.4. YOUNG ADULT MALE RESERVISTS

5.4.1. Smokers vs. non-smokers

Mean age (SD) of study participants was 25 (5) years (age range 18-48 years). In serum lipids, smokers had 6 % less HDL-c and 17 % more TG than non-smokers ($p<0,01$ for both). In oxLDL, oxLDL/LDL-c, oxLDL/HDL-c, smokers' values were 8, 6 and 14 % higher than those of non-smokers, respectively ($p<0,05$ for all). The concentration of serum testosterone was 10 % higher, whereas IGF-1 was 6 % lower among smokers ($p<0,001$ for all). The concentration of FT and SHBG did not differ between smokers and non-smokers. From the cytokines IL-6 and TNF-α, IL-6 was 31 % higher in smokers ($p<0,001$). Smokers had a 2 % greater waist circumference ($p=0,012$).

5.4.2. Co-existence of waist circumference, smoking and oxidized LDL

A shift to a greater WC groups corresponded to an increasing prevalence in smoking. In the three WC groups of < 90 cm, 90-99,9 cm and > 100 cm we used, the frequencies of smoking were 36, 43 and 45 %, respectively. In the WC group of < 90 cm, smokers had 1, 5, 8 and 9 % less glucose, HDL-c, IGF-1 and leptin than non-smokers,

respectively ($p<0,05$ for all). For smokers, the concentrations of both TG and serum testosterone were 10 % higher than for non-smokers, respectively ($p<0,05$ for all). In the WC group of 90-99,9 cm, smokers had 14, 13 and 17 % higher concentrations of oxLDL, oxLDL/LDL-c and oxLDL/HDL-c than non-smokers, respectively ($p<0,01$ for all, see fig. 19). Smokers in this group also had 21 and 20 % more triglycerides and serum testosterone, and 9 and 31 % less IGF-1 and leptin than non-smokers, respectively ($p<0,05$ for all). In the WC group of > 100 cm, smokers had 14, 19 and 17 % higher concentrations in oxLDL, oxLDL/HDL-c and triglycerides than non-smokers, respectively ($p<0,01$ for all, see fig. 20). For results in serum testosterone, IGF-1 and IL-6, see table 9. Further details are presented in the original publication no. V.

5.4.3. Correlations

Throughout the list of study parameters, the association in smokers was stronger than in non-smokers. Triglycerides had the strongest correlation to oxLDL (correlation coefficients: 0,68 for non-smokers, 0,76 for smokers). For HDL-c and TNF-α, the statistical significance of the correlation coefficient increased from non-significant in non-smokers to significant in smokers. Age, IGF-1 and IL-6 were the only parameters that did not correlate with oxLDL in neither smokers nor non-smokers.

5.4.4. High vs. low serum testosterone and smoking status

Among smokers, those with serum testosterone below 15 nmol/l, had 11, 20 and 21 % higher levels oxLDL, oxLDL/HDL-c and TG than those with serum testosterone equal or greater than 15 nmol/l ($p<0,05$ for all, see fig. 21 and 22). In the lower testosterone group, waist circumference, BMI and leptin were 9, 10 % and 29 % higher ($p<0,05$ for all).

Among non-smokers, subjects of the lower testosterone group had 6 % more oxLDL, 15 % greater ratios of oxLDL/HDL-c, 5 % more total cholesterol, 8 % less HDL-c, 10 % more TG and 7 % more LDL-c, than subjects in the higher serum testosterone group ($p<0,05$ for all). Waist circumference, BMI and leptin were 6, 7 and 35 % higher in the lower testosterone group ($p<0,001$ for all). Furthermore, subjects in the lower testosterone group were 6 % older than those in the higher testosterone group ($p=0,001$).

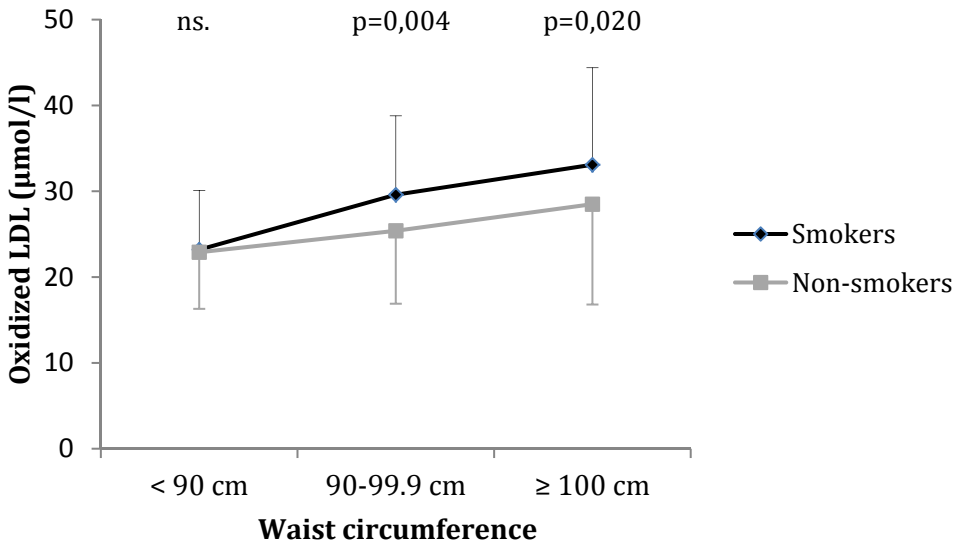


Figure 19. OxLDL according to waist circumference. Further details are presented in the original publication no. V.

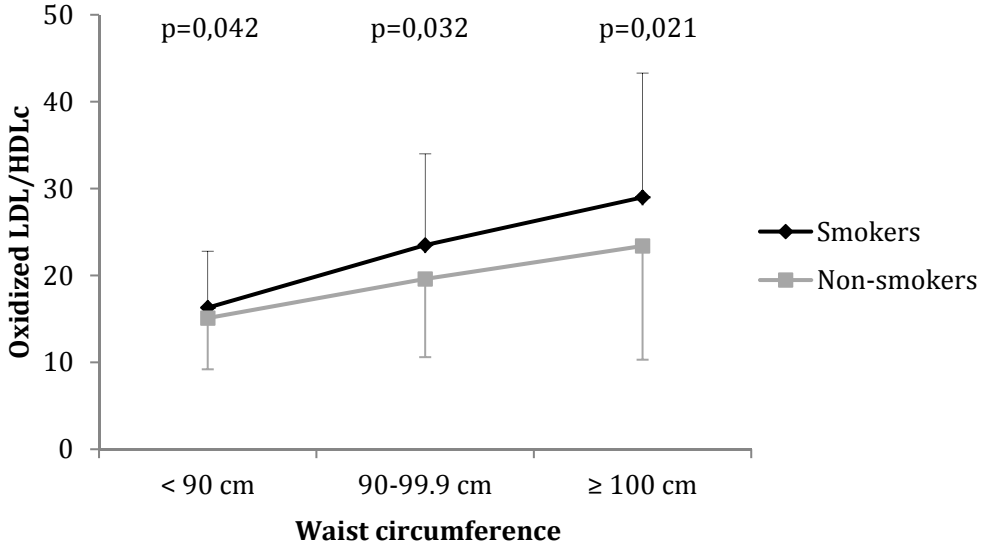


Figure 20. OxLDL/HDL-c according to waist circumference.

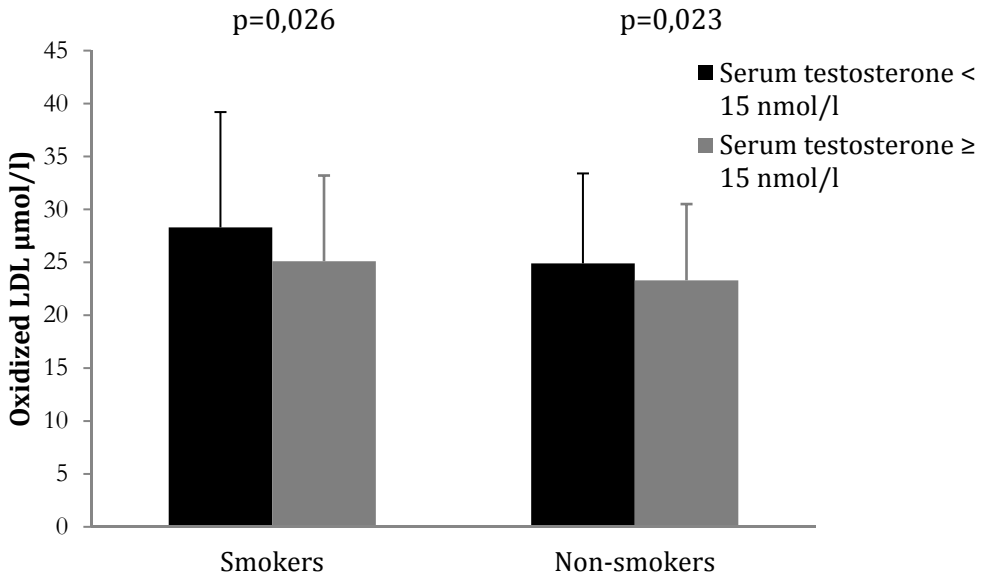


Figure 21. OxLDL in smokers and non-smokers by their serum testosterone concentration.

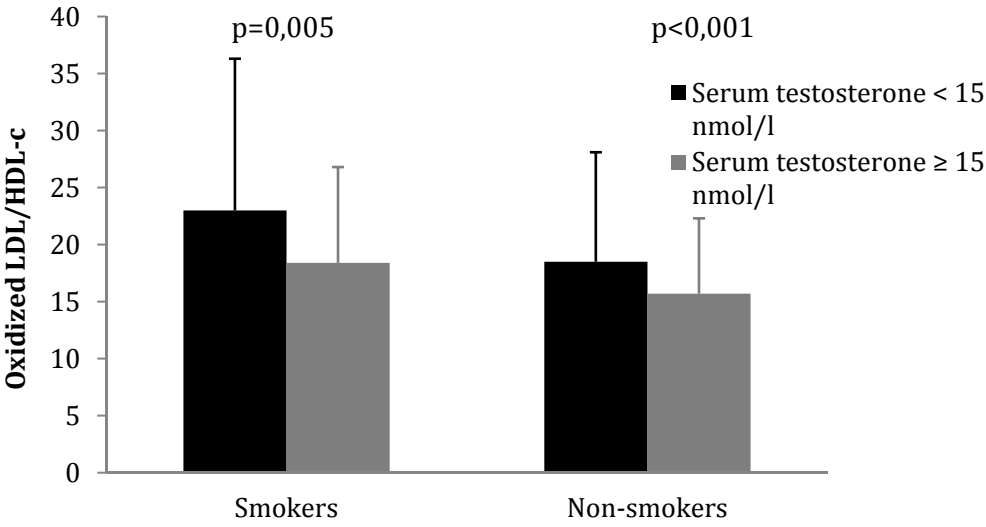


Figure 22. OxLDL/HDL-c in smokers and non-smokers by their serum testosterone concentration.

Table 9. Serum testosterone, insulin-like growth factor-1 and interleukin-6 according to waist circumference in smokers and non-smokers. Further details are presented in the original publication no. V.

	Classes of waist circumference		
Variable	**< 90 cm (n=590)**	**90-99.9 cm (n=160)**	**≥ 100 cm (n=92)**
Serum testosterone (nmol/L)			
Smokers	19.5 (5.6)	18.7 (4.7)	13.7 (4.0)
Non-smokers	17.6 (4.3)	15.0 (4.3)	13.5 (4.0)
P.	<.001	<.001	0.730
IGF-1 (nmol/L)[a]			
Smokers	30.2 (7.3)	29.4 (6.9)	29.2 (7.6)
Non-smokers	32.5 (7.6)	31.9 (7.5)	26.3 (6.1)
P.	<.001	0.002	0.166
IL-6 (pg/ml)[b, c]			
Smokers	0.87	1.06	1.52
Non-smokers	0.64	0.84	1.02
P.	<.001	0.340	0.095
HDL-c (mmol/L)			
Smokers	1.51 (0.3)	1.35 (0.3)	1.25 (0.3)
Non-smokers	1.58 (0.3)	1.37 (0.3)	1.33 (0.4)
P.	0.028	0.867	0.299
Ox-LDL (µmol/l)			
Smokers	23.2 (6.7)	29.6 (9.2)	33.1 (11.3)
Non-smokers	22.9 (6.6)	25.4 (8.5)	28.5 (11.7)
P.	0.526	0.004	0.020

a, insulin-like growth factor

b, interleukin 6

c, expressed as median

6. DISCUSSION

Most importantly, this thesis provides a window indicating the role of oxidative modification in LDL lipids in different metabolic milieus associated with atherosclerosis. The main focus was on lifestyle factors including weight loss, weight maintenance, insulin resistance and abdominally centered obesity. Additionally, a perspective combining smoking and serum testosterone concentration together with oxidized LDL concentrations is proposed. Finally, findings concerning oxidized LDL lipids proportioned to HDL-c in all-cause death – and therefore, longevity and general health – are presented and discussed.

Obesity is currently considered an epidemic, the reversal of which has turned out to be a major challenge. The health-impairing effects of obesity form a plethora of different risk factors. The proposed underlying causes characterize the problem as something considerably more complex than just an issue of energy intake surplus: genetic, socioeconomic, behavioral, ecological and political/capitalistic perspectives have been introduced (Mitchell et al. 2011; Wells, 2012; Kong et al. 2013).

Further extending the panorama of atherosclerotic implications regarding LDL oxidation from obesity to smoking introduces a risk factor, which despite the public preventive efforts, still is a considerable contributor to cardiovascular diseases. The overall prevalence of daily smoking among Finnish adult people (aged 15 years or more) is still 19 percent (OECD Health Data 2012, Eurostat Statistics Database). Recently, it was shown that smoking contributed to the majority of coronary heart disease cases in all age groups (Tolstrup et al. 2013). Therefore, as in this thesis, smoking was involved in two of five original publications (no. III and V) as an inherent part of the research setting, it is important to understand its various metabolic implications. Figure 23 gives a schematic representation of both the interconnectedness between abdominal obesity, impaired insulin action, low HDL-c, increased oxidative stress, inflammation and the contributing role of smoking in all of these. Figure 23 may also help in outlining the findings from original publications I and II concerning the interplay between weight maintenance, insulin action and oxidative stress.

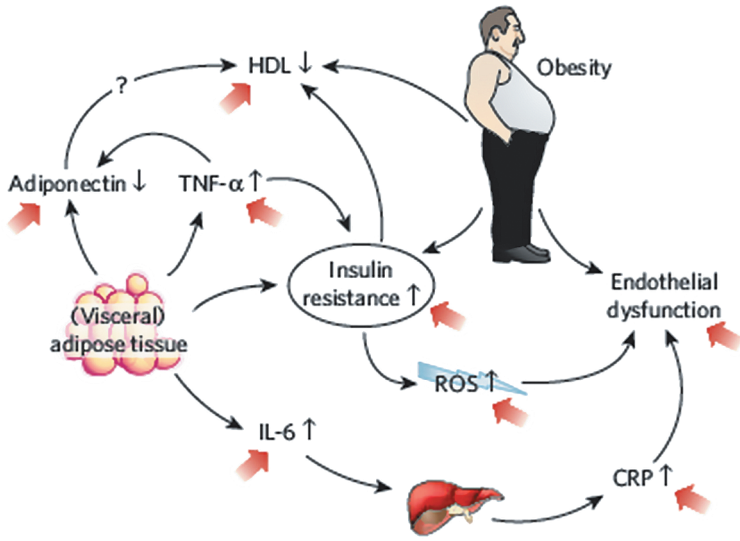


Figure 23. An outline of the inflammatory, oxidation, lipoprotein and hormonal implications involved in smoking and abdominal (visceral) obesity. Red arrows indicate the accelerating effect of smoking on these complications relevant in central adiposity. HDL, high-density lipoprotein; ROS, reactive oxygen species; IL-6, interleukin-6, CRP, c-reactive protein. Adapted with the publisher's permission from van Gaal et al. 2006.

Lifestyle modification has a beneficial influence on several risk factors at once, while pharmacotherapy usually targets only one. Therefore, therapy and intervention targeted at adjusting elements physiologically and automatically already involved in life should be prioritized over – or at least not directly paralleled to – other therapeutic tools (Frohlich and Al-Sarraf, 2013). Considering the severity of avoidable chronic disease burden, and the alternatives in the hunt for efficient solutions, lifestyle issues cannot be over-emphasized (Ezzati and Riboli, 2013). In this respect, the heart of both the problem itself and its solution, converge: daily choices. Fortunately, new promising initiatives have emerged (Frieden and Berwick, 2012). Preventive actions decreasing risk factor burden may, additionally, be beneficial even when initiated relatively late, i.e., before 80 years (Strandberg et al. 2014).

In this thesis, topics such as weight reduction and maintenance in obesity, smoking and abdominal adiposity can all be directly connected to certain habitual lifestyle decisions. Although the particulars of the precise impact of those choices (e.g. nutritional factors, types and quantities of physical activity), remain outside the scope of this thesis, generally, these results highlight their medical importance. In a broader sense than what is scientifically immediately relevant to atherosclerosis risk, the findings of this thesis promote the meaning of health-related actions which can be performed essentially at home or nearby. That is, the importance of decisions made at outside places such as hospitals and centers, where health-care professionals typically operate, is enhanced.

This thesis offers very limited room to discuss the exact practice and challenges of lifestyle choices at daily level. The fact of the matter yet persists that as we discuss the results from a lifestyle intervention, the accessibility of means that people may have had to maintain the improved health achieved by, for example, weight loss, merit some consideration. We may, for example, be tempted to conclude, that success in weight maintenance is fundamentally a matter of personal motivation. However, in a broader sense, the health industry is, in fact, one of the biggest business branches accompanied with a flood of information with variable quality. Furthermore, a westernization trend in our culture can sustain severe threats to improving health (Oddy et al. 2013). This entity is easily perceived as confusing, because the abundance in what is most accessible and overall increasing knowledge may from time to time even reshape official guidelines and recommendations as well. Nonetheless, while WHO has estimated that by eliminating lifestyle-related chronic disease risks would provide a 80 percent prevention in cardiovascular disease including type 2 diabetes and a 40 percent prevention in cancerous diseases, there is little left to dispute (WHO statistics, 2005).

6.1. LIFESTYLE INTERVENTION IN OBESE MEN

Cardiovascular risk factors tend to exist in clusters (Kannel et al. 2002). Of these, obesity is one of the most important. Currently, obesity is common and maintenance of weight loss over the following years is relatively rare (Sarlio-Lähteenkorva et al. 2000). Simultaneously, the scientific evidence presenting the health benefits of weight loss in originally obese/overweight individuals is constantly increasing in both amount and variety (Salinardi et al. 2013; Penn et al. 2005 and 2013). The favorable effects of weight loss extend to oxidative stress markers as well (Keaney et al. 2003; Rector et al. 2007). Data on trends in oxidized LDL lipids following weight loss; i.e., during unsupervised weight maintenance, have been virtually absent.

Subjects and methods

The subjects comprised of 68 originally obese, middle-aged men completing the study. Weight loss was accomplished by means of a standard, manufactured two month diet regime (Nutrilett®, Leiras Ltd, Turku, Finland) whereas the 2,5 year weight maintenance was a matter of lifestyle components. Two thirds of subjects received exercise counseling for the first six months of the follow-up. A more detailed account on these methods is given elsewhere (Fogelholm et al. 2000; Borg et al. 2002).

Main findings

Everyone lost weight during the two-month period, but during the 2.5 year follow-up, the majority experienced a relapse. The most successful men (n=20/68) at the end of the study had experienced a maximum weight regain of 6.2 kg after weight loss. Success in weight maintenance was correspondingly reflected in body shape measurements and several serum lipid concentrations. Interestingly, the least successful in weight maintenance experienced a worsening trend in the quality of LDL lipids during follow-up.

Clinical relevance and perspectives

The prevalence of obesity (BMI greater than 30 kg/m^2) among 25 to 64 year old Finnish population is 19 to 20 percent for both sexes. Central obesity (waist circumference greater than 100 cm for men and greater than 90 cm for women) characterizes 30 percent of both men and women. The health-related financial burden is some 25 percent higher for the obese compared to normal weight. At the societal level in Finland the annual costs of obesity are some 330 million euros. The main causes for these are elements of habitual culture, including sedentary lifestyle, unhealthy snacks, increased meal sizes and alcohol consumption (Männistö et al. 2012). It was recently estimated, that just by managing high blood pressure, cholesterol and serum glucose alone, up to 50 to 75 percent cuts in excess coronary heart disease or stroke risks related to high BMI could be achieved (The Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration, 2014).

The benefits of losing excess weight are, therefore, elemental and largely known. However, the trends in serum lipids after weight loss (i.e., during weight maintenance) have been far less explored. Of particular interest is the oxidative stress environment, previously shown to have, *via* oxidation of LDL, a significant role in the development of an arterial plaque in atherosclerosis (Meisinger et al. 2005). This study sheds more light on the actual metabolic findings concerning the importance of weight maintenance in cardiovascular health. Particularly, the quality of LDL lipids in relation to the success, or lack thereof, in weight maintenance is a noteworthy message. The worsening trend in LDL lipids among the unsuccessful men (S2) during follow-up may signal a particular cardiovascular shortcoming related to weight regain.

Two thirds of the subjects received exercise counseling for the first six months following weight loss. Hence, as one third of the participants were not introduced to exercise as a means of weight maintenance, participants from the two exercise groups may have been over-represented among the successful men (S1). Exercise training has been observed to have a favorable effect on oxidation of LDL lipids (Vasankari et al. 1998; Thomas et al. 2010). In this sense, the controls would have had a disadvantageous position concerning the prospects of weight maintenance in a way that may have a role in the trends of serum lipids as well. However, as both S1 and S2 had a comparable 30 to 40 percent of subjects from the control group, this is an unlikely scenario.

The big picture related to the harmfulness of visceral adiposity has has become more detailed during the past decade. Practically speaking, we know, for example, that excess fat juxtaposed to liver, may respond to the carbohydrate content in diet. An excessive amount may promote liver enlargement and fat deposition, the both of which can be defeated by restricting either calories or carbohydrates (Bian et al. 2014). Considering the previous efforts in aiming at reduced salt intake, a similar task has now been introduced to internationally target sugar contents in various groceries (MacGregor and Hashem, 2014).

As far as nutrition is concerned, to tackle the trends and challenges widely apparent particularly in the developed nations, accumulating evidence points to the beneficial role of adopting the Mediterranean Diet (Estruch et al. 2013; Koloverou et al. 2014). Broadly speaking, there is a considerable momentum encouraging both individuals and collective forces toward strategies that target particular groups of foods (for example, unhealthy snacks and beverages). Importantly, already a daily imbalance of 50 to 100 kcal in energy intake may be the sufficient limit capable of turning the course in weight management. Obviously, depending on the circumstances, this can be either a solace or a warning, indicating the delicacy of the balance (Mozaffarian et al. 2011).

6.2. INSULIN METABOLISM AND OXIDIZED LDL LIPIDS

To date, there has been only limited data investigating the long-term effects of weight maintenance on oxidized LDL lipids. Additionally, the connection between insulin metabolism to oxidation of LDL lipids has previously not been explored in a prospective intervention and follow-up –setting. Recently, it has been suggested that obesity and insulin resistance seems to have a prominent role in the progress of oxidative stress (D'Archivio et al. 2012).

Subjects and methods

The study participants and methodology are explained above (see ch. 6.1.1). Data on insulin and glucose concentrations was obtained from 67 men completing the study. HOMA was used as the indicator of insulin sensitivity/resistance.

Main findings

The fourth HOMA-quartile had consistently higher concentrations of oxLDL and oxLDL/HDL than the first fourth. An increase in waist circumference by 10 cm was paralleled by a 11 to 55 percent increase in insulin concentration and 25 to 30 percent increase in oxLDL and oxLDL/HDL-c concentrations, indicating the metabolic sequels of increasing abdominal girth.

Clinical relevance and perspectives

This study is among the first to show that oxidative modifications in LDL lipids coexist with occurrence of insulin resistant states, such as obesity. Other studies with similar aims have utilized different methodology, not focusing on lipid modifications but, for example, modifications in apoB (Rector et al. 2007; Park et al, 2009). The consistency of our results adds important evidence to support the link between atherosclerosis development, dependent on lipid metabolism – particularly the compartment in LDL – and diabetic states. As obesity and disorders of the insulin metabolism are intensely connected to everyday lifestyle choices, and can be thereby maneuvered *via* improvements in, for instance, anthropometric measures, these results further stress the overall importance of lifestyle interventions. It has been discussed that considering the origins and current condition of a Western lifestyle, what may have ensued is a situation that threatens health maintenance and should not to be

ignored (Cordain et al. 2005). To defeat these challenges, an approach urging to establish the collaboration of multiple branches of medical science, has been recommended (Ohlhorst et al. 2013).

6.3. CROSS-SECTIONAL STUDY OF AGING MIDDLE-AGED MEN

Cigarette smoking is one of the most ominous of risk factors for cardiovascular diseases, including atherosclerosis (Campbell et al. 2008). Low serum testosterone has been characterized as a cardiovascular disease risk factor (Jones and Saad, 2009), and high testosterone correspondingly a beneficial contributor (Firtser et al. 2012) although the connection between testosterone and vascular health has been described as neither consistent nor simple (Kelly and Jones 2013; Monroe & Dobs, 2013). Low testosterone has been identified as a risk factor of all-cause mortality (Lehtonen et al. 2008, Haring et al. 2010). However, the decline in serum testosterone is not an inevitable companionship arriving with aging. Lifestyle factors, especially smoking, have a considerable role (Shi et al. 2013). Additionally, weight loss may mediate improvements extending to testosterone metabolism as well (Niskanen et al. 2004).

Subjects and methods

Sufficient data was available from 165 men. Lipoproteins and sex hormones were compared between smokers and non-smokers in a cross-sectional setting. Particularly, the joint effect of smoking plus hypotestosteronaemia (cut-point was 15 nmol/l) was investigated.

Main findings

Smokers with serum testosterone concentration ≤ 15 nmol/l had 30 percent more oxLDL than smokers with serum testosterone greater than 15 nmol/l . Among non-smokers, when divided into two subgroups in similar manner, the lower testosterone – group had 11 percent higher levels of both oxLDL and oxLDL/LDL-c –ratio.

Clinical relevance and perspectives

This study indicates that smoking and low testosterone contribute to cardiovascular health as measured by circulating oxidized LDL lipids. Others have also found that lifestyle factors may have a significant role from the male health (androgens, erectile function) perspective. Smoking impairs vascular health *via* both arterial and venal mechanisms (Elhanbly et al. 2004). Oxidative stress, in general, and dyslipidemia seem to be involved in erectile dysfunction (Vrentzos et al. 2007; Zhang et al. 2011). Maintenance of a low cardiovascular disease risk profile is strongly advised as a better tool for optimal health than androgen replacement therapy (Haring et al. 2012). Additionally, Kratzik et al. (2009) found that regular physical activity of 1000 kcal/week – for example, gardening for 30 to 45 minutes daily – is an amount sufficient enough to reduce the risk to develop erectile dysfunction. Furthermore, in discussing testosterone-related atherosclerosis risk, a preference for exercise promoting

cardiorespiratory fitness in particular, may offer an additional advantage (Kosola et al. 2013a,b).

Smoking may exert a blocking effect on some enzymes at the adrenal cortex (Hautanen et al. 1993), possibly accounting for the increased levels of adrenal androgens in smokers in some studies. Overall it seems, judging by the discrepancy of studies thus far (Field et al. 1994; Vermeulen 1996; English et al. 2001; Gyllenborg et al. 2001; Allen et al. 2002), the metabolic connection between smoking and sex hormones remains largely unrevealed. The results presented herein, in light of previous results in their heterogeneity, cannot be formulated to majestic conclusions of causal relationships, either. What can, however, be cautiously interpreted, is that with the health impairing dimensions of smoking, an involvement of several metabolic axes, endocrine function included, seems apparent. For a more sophisticated analysis, a prospective study design would be the least of further requirements that were not available for our analyses. Longitudinal perspective would provide, for instance, a multiplicity of hormone measurements, thereby decreasing the errors related to a single analysis of a steroid hormone with the challenges of diurnal variation in their endogenic excretion.

To a considerable extent, the exact underlying mechanisms and key contributors, concerning potential atheroprotective or atherogenic properties of testosterone metabolism, are presently considered as topics requiring more investigation (Kelly and Jones, 2013).

Middle-age may be a pivotal era in human life in the sense that issues concerning longevity and quality of upcoming senior years are of concern. As women undergo menopause, a term called ‘andropause’ has been introduced as an equivalent male corollary of approaching the sixth decade in life (Vermeulen, 1993). Interest in agents that promote longevity, increasing lean mass and attempting to defeat frailty, is a modern trend and can be easily greeted with warm regards, as the increasing life expectancy tends to accumulate the overall burden of chronic diseases as well (Blackman et al. 2002). Therefore, tools or procedures to improve quality of life often collect immediate attention, particularly if they are escorted by increase in lean body mass at the expense of fat mass (Blackman et al. 2002).

However, considering the already present epidemic nature of diabetic conditions aside the commonness of obesity, a further potential risk of accelerating impairment of insulin metabolism is, indeed, noteworthy. As presented in chapter 2, the contemporary Western way of life is already a burden on several endocrine and cardiometabolic axes. We may have crossed a line, beyond which an additional risk impairing insulin action may be something we simply cannot afford anymore. In other words, we may have the interest, the markets and the demand for the drugs, but not for the corollary side-effects. Future studies and trials with pharmacological agents probably further elucidate the consequences of using different hormonal supplements in treating and managing lifestyle-related conditions.

Erectile dysfunction has been observed as one of the first signs of atherosclerotic disease (Billups et al. 2005; Yao et al. 2013), and linked to, for example, lower arterial elasticity (Pohjantähti-Maaroos and Palomäki, 2011). Furthermore, decreased sexual desire and low free testosterone may be associated, possibly involving mental depression as well (Hintikka et al. 2009). In light of this apparent biological relevance of testosterone function to the quality of life, it was therefore additionally investigated, how the men in our study differed by their subjectively reported potency data.

Men who characterized themselves as having extremely severe decreases in potency (n=15) compared to men who had none (n=51) in a 1 to 5 scale, were eight years older (p=0,003) and at this age (62 vs. 54 years) had 20 percent less serum testosterone (p=0,034) and 15 percent less free testosterone (p=0,043). Accordingly, the concentration of LH was increased by 27 percent (p=0,016), potentially reciprocating to an existing higher demand for stimulatory effect. When the two most dysfunctional groups were pooled together (n=34), only LH and age remained significant (p<0,05).

In these analyses, differences in oxLDL and oxLDL/HDL-c were not significant. This is quite an expected result, as steroid hormones naturally play a more direct mechanistic role in the physiology behind potency than markers of circulating lipids. A cross-sectional study design may further challenge the possibilities to detect a connection between a lipid risk factor and erectile capability. However, considering the role of serum total testosterone concentration in male health, longevity and cardiovascular disease risk throughout adult lifespan, a broader perspective is useful even in – or particularly in – the case of more specific main outcome measures.

By this finding it may be suggested that subjectively experienced, distinct erectile dysfunction could appear when serum testosterone reaches the vicinity of 12 nmol/l. Indeed, recently it was found that it may be reasonable to revise the definition for hypogonadism (Finkelstein et al. 2013). They also found that sexual desire appeared diminished at levels 7 to 14 nmol/l, and the severity of which depended on concentration of estradiol. In our study, no differences were observed between the two potency groups in estradiol. A younger study population (mean ages 30-40 years) and overall slightly larger sample sizes may account for this difference, as indeed aging tampers androgen metabolism. In fact, a significant proportion of men aged over 60 years may have serum testosterone levels considered to be hypogonadal (Harman et al. 2001). Additionally, the reproductive health of recent generations show a decreasing trend compared to their older predecessors with a corresponding age (Perheentupa et al. 2013). These findings have raised concern whether a type of peak in reproductive competence has been crossed (Andersson et al. 2008). Considering the reflective role in early detection of cardiovascular diseases, it has been proposed that queries about erectile function should be unselectively targeted to all men from 25th year on (Billups et al. 2005).

6.4. SURVIVAL STUDY OF ELDERLY MEN AND WOMEN

There are no pre-existing studies investigating the possible prognostic role of circulating oxidatively modified LDL lipids related to all-cause mortality. This is important considering the substantial role of cardiovascular diseases in contributing to global morbidity and mortality statistics. Additionally, the purpose to combine circulating lipid metabolism to mortality connects the essentiality of vascular health to overall health, which – in light of the very commonness of cardiovascular disorders – cannot be understated. Many human tissues or organs may be at least partly removed, or replaced by a donor, without threatening life, but circulation of blood is irreplaceable, and the tissue demand for it is unavoidable.

In light of the essentiality of circulation, the scientific meaningfulness to increase understanding on how to preserve its optimal function, is apparent. Accordingly, the rationale to evaluate oxLDL – alone and with other lipid classes against all-cause mortality – is obvious. Nevertheless, it could be argued, that cardiovascular risk factor research focusing on the elderly is pointless, since there is only relatively little time left to take preventive or therapeutic action.

However, as noted earlier, the value of a risk factor may vary depending on the age group of the target population (Strandberg et al. 2014). This provokes the importance of cardiovascular research to fully cover the entirety of a lifetime. By gaining knowledge of risk factors in the elderly, we may improve our understanding on how to view their role in disease prevention amidst other risk factors in the earlier years, as well. A phenomenon like this is logical considering the intricacy and complicated nature of lipid metabolism in its connectedness to nutritional, hormonal, anthropometric and physical activity measures. All of these aspects are involved in key events of our daily lives and vary accordingly by increasing maturation and age. In disease development, time, frequency and space are all constants and inevitable forces.

Importantly, it should be remembered that research on a risk factor with possible prevention in mind, does not provide a manual to a cordless system where metabolic consequences could, by default, be compartmentalized to e.g., a single organ. Rather, in reality, an investigation of one single risk factor translates to the co-dependence of many interacting elements in a larger biologic system.

The obvious benefit regarding research around the potential prognostic value of oxLDL is that it is simple to measure. Secondly, the ways to control oxLDL concentrations, with the current level of knowledge, can be efficiently and safely implemented to our daily lives *via*, for instance, healthy nutrition (Ahotupa et al, 2010) or losing excess weight (see ch. 5.1 and original publication no. I). This naturally, assimilates effortlessly to the statistics by WHO, indicating the role of lifestyle factors in current mortality rates (WHO statistics, 2011).

Subjects and methods

Of the 1596 elderly inhabitants in Lieto, 1260 subjects (79%) participated and gave their informed consent, which consisted of 533 men (42%) and 727 women. The originally cross-sectional data was completed with a review of medical records in January 2009. The mean (SD) age of death was 83 (7.2) years. Survivors by the end of 2008 were 81 (4.8) years old. Of the deceased, 36 % had died of atherosclerotic and/or ischemic cardiovascular diseases.

Main findings

The survivors had seven to ten percent less oxLDL/LDL-c and oxLDL/HDL-c and five percent more LDL-c and HDL-c than the deceased. OxLDL/LDL-c, oxLDL/HDL-c and oxLDL/apoA1 were significant predictors of all-cause mortality, and these associations remained significant after controlling for age, sex, BMI, smoking, blood pressure and diabetes. As anticipated, HDL-c and apoA1 had a significant protective effect ($p<0.05$ for all).

Clinical relevance and perspectives

What has been the reality for some lipoprotein variables such as total cholesterol is that aging may alter its predictive use in cardiovascular disease risk assessment (Krumholz et al. 1994) but the same does not seem to apply for parameters reflecting oxidation of LDL lipids and those indicating anti-atherogenic actions (HDL-c, apoA1). Importantly, these results can be interpreted with special confidence because of the fact that the study subjects are generally representative of an elderly population. These results also align with current concepts of the importance of lipoprotein actions as a whole, involving both atherogenic and anti-atherogenic mediators. Our findings encourage the simultaneous investigation and consideration of HDL-c and oxLDL in assessing the potential predictive value.

What should be contemplated with intrigue and where caution may also be advisable, is around the fact that the subjects in this study have sustained a lifespan with environmental features that will not be repeated for their future generations. Firstly, they have generally lead a life, which includes an environment with agricultural emphasis on a number of lifestyle sectors of several decades' duration. Secondly, all subjects have survived the Finnish societal dimensions of at least one World War, the presence and aftermath of which, naturally, have remarkable consequences on many aspects of health – both short- and long-term. Thirdly, and consequentially from the two earlier notions, the majority of their lives has most likely been lead under circumstances with considerably challenged availability on pharmacotherapy for different illnesses. For example, the prevalence of lipid-lowering therapy for the entire 1260 inhabitants was remarkably low – only six percent of subjects (80 subjects, 40 men, 40 women). This is especially intriguing, as the reputation of Finland as a leader in cardiovascular mortality originates as far back as the 1960's (Keys et al. 1970 and 1986).

It could be argued that these results therefore are no longer valid in front of today's treatment protocols with highly expanded pharmacotherapy industry. However, it could as well be argued, that this study population offers an exceptional opportunity to explore all-cause mortality risk without previous history of modern pharmacotherapy and its risk modifying potential. In other words, it can be concluded that this study population represents mortality with a noteworthy lifestyle emphasis and without the shaping effect of medicinal treatments confounding the horizon.

For this study, as it includes people in the evening of their lifespan, the most ill have deceased the earliest, and the healthiest subjects have remained. This is something that cannot necessarily be said anymore, as current treatments have prolonged and enabled the survival of patients with conditions that used to be fatal in a more straightforward fashion.

The survived and the deceased have been selected by nature to either group. By today, and ever since the industrialization, pharmacological industry and medical treatment protocols have intervened this course of events in a unforeseen manner. By continuance of this current trend, people who survive, are more and more likely to do so *via* assistance of pharmacotherapy, and consequentially, fatal outcomes occur regardless.

6.5. YOUNG ADULT MALE RESERVISTS

Metabolically, abdominal obesity is particularly harmful, acting *via* a multiplicity of different pathways (Pouliot et al., 1994; Wang et al. 2005). One relatively novel dimension in this entity is the oxidative stress milieu. It has been proposed that the oxidation events relevant in atherosclerosis development may be a response to the underlying inflammatory events (Stocker and Keaney, 2004). Indeed, inflammatory processes play a substantial role in obesity-related disadvantages (Lumeng and Saltiel, 2011). The scarce data thus far investigating oxidative stress markers in obesity further suggests the especially detrimental nature of central fat accumulation (Pihl et al. 2006, Weinbrenner et al. 2006). It has been observed that the development of atherosclerosis risk may initiate as early as childhood (Juonala et al. 2013). Some promising findings indicate that a healthier lifestyle, including the adoption of the Mediterranean diet (Urpi-Sarda et al. 2012) and reduced waist circumference, is accompanied with decreased levels of inflammatory markers, such as IL-6 (Richard et al. 2013). What has been less explored, however, has been whether or not oxidatively modified LDL lipids might interact in circumstances with joint effects of both smoking and abdominal obesity.

Subjects and methods

A total of 846 participants, aged 18 to 48 (mean age 25,1 years), volunteered for the study. The data collection took place during the year 2008. The 323 smokers (38%) men smoked regularly the remaining non-smokersreported never to have smoked regularly.

Main findings

A shift to a greater WC group corresponded to an increasing prevalence in smoking (from 36 to 45%) . This is expected as risk factors of lifestyle origin tend to exist in clusters. In the WC group of less than 90 cm, smokers had one, five and eight percent less glucose, HDL-c and IGF-1 than non-smokers, respectively. In the WC groups of 90-99.9 cm and greater than 100 cm smokers had consistently 14 to 19 percent higher concentrations of oxLDL and oxLDL/HDL. Generally, adding a smoking habit to an increasing abdominal girth seems to burden the metabolic milieu comparable to having several more centimeters of excess waist.

Clinical relevance and perspectives

In light of the enormous weight of evidence, quitting smoking has been, rightfully so, denoted as the single most efficient task to improve cardiovascular disease risk factor profile (Messner and Bernhard, 2014). Despite the overwhelming amount of data and the public programs targeted at cutting down smoking habit, unfortunately this issue is still contemporary: second-hand smoke alone has been estimated to kill some 600 000 people annually (Messner and Bernhard, 2014). Additionally, in Finland, of particular concern is the notion that among pregnant women, unlike in, for example, Sweden, the occurrence of smoking has not decreased during the last two decades, but instead, has frozen at 15 percent (Rogers, 2009; Ekblad et al. 2013). For men, however, the popularity of smoking has been on a decreasing trend (Heloma et al. 2004), currently being some 25 percent (Jousilahti et al. 2012).

Considering the androgenic perspective in smoking, interestingly it was recently discovered that maternal smoking is associated with impairments in the son's testicular development and semen quality (Virtanen et al. 2012). Bearing in mind the persistence in smoking habit among the Finnish population, increasing scientific data on the related adverse effects should offer all the more rationality to expect a more favourable future. However, a particularly perilous trend is the commonness of snuff use in young Finnish boys: among 18-year-old boys the prevalence of snuff use is seven-fold (*ca.* 12-14%) compared to the situation 30 years ago (Raisamo et al. 2011). Snus ('moist snuff') users have an approximately 13 percent higher (i.e. moderately increased) cancer-related mortality compared to non-users (Nordenvall et al. 2013). One main concern is the prolonged exposure of nicotine that aggravates the development of severe addiction (Wickholm et al. 2012). Smoking elicits a response characterized as alternated HPA-axis, implicating changes in cortisol action. It propagates low-grade inflammation, and thereby may cause further aggravation in atherosclerosis (Rohleder and Kirschbaum, 2006).

It appears that at the individual level, the ice-breaking factors that finally could turn the course in popularity of smoking – permanently and for both sexes – are not scientific but rather social, psychological or traditional. Cultures, where tobacco products are an implicit part of otherwise seemingly healthy lifestyle, such as playing ice-hockey, are especially worrisome for these participating young

men. It is therefore obvious that as our national goal "Non-smoking Finland by 2040" will be a multi-faceted challenge (Wickholm et al. 2012).

In recent years, the metabolic details involved in abdominal obesity have started to become more and more elucidated. Currently, adipose tissue – especially viscerally accumulated – is considered an organ with endocrine properties (Galic et al. 2010) and the data on the pathophysiology of obesity is becoming increasingly sophisticated (Tchernof and Després, 2013). For example, in cases where a metabolic syndrome – type of cluster of different cardiovascular risk factors is developing, inflammation of the adipose tissue might play a role (Naukkarinen et al. 2014).

This is of substantial relevance in light of obesity prognosis for the future in Finland. Between 1986 and 2006, among 12-year old children, the prevalence of overweight (BMI above 25 kg/m^2) increased from 13 percent to 19 percent for girls and 24 percent for boys. There was also a significant difference in overweight prevalence according to rural or urban living environment, favoring urban areas (Vuorela et al. 2009). Obviously, this trend needs to be reversed as soon as possible (Kiukaanniemi et al. 2013).

Bearing these in mind the rationale to further explore the detrimental nature of both smoking and obesity, as outlined here, seems evident. The results presented above suggest that abdominal obesity is further complicated by concomitant smoking habit, when considered from the perspective of atherosclerosis development. Or, alternatively, that a smoking habit is associated with a worsening in both visceral adiposity and concentrations of oxLDL, as the prevalence of smoking increased by growth in waist circumference. It should be noted, however, that a single measurement of waist girth, measured in centimeters, although done professionally, does not differentiate between visceral and peripheral adipose tissue. Some have tried to improve the accuracy of waist line measurement by defining a condition called 'hypertriglyceridemic waist', where waist circumference is accompanied by concentration of serum triglycerides (Lemieux et al. 2000).

We did not explore this condition in our data, as in all our studies presented above, oxLDL is already strongly correlated with triglycerides (see ch. 5.4). In other words, subjects with high concentrations of oxLDL, most likely share an increased level of triglycerides as well. Furthermore, the results summarized in this chapter are in line with results presented in ch. 6.2, where increasing waist by 10 cm in middle-aged men was associated with an increase in oxLDL and oxLDL/HDL-c.

Chronic smoking diminishes the adrenocortical response to stress, and after cessation, the system's recoil back to physiological balance may be a lengthy process (Berlin, 2009). The interrelationship between disturbed glucocorticoid action and development of excess visceral fat deposition (Lee et al. 2014) warrants that challenges in HPA-axis via a smoking habit may therefore in some cases compromise abdominal leanness, or the health of internal organs, as well. After all, visceral obesity is sometimes titled as "functional hypercortisolism" (Pasquali and Vicennati, 2000). However, a detailed interconnectedness between

these factors remains to be elucidated, as the intricacies behind endocrine consequences perpetrated by smoking remain largely yet unknown. Additionally, no cortisol measurements were included in the study design, which could've provided a framework to demonstrate or discuss possible relevance. Also, our cross-sectional study design allows no causal brilliance. Traditionally, discussions over the exact culprit(s) responsible for the plethora of various consequences of smoking have been centered on the effects of nicotine (Rohleder and Kirschbaum, 2006).

This study, indicating some of the complications involved in central obesity, aligns with previous works promoting the importance of adopting healthy lifestyle. Prevention of obesity in young adulthood is an investment to the future, paying itself back as dividends in the form of, for example, decreased atherosclerosis risk later in life (Reis et al. 2013).

7. SUMMARY AND CONCLUSIONS

~ Weight reduction of 14 percent caused a considerable reduction (27%) in the concentration of oxLDL in middle-aged, obese men. This reduction persisted alongside successful weight maintenance, if regain in weight remained below six kilograms per two years of follow-up.

~ In both smoking and non-smoking middle-aged men, low serum total testosterone (≤ 15 nmol/l) was associated with significantly higher concentrations of oxLDL than in men with normal/high serum total testosterone. Low serum total testosterone was associated with a generally worse lipid profile than normal/high serum total testosterone. Additionally, smokers had more oxLDL and less HDL-c than non-smokers.

~ In the most insulin resistant middle-aged men, the concentrations of oxLDL and oxLDL/HDL were the highest during a two-year follow-up after weight reduction. Similarly, the most insulin sensitive of men had the lowest concentrations of oxLDL. An increasing severity in insulin resistance was paralleled by a general worsening of anthropometric and serum lipid profile.

~ OxLDL, when proportioned to LDL-c, HDL-c or apoA1, maintained its predictive value as a risk factor of all-cause mortality in a population aged 64 years or more. These findings persisted after controlling for age, sex, BMI, smoking, blood pressure and diabetes. The concentrations of those surviving a 10-year period were significantly higher in oxLDL/LDL-c and oxLDL/HDL-c compared to subjects, who had deceased.

~ In young adult men, an increasing abdominal girth in smokers led to higher concentrations of oxLDL and oxLDL/HDL-c than among non-smokers. A 10 cm increase in waist circumference corresponded to a 11 to 55 percent increase in insulin concentration and 25 to 30% increase in oxLDL and oxLDL/HDL-c.

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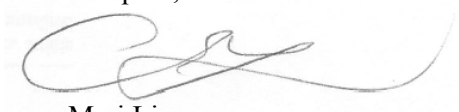
Tampere, Novermber 2014



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