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OXIDIZED LDL AND PHYSICAL FITNESS IN HEALTHY YOUNG MEN: ASSOCIATIONS WITH BODY COMPOSITION, SMOKING, METABOLIC SYNDROME AND ANDROGEN STATUS

by

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ABSTRACT

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Oxidized LDL and physical fitness in healthy young men: associations with body composition, smoking, metabolic syndrome and androgen status.

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Background: In the past, oxidized low density lipoprotein (ox-LDL) has been associated with an unbeneficial lipid profile. This atherogenic lipid profile increases the risk of atherosclerotic cardiovascular diseases. Physical fitness has substantial effect on serum lipoprotein concentration as well as body composition and humoral responses, however interrelationships between ox-LDL and physical fitness have not been widely examined in a nationally representative sample.

Aims: This thesis evaluates how cardiorespiratory and muscular fitness associate with ox-LDL lipids and how the other known risk factors of atherosclerosis might alter these associations.

Subjects and Methods: The study cohort consisted of 846 healthy young males (mean age 25.1, SD 4.6) who were gathered by voluntary nationwide recruitment. Each participant conducted a series of physical fitness tests (cardiorespiratory and muscular fitness) and answered a detailed questionnaire that included lifestyle habits (i.e. smoking and leisure-time physical activity). Venous blood samples including ox-LDL and serum lipids were also collected.

Results: Higher levels of ox-LDL were found in overweight and obese men, however, high cardiorespiratory fitness seemed to protect the overweight from high levels of ox-LDL. Young men who smoked and had poor cardiorespiratory or muscular fitness possessed a higher concentration of ox-LDL lipids when compared to comparable levels of cardiorespiratory or muscular fitness non-smoking young men. Metabolic syndrome was associated with increased levels of ox-LDL and high levels of ox-LDL combined with poor cardiorespiratory and abdominal muscle fitness seems to predict metabolic syndrome in young men. Also, participants with poor cardiorespiratory fitness and low levels of testosterone had higher levels of ox-LDL when compared to participants with high cardiorespiratory fitness / low testosterone as well as those with poor cardiorespiratory fitness / high testosterone.

Conclusions: Good cardiorespiratory and muscular fitness protects young men from increased levels of ox-LDL lipids. This association was discovered in young men who were categorized as being overweight, smokers, metabolic syndrome or with low levels of testosterone. Being fit seems to prevent higher levels of ox-LDL, even in young healthy men.

TIIVISTELMÄ

Jussi Kosola

Hapettuneet LDL rasvat ja fyysinen kunto terveillä nuorilla miehillä - yhteydet kehon koostumukseen, tupakointiin, metaboliseen oireyhtymään sekä mieshormoneihin. Department of Health Exercise; Department of Physiology; Paavo Nurmi Center, University of Turku, Turku, Finland. Annales Universitas Turkuensis, Medica-Odontologica, Turku, Finland, 2013.

Tausta: Hapettuneet LDL rasvat (ox-LDL) ovat avainasemassa valtimonkovettumataudissa. Valtimoissa kiertävät LDL-rasvat hapettuvat valtimon seinämissä, jolloin seinämissä muodostunut ox-LDL aiheuttaa stressitilan. Tämä kohonnut hapettunut stressi kulminoituu kolesterolin kerääntymisenä valtimon seinämiin ja edelleen valtimoa tukkivaan kalkkeutuneeseen plakkiin. Fyysisen kunnon tiedetään vaikuttavan elimistön rasva-arvoihin laskemalla haitallisten kolesterolia valtimoon tuovien LDL-rasvojen määrää ja samalla nostamalla kolesterolia valtimon seinämistä poisvievien HDL-rasvojen määrää. Ylipaino, tupakointi, metabolinen oireyhtymä sekä matalat mieshormonitasot ovat kaikki rinnastettu kohonneisiin LDL- ja mataliin HDL-rasvojen pitoisuuksiin aiemmin.

Tavoite: Tutkia laajalla kansallisella aineistolla nuorten miesten ox-LDL-pitoisuuksia eri fyysisen kunnon ryhmissä sekä kartoittaa kehonkoostumuksen, elämäntapojen, tupakoinnin sekä aineenvaihdunnan häiriöiden yhteyksiä ox-LDL-pitoisuuksiin.

Menetelmät: 846 nuorta miestä (keski-ikä 25.1, keskihajonta 4.6 vuotta) osallistuivat vapaaehtoisina Puolustusvoimien kertausharjoitusten yhteydessä kerättävään tutkimusaineistoon. Kukin osallistunut suoritti maksimaalisen hapenottokyvyn sekä lihaskunnon testauksen, ja heistä kerättiin laskimoverinäytteet sisältäen ox-LDL-pitoisuuden sekä muita veren rasva-arvoja. Ennen kuntotestejä ja verinäytteenottoa osallistujat kävivät rutiini terveystarkastuksen, johon kuului myös kirjallinen sekä suullinen haastattelu.

Tulokset: Ylipainoisilla sekä lihavilla nuorilla miehillä havaittiin korkeammat ox-LDL-rasvojen pitoisuudet verrattuna normaalipainoisiin. Kuitenkin hyvä kestävyyskunto näytti suojaavan myös ylipainoisia nuoria miehiä kohonneilta ox-LDL-pitoisuuksilta. Huonon kestävyys- ja lihaskunnon omaavilla tupakoivilla nuorilla miehillä on korkeammat ox-LDL-pitoisuudet verrattuna parempi kuntoisiin nuoriin miehiin. Lisäksi heikon lihaskunnon alaryhmässä tupakoijilla oli merkittävästi korkeammat ox-LDL-pitoisuudet. Nuorilla miehillä, joilla täyttyvät metabolisen oireyhtymän kriteerit, on korkeammat ox-LDL-pitoisuudet verrattuna nuoriin miehiin ilman metabolista oireyhtymää. Myös korkea ox-LDL-pitoisuus yhdessä heikon kestävyyskunnon sekä vatsalihasten lihaskunnon kanssa voivat ennustaa metabolista oireyhtymää. Matalat testosteronipitoisuudet ovat yhteydessä kohonneisiin ox-LDL-pitoisuuksiin. Hyväkuntoisilla matalan testosteronin miehillä on kuitenkin yhtenevät ox-LDL-pitoisuudet hyväkuntoisten korkean testosteronin miehiin verrattuna.

Johtopäätökset: Hyvä kestävyys- ja lihaskunto suojaavat nuoria miehiä kohonneilta ox-LDL-pitoisuuksilta. Ylipaino, tupakoiminen, metabolinen oireyhtymä sekä matalat testosteronipitoisuudet ovat yhteydessä kohonneeseen ox-LDL-pitoisuuteen. Hyvä kunto suojaa yllämainituilta riskitekijöiltä.

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LIST OF ABBREVIATIONS

ACCF / AHA	American College of Cardiology Foundation and the American Heart Association
BMI	Body Mass Index
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
FTES	Free testosterone
HDL	High density lipoprotein
IDF	International Diabetes Federation
il-6	Interleukin-6
IPAQ	International Physical Activity Questionnaire
LDL	Low density lipoprotein
LTPA	Leisure-Time Physical Activity
MetS	Metabolic syndrome
MFI	Muscle fitness index
NIH	National Institute of Health
ox-LDL	Oxidized LDL lipids
ROS	Reactive oxygen species
TES	Testosterone
TNFα	Tumour necrosis factor alpha
USDHHS	U.S. Department of Health and Human Services
WHO	World Health Organization
VLDL	Very low density lipoprotein
VO_2max	Maximal oxygen uptake

LIST OF ORIGINAL COMMUNICATIONS

This academic dissertation if based on following original research papers. Referred papers are indicated by Roman numerals (I-IV):

I) **Kosola J**, Ahotupa M, Kyröläinen H, Santtila M, Vasankari T. Both poor cardiorespiratory and weak muscle fitness are related to a high concentration of oxidized low-density lipoprotein lipids. Scandinavian Journal of Medicine & Science in Sports, 2012; 22: 746-755.

II) **Kosola J**, Ahotupa M, Kyröläinen H, Santtila M, Vasankari T. Good aerobic or muscular fitness protects overweight men from elevated oxidized LDL. Medicine and Science in Sports and Exercise, 2012; 44: 563-8.

III) **Kosola J,** Vaara J, Ahotupa M, Kyröläinen H, Santtila M, Oksala N, Mustafa A, Vasankari T. Elevated concentration of oxidized LDL together with poor cardiorespiratory and abdominal muscle fitness predict metabolic syndrome in young men. Metabolism, 2013, doi: 10.1016/j.metabol.2013.01.013

IV) **Kosola J,** Ahotupa M, Kyröläinen H, Santtila M, Vasankari T. Young men with poor cardiorespiratory fitness combined with lower testosterone have high levels of oxidized LDL lipids - being fit prevents this relationship. [Submitted].

1. INTRODUCTION

As cardiovascular diseases and diabetes are the number one cause of mortality worldwide, more information regarding the pathophysiology of such disease is necessary (World Health Organization [WHO], 2012). The major cause of cardiovascular disease is the formation of atherosclerosis in the arterial endothelium. The atherosclerotic cascade culminates in a rigid plaque which utterly impairs the vasodilation function of the artery (American College of Cardiology Foundation and the American Heart Association [ACCF / AHA], 2011). This atherosclerotic plaque is formed from cholesterol particles which are delivered from the lumen and due to the harmful reactions of oxidative stress towards cholesterol carrying lipoproteins, the vicious circle of plaque formation begins (Escarqueil-Blanc, 1994; Salvayre et al., 2002). In these oxidative stress reactions, oxidized LDL lipids (ox-LDL) are considered as major contributors (Stocker & Keaney, 2004). An increased concentration of cholesterol delivering circulating lipoprotein particles (mainly low density lipoprotein [LDL]) is considered as one of the major risk factors behind cardiovascular diseases, and poor physical fitness and overweight have been acknowledged to have a significant impact on the levels of circulating lipoproteins (Slentz et al., 2007; Lee et al., 2007).

Poor physical fitness has been widely accepted as predisposing factor of cardiovascular diseases (Blair et al., 2001; Sandvik et al., 1993), and is also related to all-cause mortality (Lee, CD et al., 1999). On the other hand, good physical fitness has positive effects on lipid metabolism by way of decreasing the atherogenic levels of total cholesterol lipoprotein particles (mainly the subclasses of different LDL lipids), and increasing antiatherogenic high density lipoprotein (HDL) (Carnethon et al., 2005a). However, new novel biomarkers such as ox-LDL, are considered to be more sensitive for evaluating preclinical atherosclerosis, but have not been evaluated thoroughly in terms of physical fitness.

Especially, the Western lifestyle which includes a vast amount of physical inactivity leading to poor fitness is piling up multiple risk factors for cardiovascular disease. A sum of these atherogenic risk factors was collected and the condition was firstly named as MetS in 1988 (Reaven et al., 2005). The main reason for metabolic syndrome (MetS) is considered to be insulin resistance which increases highly in abdominal obesity (International Diabetes Federation [IDF], 2006). Furthermore, abdominal obesity has recently been deemed to be associated with an increased oxidative stress, systemic inflammation and poor physical fitness. Thus the evaluation of these individual components in young men seems a highly relevant research goal in order to determine the interrelationships of different components of MetS and the earlier

mentioned novel risk factors of cardiovascular diseases (Kern et al., 2001; Fischer et al., 2006).

Finally, testosterone (TES) seems to have a role in the formation of atherosclerosis and serum lipoprotein content. TES inhibits nitric oxide reactions in the endothelium and has also been associated with increased oxidative stress (Bernini et al., 2006; Shijun et al., 2008; Zhang, 2011). Moreover, low levels of TES have independently been associated with increased levels of ox-LDL (Linna et al., 2008). Both ox-LDL and physical fitness have numerous independent effects on endothelial function, yet no studies have been concluded in order to assess the associations between TES, ox-LDL and physical fitness.

In this thesis, the main objectives were to investigate the interrelationship between ox-LDL and physical fitness and how it is affected by known cardiovascular risk factors (overweight, smoking, MetS and low levels of TES) in young healthy Finnish men.

2. REVIEW OF LITERATURE

2.1. CARDIOVASCULAR DISEASES DUE TO ATHEROSCLEROSIS

2.1.1. Atherosclerosis relation to cardiovascular diseases

Atherosclerosis affects many of the hemodynamic organs. The plaque formed inside the intima of affected arteries causes decreased blood flow to the organ which slowly diminishes the functions of the target organ (ACCF / AHA, 2011). Acute symptoms and even death can occur if the plaque erupts and causes a blockade in artery leading to ischemia of the target tissue such as cardiac muscle (ACCF / AHA, 2011). The mechanisms behind different atherosclerotic cardiovascular diseases are however the same whether the target arteries are coronaries of the heart, peripheral arteries of the limbs, arteries of the kidneys or cerebral arteries. In recent years, more and more attention has been focused on the diagnosis and treatment of asymptomatic atherosclerosis using methods such as intima-media thickness which relates to elevated levels of atherogenic lipoproteins (Schermund et al. 2002; Boquist et al., 1999; Salonen & Salonen, 1991).

2.1.2. Effects on the endothelium in atherosclerosis

The atherosclerotic cascade which leads to plaque formation starts from the damaged endothelial cells of artery walls (Libby, 2011). The damaged cells entice leukocytes which in-turn attract inflammatory compounds to the vessel wall, leaving the damaged endothelium in a state of chronic inflammation and under stress of radical compounds (Shibata & Glass, 2008; Packard & Libby, 2008). These radical compounds are cell toxic and modify the haemostasis of arterial intima cells leading to the further release of inflammatory cytokines (Pohlman & Harlan, 2000; Madge & Pober, 2001). Lipid carrying lipoproteins such as LDL are taken from blood flow into the vascular intima, where they undergo harm by oxidative stress. Especially, LDL lipids are modified into reactive compounds (Stocker & Keaney, 2004). After this modification, the leukocytes (mainly macrophages) in the wall of endothelium digest ox-LDL, so turning the macrophages into bloating foam cells (Gerrity, 1981a). The final step of the cascade is the actual macrovascular intima thickening in the form of an atherosclerotic plaque which contains a necrotic core of macrophages (Gerrity, 1981b; Seimon & Tabas, 2009).

2.2. OXIDIZED LDL AND LIPID METABOLISM IN THE FORMATION OF ATHEROGENIC PLAQUE

2.2.1. Oxidative stress and atherosclerosis

The exact etiology behind the plaque formation in atherosclerosis has not been fully discovered. However, different hypothesis have been suggested including a theory of oxidative stress (Stocker & Keaney, 2004). In the past decade, more detailed information about the role of reactive oxidative species (ROS) has been reported in regard to atherosclerotic plaque formation (Pohlman & Harlan, 2000; Cai & Harrison, 2000). ROS are by-products of cellular metabolism which are capable of damaging macromolecules, such as lipoproteins, via oxidative reactions (Thannickal & Fanburg, 2000). These oxidatives are suggested to play a major role in intermitting cellular signals between the cells of arterial intima and the lipoprotein particles of the arterial lumen leading to cardiovascular diseases such as coronary artery disease (Pohlman & Harlan, 2000; Kiechl et al 2007; Tsimikas et al. 2010). One group of lipoprotein particles is LDL which are mainly responsible for delivering atherogenic lipids into the wall of vessel (Libby et al., 2000). ROS turns LDL into a more reactive lipoprotein called ox-LDL and the concentration of LDL is associated with clinically used ox-LDL biomarkers (Fraley et al 2009; Jessup & Kritharides, 2000). The ox-LDL lipids are closely related to the earlier mentioned creation of foam cells and macrophage activity (Jessup & Kritharides, 2000). It is however important to mention, that LDL does not turn directly into ox-LDL, in fact, ox-LDL lipids are a heterogeneous group of lipoproteins which are in a different phase of oxidation (Jessup & Kritharides, 2000; Miller et al., 2003). Firstly LDL turns into minimally oxidized LDL whose lipoprotein carrier (apolipoprotein B) is not yet damaged and therefore is not capable of foam cell formation (Jessup & Kritharides, 2000; Miller et al., 2003; Berniler et al., 1990). This minimally oxidized LDL can stimulate more macrophages from blood stream into endothelia via inflammation reactions (Berniler et al., 1990). After sufficient oxidation of LDL particles, this minimally oxidized LDL turns into ox-LDL, which has the greatest potential for atherosclerosis formation (Vasankari et al., 2001a; Toikka et al., 2000; Raitakari et al., 2001; Toikka et al., 1999).

2.2.2. Ox-LDL actions in endothelia

Damaged endothelium attracts inflammatory cells, which modify LDL lipids into reactive ox-LDL lipids (Tsimikas & Miller, 2011; Jessup & Kritharides, 2000). These oxidized compounds are prominent for binding into the “scavenger” receptor’s macrophages leading to the digestion of ox-LDL (Sawamura et al., 1997; Jessup & Kritharides, 2000; Greaves & Gordon, 2009). This reaction becomes a continuum where more inflammatory cytokines (Boring et al., 1998; Mach et al., 1999) are released by macrophages due to ox-LDL digestion, resulting in increased macrophage homing to damaged intima and ox-LDL digestion (Stocker & Keaney, 2004). These ox-LDL lipids

turn the macrophages into foam cells which eventually go through apoptosis or necrosis (Escarqueil-Blanc, 1994; Salvayre et al., 2002). Finally, a thick calcium and fibrin containing plaque is formed inside the intima wall of the artery (Wexler et al., 1996; Newby & Zaltsman, 1999; Sukhova et al., 1999; Libby & Simon, 2001). When this continuum of intima damage, foam cell activation and plaque formation has reached its peak, macrovascular rupture of the intima occurs and causes a possibly fatal thrombus in the arterial lumen (Libby et al., 2002; Libby & Simon, 2001).

2.2.3. Lipid metabolism, hypercholesterolemia and atherosclerosis

Increased serum total cholesterol (hypercholesterolemia) has been identified as the main reason of atherosclerosis as much as over two decades ago (Gotto & LaRosa, 1990). However, the treatment of atherosclerotic cardiovascular diseases - the actual end-point of increased serum cholesterol, is still under some doubt (National Institute of Health, 2002 and 2004). According to an expert panel of the National Cholesterol Education Program (National Institute of Health, 2002 and 2004), classifications of unhealthy cholesterol levels include: total cholesterol ≥5.17 mmol/L, LDL cholesterol ≥2.59 mmol/L, HDL cholesterol <1.03 mmol/L and triglycerides ≥ 1.69 mmol/L. Moreover, the ratios between total cholesterol, LDL cholesterol and HDL cholesterol have been noticed to be associated with an increased risk of acute myocardial infarction and ischaemic cardiovascular diseases (Burku et al., 1997; Barter et al., 2007; Prospective Studies Collaboration, 2007; Pedersen et al., 1994).

The LDL lipoprotein complex which contains apolipoprotein B isconsidered as the most ominous lipoprotein causing atherosclerosis (National Institute of Health, 2002; Hevonoja et al., 2000). Reviewed on many occasions, the level of LDL is the main target of therapeutic interventions for atherosclerotic cardiovascular diseases (National Institute of Health, 2002). These LDL lipids are ultimately turned into atherogenic agents through oxidation (section 2.2). Especially, LDL lipids with a high content of triglycerides can easily oxidize and are therefore correlated with the formation of atherosclerosis (Regnstrom et al., 1992). Even the raised levels of LDL in adolescents are interestingly found in middle-aged individuals with increased intima-thickness of the common carotid artery (Raitakari et al., 2003).

HDL serve a reverse role to that of LDL, where HDL lipids carry atherogenic intima-media cholesterol away from cellular matrix back to blood stream (Lewis & Rader, 2005; Stein & Stein, 1999). HDL lipids consist of a subpopulation of different sized HDL lipids (Rye et al., 2009) and have many anti-atherogenic functions such as enhancing endothelium repair and function, as well as inhibiting monocyte binding into the endothelium (Tso et al., 2006; Mineo et al., 2006: Murphy et al., 2008; Rye et al., 2009; Nofer et al., 2002). Adding to these beneficial roles, HDL has anti-thrombotic and anti-inflammatory properties, so preventing ischemic thrombus formation (Mineo et al., 2006; Cockeril et al., 1995). As a risk factor for pathological cardiovascular

conditions, HDL has proven to be an independent cardiovascular disease risk factor and levels of below 1.04 mmol/L have been related to an increased onset of metabolic syndrome (MetS) and acute myocardial infarct (Ford et al., 2002; Burku et al., 1997; Barter et al., 2007; Gordon et al., 1989a). HDL particles have an antioxidant capacity and inhibit atherogenic LDL oxidation and reduces the biological activity of pro-inflammatory minimally ox-LDL (Negre-Salvayre et al., 2006; Watson et al., 1995).

Serum triglycerides are less researched when compared to the serum levels of LDL and HDL lipids, however hypertriglyceridemia is an independent risk factor of coronary artery disease and cardiovascular diseases caused by atherosclerosis (Fruchart et al., 2002; Germing et al., 1996; Gotto, 1998). High-triglyceride containing lipoprotein fragments are suggested to be the main reason of hypertriglyceridemia caused atherosclerosis (Lippel et al., 1981). With interrelationships with endothelial actions, high triglyceride levels are constantly seen in lifestyle diseases such as insulin resistance and metabolic syndrome, increasing the risk of metabolic syndrome over levels of 1.69 mmol/L as well as a higher prevalence of atherosclerotic cardiovascular diseases (Wallenfeldt et al., 2005; Ford et al., 2002; National Institute of Health, 2002).

2.3. POOR PHYSICAL FITNESS AND PHYSICAL INACTIVITY AS RISK FACTORS OF ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASES

2.3.1. Physical inactivity, atherosclerosis and clinical disorders

In 2011, physical inactivity had reached number four in the World Health Organization (WHO) cause of mortality list and over it was cited that 1.9 million people die annually because of physical inactivity (WHO, 2011). As atherosclerosis related cardiovascular diseases maintained the number one position, further evidence was shown that physical activity can influence upon the protection from cardiovascular diseases, both as a primary and secondary prevention measure (Lavie et al., 2009; Hobbs, 2004). Physical activity has positive effects on blood pressure by lowering high systolic or diastolic pressure and offering protection from hypertension, whilst also benefiting heart rate recovery after exercise (National Institute of Health [NIH], 2004; Whelton et al., 2002; Carnethon et al., 2005b). Moreover, physical inactivity is widely associated with an increased risk of cardiovascular diseases and is one of the hallmark lifestyle attributes in weight control and the reduction of weight in those who are overweight (WHO, 2002, 2005, 2007-2009). These beneficial effects seems to be dose related whereas higher activity levels are associated with higher cardiovascular health (Shiroma et al., 2010; U.S. Department of Health and Human Services [USDHHS], 2008). Regular physical activity improves endothelial function and prevents atherosclerosis formation. Leisure-time physical activity (LTPA) also correlates with arterial elasticity which somewhat associates with atherosclerosis (Zureik et al., 2002; Schmidt-Truckäss et al.,

1999). In adolescents, the progression of intima-media thickness can be attenuated by being more active (Pahkala et al., 2011). Physical activity is considered to be beneficial for asymptomatic and symptomatic atherosclerosis disease and different guidelines for regular physical activity have been established (ACCF/AHA, 2006; Haskell et al., 2008). Being active also decreases obesity and obesity related comorbidies (Grundy et al, 1999) and physical activity improves the function of endothelium and vasodilation in atherosclerotic compromised arteries (DeSouza et al., 2000). Furthermore, low grade systemic inflammation has been associated with atherosclerotic cardiovascular diseases and physically active individuals are proven to possess lower concentrations of inflammation markers such as C-reactive protein (CRP), tumour necrosis factor-alpha (TNFα) and interleukin-6 (il-6) (Panagiotakos et al., 2005; Elosua et al., 2005).

2.3.2. Effects of cardiorespiratory and muscular fitness on atherosclerosis and health

In the late 1980s, the abnormal function of calcified coronary arteries during peak exercise was discovered (Gordon et al., 1989b). From these studies, the inverse associations of cardiorespiratory fitness and atherosclerosis related diseases is well-documented in large scale international epidemiological studies (Table 1.)

Table 1. Large scale international epidemiological studies related to cardiorespiratory fitness (VO_2max) and atherosclerosis (N=number of participants).

Year published	Design	Physical fitness test	Measurement of atherosclerosis	N	age (years)	Reference
1989	cross-sectional	bicycle ergometer	angiography, vascular lumen stenosis	21	54±7	Gordon et al.
1995	cross-sectional	bicycle ergometer	Ultrasound of intima-media thickness	163	50-60	Rauramaa
2001	longitudinal	bicycle ergometer	Ultrasound of intima-media thickness	854	42-60	Lakka et al.
2002	cross-sectional	treadmill	Ultrasound diameter of arterial lumen	351	13-16, 36	Ferreira
2003	longitudinal	treadmill	Ultrasound diameter of arterial lumen	154	13-16, 21-32	Ferreira
2009	cross-sectional	treadmill	Ultrasound of intima-media thickness	9871	40-81	Lee et al.

Low cardiorespiratory fitness is also precursor of a cardiovascular disease, mortality and lifestyle related diseases such as insulin resistance and metabolic syndrome (Anderssen et al., 2007; Blair et al., 1996; Orakzai et al., 2006; Laaksonen et al., 2002). Muscular fitness and strength training are related to these insulin resistance based metabolic disorders including obesity and metabolic syndrome (Strasser et al., 2010). As insulin resistance is constantly increasing in Western Society leading to increased

levels of fasting glucose, metabolic syndrome and type 2 diabetes, resistance training (leading to better muscular fitness [Bemben et al., 2000]) is suggested in order to correct the impaired glucose uptake of the tissues. For type 2 diabetics who are already insulin resistant, progressive resistance training seems to balance long term glucose homeostasis, lower high waist circumference and lower systolic and diastolic blood pressure (Arora et al., 2009; Castaneda et al., 2002; Kelley et al., 2000). Interestingly, with only minor effects on lipids, high muscular strength could attenuate the risk for cancer (Ruiz et al., 2009), while both individual poor grip strength and abdominal fitness seem to predict higher mortality rates (Katzmarzyk & Craig, 2002; Laukkanen et al., 1995; Sasaki et al., 2007).

2.3.3. Effects of physical fitness and physical activity on blood lipid metabolism

With numerous beneficial effects on cardiovascular health, being active can improve the size of lipoprotein particles in HDL, LDL and decrease serum very low density lipoprotein (VLDL)-triglyceride concentration (Slentz et al., 2007). Especially, exercise increases HDL, lowers triglycerides and apolipoprotein B in subjects with low HDL, high triglycerides and increased abdominal adiposity (Couillard et al., 2001). Detraining shows how beneficial lipid changes obtained with aerobic exercise intervention (not with resistance training) can be reduced by being sedentary (LeMura et al., 2000). However, in adolescents and children, no significant change in lipid profiles by exercise intervention has been fully established (Tolfrey et al., 2000).

Cardiorespiratory fitness is associated with the earlier mentioned atherogenic lipid profile by lowering levels of total cholesterol, LDL and triglycerides, and increasing HDL (Vasankari et al., 2000; Linna et al., 2007; Carnethon et al., 2003; LeMura et al., 2000). When maximal oxygen uptake (VO_2max) raises, the LDL complex - containing apolipoprotein B - decreases (Holme et al., 2007).

While some evidence has been gathered concerning the poor muscle fitness relation to oxidative stress and poor lipid profile, no studies have been conducted using novel new biomarkers (such as ox-LDL) for detecting the associations of muscle fitness and the possible early risk of atherosclerosis. Although having less effect on circulating lipids when compared to cardiorespiratory fitness (LeMura et al., 2000; Banz et al., 2003), resistance training can increase antiatherogenic HDL (Thompson & Rader, 2001). Also, resistance training has been shown to lower levels of total cholesterol in type 2 diabetics (Arora et al., 2009).

Unfortunately, the most atherogenic lipids produced by oxidative stress (mainly ox-LDL) have not been widely examined. A study using the diene conjugation method of determining ox-LDL indicated that exercise improves cardiorespiratory fitness and peak VO_2max, leading to decreased levels of atherogenic ox-LDL (Vasankari et al., 1998). No studies have been published however, using muscular fitness as a variable of comparison with ox-LDL.

2.3.4. Physical fitness, oxidative stress and oxidized LDL

The early evidence of exercise induced oxidative stress and ROS capabilities for tissue damage was shown in 1978 (Dillard et al., 1978). Past research suggested that the amount of stress produced by exercise is an indication of ROS species formation (Jenkins, 1988) Further research has since been conducted in order to determine the role of ROS in exercise induced oxidative stress (Finaud et al., 2006). Apparently, ROS has both positive and negative effects through exercise. In regard to positive effects; exercising with unfatigued muscles could balance the oxidation whilst training in a fatigued state could cause oxidation to be accelerated (Reid, 2001). The negative effects of ROS are mainly due to the modification of molecules into more harmful species, for example peroxidized lipids, damage to DNA and RNA and modifications in the protein amino acid profile (Finaud et al., 2006). This especially concerns lipoprotein oxidation, which can be heightened with strenuous exercising and generates instinctively reactive ox-LDL with delay (Liu et al., 1999). Yet individuals with high physical fitness (i.e. athletes) seem to compensate such oxidation via an increased high quantity of free radical scavenger enzymes which are produced during aerobic exercise (Marzatico et al., 1997; Hellsten et al., 1996). This could protect athletes from atherosclerosis despite the fact that exercise enhances the oxidation of LDL (Pincemail et al, 2000). Notably, the reactions of the endothelium and ox-LDL are of high focus in the research of atherosclerosis prevention related to physical fitness, thus high levels of ox-LDL have been proven to decrease through lifestyle interventions (Vasankari et al., 1998; Vasankari et al., 2001b) In addition to the atherosclerotic endothelial reactions of oxidative stress and ox-LDL, physical activity and physical fitness seem to protect vascular health with a mechanism affecting the impaired function of nitric oxide and low-grade inflammation (Aronson, et al., 2004; Goldhammer et al., 2005; Martinez Gomez et al., 2010; Hambrecht et al., 2000; Hambrecht et al., 2003).

2.4. SMOKING, OXIDATIVE STRESS AND OXIDIZED LDL

Smoking causes inadequate vasomotor function in arteries by vasodilation (Celermajer et al., 1993) and is associated with unbeneficial concentrations of serum HDL and triglycerides (Mammas et al., 2003). Atherogenic based cardiovascular diseases have been related to the habit of smoking and smokers have an increased risk of all-cause and cardiovascular disease mortality (Doll et al., 2004). While Kamisaki et al. (1997) found over 4000 oxidizing substances from within tobacco smoke and because of the additional substance reactions with arterial endothelium and associations with atherosclerotic morbidities, the effects of smoking on the lipoprotein profile and oxidative stress have become an increasing focus in the field of atherosclerotic research. A recent study (Talukder et al., 2010) introduces a mouse model linking smoking to increased oxidative stress, while Beck et al. (2008) suggested this same relationship with women aged over 65 years. Yanbaeva et al. (2007) also demonstrated

atherogenic low-grade systemic inflammation to be associated with smoking. Furthermore, whilst smoking associates with lipoprotein oxidatives, middle aged Finnish men who were chronic smokers demonstrated higher concentrations of ox-LDL (Linna et al., 2008). From the perspective of physical fitness, smokers tend to have poorer cardiorespiratory and muscular fitness (Conway & Cronan, 1992). Smoking also has some effect on testosterone metabolism (English et al., 2001).

2.5. ASSOCIATIONS OF METABOLIC DISORDERS, PHYSICAL FITNESS AND OXIDATIVE STRESS

2.5.1. Overweight, obesity, increased central waist circumference and lipid metabolism

Increasing overweight, obesity and waist circumference seems to be the plague of the 21st century. WHO describes overweight, obesity and increased waist circumference as follows: overweight (25.0-29.9 kg/m2), obese (≥ 30.0 kg/m2) and an increased waist circumference (> 102 cm) (NIH, 1998). Obesity is linked to numerous harmful effects on the human body and one of the end-points is calcified arterial veins *a.k.a.* atherosclerosis (Lee et al., 2007). There is increasing evidence towards the harmful effects of obesity and a number of studies have demonstrated the increased risk of type 2 diabetes, hypertension, and cardiovascular disease, as well as mortality associations (NIH, 1998; Hubert et al., 1983).

Compelling evidence has been produced that human adipose tissue has an active role in modulating the oxidative stress and chronic inflammation that may lead to atherosclerosis. While oxidative stress is highly relevant in modifying lipoprotein into a more atherogenic form, it also compromises endothelial function. Obesity causes arterial wall dysfunction by impairing vasodilatation through increased oxidative stress (Steinberg et al., 1996; Perticone et al., 2001) and in contrast, a 6 week intervention of weight reduction with a very low calorific diet enhanced the endothelial function in overweight adults (Raitakari et al., 2004). Furthermore, with small numbers of subjects, Urakawa et al., (2003) published a significant correlation between adiposity and elevated oxidative stress.

In the Bogalusa Heart Study, clinical fatty streaks and fibrous atherosclerotic plaques were found in post mortem arterial samples taken from overweight children and young adults, indicating a clear correlation between obesity and atherosclerosis (Berenson et al., 1998). In addition to this, overweight and obesity are widely associated with a harmful lipid profile - high levels of atherosclerotic total cholesterol, LDL, triglycerides and low levels of antiatherosclerotic HDL (Brown et al., 2000; Denke 1993; Berenson et al., 1998). Rationally, weight loss through dieting lowers the levels of total cholesterol, LDL and triglycerides (Dattilo & Kris-Etherton, 1992), and when

weight loss was enhanced by adding an exercise regime into the weight loss intervention, the HDL was also raised (Leon & Sanchez, 2001).

The formation of atherosclerotic plaque and endothelial dysfunction derive from numerous molecular reactions where the oxidation of lipids and systemic inflammation play key roles in the overweight and obese. Few studies have found significant relationships between oxidative modified LDL (ox-LDL), and overweight and obesity. Both an increased waist circumference and high BMI are independently associated with increased levels of ox-LDL (Weinbrenner et al., 2006; Holvoet et al., 2001). However, shorter lag times of LDL oxidation have been reported in obese children and morbidly obese adults when compared to normal weight individuals, which might in-turn indicate a more potent oxidation of LDL lipids in the obese (Van Gaal et al., 1998; Mohn et al., 2005).

2.5.2. Metabolic syndrome relation to lipids, oxidized LDL, atherosclerosis and physical fitness

Metabolic syndrome (MetS) is defined as a cluster of risk factors of atherosclerotic cardiovascular disease and as a combination of increased waist circumference or high BMI, together with hypertension, lipid abnormalities and insulin resistance (IDF, 2006). Lipid abnormality includes raised levels of triglycerides and low levels of HDL, while a raised fasting glucose of plasma is considered to determine the presence of insulin resistance (Reaven, 2005). Due to these cascading multiple cardiovascular disease risk factors, MetS has been associated with atherosclerosis and with increased intima-media thickness (Mattson et al, 2008; Hassinen et al., 2006).

While MetS has numerous connections with different lipoprotein particles, notably, oxidative stress has been associated with MetS components (Halle et al 1999; Park et al., 2009). Moreover, ox-LDL – one of the key players in the formation of atherosclerotic plaque – has been appointed as a predictive risk factor of MetS in a large scale Finnish population study (Koskinen et al., 2011). Ox-LDL seems to play a substantial part in the pathogenesis of insulin resistance and Holvoet et al., (2008) have suggested the causality between increased ox-LDL and MetS. Originally demonstrated in 1988 as syndrome X by Reaven et al., MetS has been contrasted in different lifestyle-related diseases such as type 2 diabetes and fatty liver (Lakka et al., 2002; Kotronen & Yki-Jarvinen, 2008). Especially, this obesity linked syndrome has been associated with cardiovascular diseases and unhealthy vascular endothelium. Adding the fact that MetS has been related to an increased risk of mortality (Lakka et al., 2002), more research should be focused in finding the different risk factors of this vicious syndrome (IDF, 2006).

MetS has a considerable linkage to an unhealthy lipid profile leading to atherosclerosis, however, in the past decade, more evidence has been shown for the protective capabilities of physical fitness and activity in regard to MetS and its components

(Cornier et al., 2008; Stewart et al., 2005). Yet, clear consensus has not been established as to which is more beneficial for attenuating MetS – physical fitness or physical activity (Blair et al., 2001). Evidently though, physical fitness and activity have significant roles in the development and affect of insulin resistance as well as in balancing the glycaemic state, both of which are related to oxidative stress (Ingelsson et al., 2009; Lin et al., 2010; Mohebbi et al 2011; Maiorana et al., 2002; Houstis et al., 2006).

2.5.3. The risk of atherosclerosis and systemic inflammation, interleukin-6 and tumour necrosis factor alpha

Increased systemic inflammation is recognized in atherosclerosis and acute plaque rupture (Libby, 1995; Moreno et al., 1994). Markers such as C-reactive protein (CRP), interleukin-6 (il-6) and tumour necrosis factor alpha (TNFα) are all markers of systemic inflammation (Tedgui & Mallat, 2006). Also systemic inflammation (as one part of the atherosclerosis cascade) is increased in obesity, where the inflammatory cytokines il-6, TNFα and CRP are all elevated (Berg & Scherer, 2005; Saito et al., 2003; Kopp et al., 2003; Davi et al., 2002; Valle et al., 2005).

Il-6 is a cytokine which is mainly produced in monocytes / macrophages in vascular endothelial cells while some extraction is done by adipose tissue (Akira et al., 1993; Seino et al., 1994; Rus et al., 1996; Mohamed-Ali et al ., 1997). An elevated concentration is linked to obesity, type 2 diabetes and atherosclerosis and moreover, levels of il-6 predict total and cardiovascular mortality (Kern et al., 2001; Pradhan et al., 2001; Yudkin et al., 2000; Ridker et al., 2000a; Ridker et al., 2000b). Interestingly, il-6 is independently related to obesity and inactivity (Fischer et al., 2006). Altogether and taking perspective of atherosclerosis, the endothelium cells imprisoned by atheroma have increased expression of the il-6 transcripting gene and the high concentration of il-6 is a mediator in chronic inflammation (Seino et al., 1994; Rus et al., 1996). In terms of physical exercise and il-6, the contracting of skeletal muscles produces il-6 into the blood flow appearing as an increased inflammation response (Ostrowski et al., 1998). When comparing the two inflammation cytokines il-6 and TNFα, il-6 is considerably more related to subcutaneous adipose tissue, obesity and acute exercise in comparison to TNFα (Ostrowski et al., 1998; Mohamed-Ali et al., 1997; Fischer et al., 2006).

TNFα is a pro-inflammatory cytokine which promotes endothelial damage and dysfunction (Pober & Cotran, 1990; Madge & Pober 2001a). In intracellular signalling, TNFα initiates apoptosis (Madge & Pober, 2001b). High levels of TNFα have been associated with high prevalence of atherosclerosis in early atherosclerosis formation (Bruunsgaard et al., 2000; Skoog et al., 2002). In atherosclerotic cascade, pro-inflammatory TNFα is produced from activated endothelial macrophages and furthermore, TNFα prospects more cells of the inflammatory cascade into damaged

endothelium where atherogenic plaque is formed (Hansson, 2001). TNFα also promotes foam cell formation by disturbing the ability of LDL attenuation in macrophages (Tedgui & Mallat, 2006). As with other considered risk factors of cardiovascular disease and atherosclerosis, TNFα has a wide range of interactions with adipose tissue where it intermits different cellular signals in order to balance energy expenditure (Argilés et al., 1997). Therefore both cachexia and obesity are linked to contrasting levels of TNFα (Argilés et al., 1997). TNFα blocks insulin receptor activity which increases insulin resistance leading to a higher risk of MetS and type 2 diabetes (Hotamisligil et al., 1994; Dandona et al., 2005; DeFronzo & Ferrannini, 1991). TNFα possesses a link between different lipids: LDL lipids associates with TNFα while HDL markedly prevents the adhesion of the monocyte TNFα receptor (Skoog et al., 2002; Park et al., 2003). Also, triglycerides and TNFα correlates with each other, thus TNFα induces hypertriglyceridemia (Popa et al., 2007). Furthermore, in terms of atherogenicity, TNFα is associated with highly atherogenic ox-LDL, whereas ox-LDL induces the release of TNFα from inflammatory cells (Frostegård et al., 1997; Jovinge et al., 1996).

2.6. TESTOSTERONE ASSOCIATIONS WITH LIPIDS AND ENDOTHELIUM HEALTH

While atherosclerosis formation is not yet fully understood, testosterone seems to play some role in plaque formation. From a clinical perspective, low levels of testosterone have been related independently to atherosclerotic cardiovascular morbidity and mortality as well as with increased intima-media thickness which is again a sign of atherosclerosis formation (Malkin et al., 2010; Phillips et al., 1994; Mäkinen et al., 2005). Low levels of testosterone are associated with clinically relevant diseases such as the notorious MetS, type 2 diabetes and a large range of different cardiovascular diseases (Jones, 2007). The specific molecular mechanism is unclear, however, low levels of testosterone attenuates the healthy vasodilation of arteries through oxidative stress reactions, poor formation of nitric oxide and increased systemic inflammation (Bernini et al., 2006; Shijun et al., 2008; Zhang, 2011). Fortunately, in contrast, higher levels of endogenous testosterone are associated with a healthier lipid profile and testosterone seems to have positive relationship with cholesterol transportation by enhancing antiatherogenic HDL lipids and correcting systemic inflammation in low testosterone individuals, however, inconsistent data has been published (Khaw & Barret-Connor, 1991; Corcoran et al., 2010; Dai et al., 1984). Higher testosterone levels also are associated with a lower concentration of triglycerides (Gyllenborg et al., 2001) and more balanced blood glucose (Haffner et al., 1988). Some evidence has been reported for the negative correlation between testosterone and systolic and diastolic blood pressure in 30-79 year old men (Khaw & Barret-Connor, 1988). The association between testosterone and oxidative stress or oxidized lipoprotein particles has not been widely examined. Especially, interests concerning ox-LDL in the role of

atherosclerosis formation has been recently increasing, although information regarding the testosterone relation between ox-LDL and atherosclerosis is still lacking. Barud et al. (2002) showed new insights for an inverse relationship between total testosterone and anti-oxidized LDL antibody levels in ageing males, and Linna et al. (2008) showed significantly higher levels of ox-LDL in tobacco smoking men with testosterone levels under 15 nmol/L. These studies however do not fully explain the relationship between heightened levels of ox-LDL and low levels of testosterone, thus there is adequate reason for further declarative research. As for physical fitness and testosterone, acute exercising (both cardiorespiratory and resistance) increases testosterone, and their associations seem consistent (Grandys et al., 2009; Santtila et al., 2010; Kraemer et al., 1998).

2.7. METHODS OF DETERMINING OXIDIZED LDL

Methods of quantitative analysis for the determination of ox-LDL and oxidative stress have been created, such as procedures using enzyme-linked immunosorbent assays (ELISA) and diene conjugation (Itabe & Ueda, 2007). These ELISA and the presented diene conjugation methods have replaced previously used *in vitro* ox-LDL assays which used copper exposure catalyzing oxidation (Tsimikas, 2006).

In more detail, the diene conjugation method uses the identification of conjugated diene double bonds which are present in all polyunsaturated fatty acids (Ahotupa et al., 1996). The procedure consists from the isolation of the lipoprotein fraction, extraction of lipoprotein lipids and spectrophotometric analysis of the conjugated dienes in the lipoprotein lipids. Compared to other methods, the diene conjugation method does not require ultracentrifugation which could damage the actual LDL fractions (Ahotupa et al., 1996). Regarding the methodology aspect of determining the concentration of ox-LDL, the diene conjugation method is proven to be validated *in vivo* and has been well recognized in Finnish and European population based samples in detecting clinical atherosclerosis (Vasankari et al., 2001a; Nyyssonen et al., 2012).

In ELISA, the method is based on the recognition of monoclonal antibodies which are detected whit oxidation-specific epitopes. Three different ELISA are widely used to determine the concentration of ox-LDL: ox-LDL-4E6 (standard and competition), ox-LDL-DLH3, ox-LDL-E06 (Fraley & Tsimikas, 2006). These ELISA assays have been associated with atherosclerosis and high oxidative stress related lifestyle diseases such as coronary heart disease and metabolic syndrome and its components (Weinbrenner et al., 2003; Holvoet et al., 2001; Sigurdardottir et al., 2002; Shimada et al., 2004; Tsimikas et al., 2005). Curiously, Braun et al., (2005) reported insignificant relationships between ox-LDL and coronary artery disease using a competitive ELISA test (competitive ox-LDL-E46).

Although these above mentioned procedures are highly correlated with clinical atherosclerosis formation, they are used solely in investigation purposes due to the heterogeneity in reference values.

2.8. SUMMARY OF GENERAL PHYSICAL FITNESS TESTING

Physical fitness can be categorized for endurance, strength and mobility. From these different components endurance (cardiorespiratory fitness), and strength (muscular endurance and strength and power) seem to have beneficial associations with lifestyle related diseases (Table 1, NCEP 2002, Artero et al., 2012). Majority of large epidemiological research based on cardiorespiratory fitness testing is based on bicycle ergometer and treadmill testing where participants are stressed until exhaustion (Table 1). However, different field testing can also be conducted especially in youth (Castro-Piñero et al., 2010).

Comparing to cardiorespiratory fitness testing, muscular fitness testing is based on variety of test set-ups (Knapik et al., 2009). The different muscle groups are mostly tested based on the anthropometrics where lower and upper body are tested separately (Markovic & Slobodan, 2003; ACSM, 2000). In Nordic countries, a guideline has been assembled by Malmberg (2011) which has overviewed the different protocols of physical fitness testing in Nordic armed forces. As for muscular fitness, the presented guideline shows testing protocols for upper and lower muscular endurance which have been nationally validated.

Internationally, Leisure-time Physical Activity is determined by long and short International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003). With short IPAQ, individual LTPA could be analyzed from last activity of 7 days. IPAQ is also used as reference for other LTPA questionnaires such as Behavioral Risk Factor Surveillance System (Brown et al., 2004), CHAMPS Physical Activity Questionnaire (Harada et al., 2001) and Physical Activity Questionnaire for Adolescents (PAQ-A). In Finland, Fogelholm et al. (2006b) have established validated LTPA questionnaire which can be used for Finnish reference.

3. PURPOSE OF THE PRESENT STUDY

The purpose of the present study was to evaluate how cardiorespiratory and muscular fitness associate with ox-LDL lipids and how the other known risk factors of atherosclerosis might alter these associations. The enquiry was conducted in four study sections:

I) The effects of both cardiorespiratory and muscular fitness on the concentrations of ox-LDL and serum lipids (LDL , HDL , total cholesterol and triglycerides) in healthy young men.

II) The concentrations of ox-LDL and serum lipids among normal weight, overweight and obese individuals and to examine whether good cardiorespiratory and muscular fitness could protect overweight and obese subjects from the atherogenic lipid profile.

III) The interrelationships of physical fitness (cardiorespiratory and muscular), ox-LDL lipids, TNFα and il-6 in otherwise healthy young men with and without MetS.

IV) How combined levels of fitness (cardiorespiratory fitness, muscular endurance, maximal muscle strength) and TES status influence ox-LDL and serum lipids in healthy young men.

4. MATERIALS AND METHODS

4.1. PARTICIPANTS

The study participants (mean age 25.1, SD ±4.6) were healthy young Finnish men who participated in compulsory refreshment courses of military training during 2008. The participants were gathered from nationwide refreshment courses which leads the cohort of the present study to be nationally representative. Invitation letters were sent to 1155 men of which 922 took part in the actual refreshment courses. From the 922 participants, 846 volunteered for the study. Of these final 846 participants, 323 (38 %) were smokers. More detailed information of the study cohort is seen in the Table 2.

Table 2. Basic characteristics of the study participants. Mean (±SD).

	Age (years)	**Weight (kg)**	**Height (m)**	**Waist circumference (cm)**	**Body mass index (kg/m^2)**	**Leisure-Time Physical Activity per week (times per week)**		
						0 or with only moderate intensity	1-2	3 or more
Cohort (n=846)	25.1 (±4.6)	80.6 (±13.4)	1.80 (±0.06)	86.3 (±10.4)	24.8 (±3.8)	266	326	253
Normal weight (n=486)	24.4 (±4.1)	72.9 (±7.5)	1.80 (±0.06)	80.2 (±5.3)	22.4 (±1.8)	150	197	149
Overweight (n=269)	26.0 (±5.1)	87.5 (±7.9)	1.80 (±0.06)	91.9 (±6.2)	26.9 (±1.4)	87	98	85
Obese (n=76)	26.5 (±5.2)	106.3 (±13.4)	1.80 (±0.06)	106.3 (±10.7)	33.0 (±3.2)	27	31	18
non-MetS (n=790)	25.0 (±4.5)	79.2 (±12.2)	1.80 (± 0.06)	85.1 (±9.3)	24.4 (±3.4)	239	308	242
MetS (n=54)	27.0 (±6.3)	100.3 (±15.0)	1.79 (±0.06)	103.2 (±10.3)	30.9 (±4.2)	27	18	9
Smokers (n=323)	24.7 (±4.0)	81.2 (±13.9)	1.80 (±0.06)	87.2 (±10.6)	25.0 (±3.9)	138	126	59
non-Smokers (n=523)	25.4 (±5.0)	80.2 (±13.1)	1.80 (±0.06)	80.7 (±13.1)	24.7 (±3.7)	127	200	193

4.1.1. Normal weight, overweight and obese participants

Weight data was missing from 4 participants and 11 participants were excluded due their low BMI (under 18.5). Thus, the total number of participants analysed in the present study was 831, which corresponds to 72 % of the invited reservists. In order to divide participants into different groups of weight, the criteria used by the WHO was

employed [BMI (kg/m2)]: normal weight, 18.5-24.99); overweight, 25-29.99; obese ≥ 30.00 (World Health Organization, 2007). The height and weight of every participant was measured. Height was measured at a precision of 1 mm and weight was measured with a commercial scale (0.1 kg precision) wearing light clothes. After measurements of the participant's height and weight, the Body Mass Index (BMI) was calculated using the standard equation: weight (kg) / height squared (m^2) (NIH, 1998). The deviation of participants for each BMI subclass was n=486 (normal weight 18.5-24.99), n=269 (overweight, 25-29.99) and n=76 (obese ≥ 30.00).

4.1.2. Participants with metabolic syndrome

MetS can be defined through the IDF criteria 2007: Waist circumference ≥ 0.94 m or BMI > 30 kg/m^2 plus two of the following four factors; raised triglycerides (≥ 1.7 mmol/L) or lipid abnormality treatment, reduced HDL cholesterol (< 1.03 mmol/L) or lipid abnormality treatment, elevated blood pressure (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg) or treatment for diagnosed hypertension and raised fasting plasma glucose (≥ 5.6mmol/L) (IDF, 2006). According to IDF criteria, the cohort was separated into participants with MetS (n=54) and without metabolic syndrome (non-MetS, n=790).

4.2. STUDY DESIGN

The subjects came to the training courses in the late afternoon before the study day. Firstly, the participants answered a detailed questionnaire of lifestyle and health containing a total of 120 questions. In addition to several other items, questions concerning the usage of tobacco products and illnesses diagnosed by a physician were included in the questionnaire. After the questionnaire, general health examination followed. The general health examination consisted of a registration of height, weight, waist circumference and blood pressure measurement. Blood pressure was determined twice from the left arm while participants were sitting still, using an Omron M6 Comfort (Omron Healthcare Europe B.V. Kruisweg 577–2132 NA, Hoofddorp, Netherlands). The blood pressure monitor cuff was placed 2-3 finger widths above the bend of arm and a 2 minute pause was taken between the first and second measurements. Fasting (12 h) blood samples were obtained from an antecubital vein at 6.30—7.30 a.m. the following morning, before a light breakfast which was followed by the fitness tests, beginning with a bicycle ergometer test.

4.3. PHYSICAL FITNESS TESTS

Both cardiorespiratory fitness (VO_2max) and muscle fitness tests were performed under supervision by professional military testing personnel (Vaara et al., 2012).

Supervisors demonstrated the standardized techniques for each test and monitored the performance technique of each participant. LTPA was inquired with questionnaire including lifestyle related questions. The amount of LTPA was determined with one closed question (1) and one open question (2): 1) How many times you conduct LTPA per week? i) none, ii) with only moderate intensity, iii) with vigorous intensity a) 1, b) 2, c) 3, and d) 4 or more times per week; 2) If answered "ii", the open question followed which determined the amount of moderate intensity per week (Fogelholm et al., 2006b). All of the physical fitness tests were carried out in one test day. The order of the physical fitness tests were as follows (time reserved for test execution): 1) general health examination + breakfast (40min), 2) maximum strength tests (grip strength test, lower and upper extremity tests) (30min), 3) bicycle ergometer (30min), 4) muscle endurance tests (sit-ups, puhs-ups, repeated squats) (30min). The participants had 1 hour rest between the tests 3 and 4.

4.3.1. Maximal oxygen uptake (VO_2max)

The predicted maximal oxygen uptake was indirectly measured using a bicycle ergometer (Ergoline® 800 S, Ergoselect® 100 K or 200 K) test using validated equations of VO2max measurements (VO_2max [$ml \cdot kg^{-1} \cdot min^{-1}$]) = ($P_{max}$ x 12.35)/body mass + 3.5 and VO_2max=[(P_{max} x 12.48)+217]/body mass, P_{max} is maximal power) (Fogelholm et al., 2006a; Santtila et al., 2013). The initial work load of the test was 50 W, and this was increased by 25 W every two minutes until exhaustion (Santtila et al., 2013).

4.3.2. Determination of muscle fitness, muscle fitness index (MFI)

For muscular fitness, the muscle fitness index (MFI) was calculated using the results of each muscle test according to the standards of the Finnish Defence Forces (Pihlainen et al., 2009) (APPENDIX section, Table). MFI was acquired using four consecutive tests: isometric grip strength, push-ups, sit-ups and repeated squats. Each individual muscle test had time limit of 60 seconds (Fogelholm et al., 2006a & 2006b; Häkkinen et al., 2010).

Maximal isometric grip strength was measured by dynamometer (Saehan Corporation, Masan, South Korea) while the participant was sitting and keeping his elbow at a 90° angle. The maximal isometric grip strength score was taken from the average of mean results of both right and left hands (Kyräläinen et al., 2008).

In order to measure the muscular fitness of upper extremity extensors, a test of push-ups was conducted. One counted push-up repetition consisted from start position (shoulder wide stance, fingers pointing forward, legs parallel) followed by a lowering of the tensioned torso (90° elbow angle) and a full extension of the arms (ACSM, 2000).

Abdominal and hip-flexor fitness was measured using repeated sit-ups. In the start position, the participant was lying on the floor, knees flexed to a 90° angle and ankles

fixed to the floor by an assistant. One repetition was fully executed when the upper body was lifted and the elbows touched the participant's knees (Viljanen et al., 1991).

Lower extremity muscle fitness was evaluated by measuring repeated squats. In the start position, feet were at a shoulder wide stance and participant flexed his knees until the thighs were on a horizontal level (Pohjonen, 2001).

Maximum strengths of lower extremities (MaxLE) and upper body (MaxUB) were determined with bilateral isometric dynamometric leg press and bench press tests (1KHz frequency AD converter, CED power 1401, Cambridge Electronic Design, Ltd., Cambridge, United Kingdom) (Vaara et al, 2012; Häkkinen & Häkkinen, 1995). Participants had 5 minute warmup and guidance for proper technique by test supervisors before maximum strengths tests. Familarization before the actual maximum efforts were guided: 2 to 3 submaximum test sets following one near maximum effort test set. After these test sets, maximum strengths were tested by supervision. In the leg press, the knee was set to 107^{o} whilst the bench press was conducted in the supine position and positioning shoulders and elbows at 90^{o}. Participants had three attempts in both maximal strength tests.

4.4. LABORATORY ANALYSIS

4.4.1. Oxidized LDL lipids

Venous blood samples were taken, following overnight fasting. Analysis of ox-LDL was based on the determination of the baseline level of conjugated dienes in LDL lipids (Ahotupa et al., 1996; Ahotupa et al., 1998). The appearance of conjugated diene double bonds is characteristic with peroxidation of all polyunsaturated fatty acids. In *in vitro* and *ex vivo* studies on LDL oxidation, diene conjugation has commonly been used as the index of LDL oxidation. The assay procedure consisted of the isolation of the lipoprotein fraction, extraction of lipoprotein lipids and spectrophotometric analysis of the conjugated dienes in the lipoprotein lipids. LDL was isolated by precipitation with buffered heparin. The isolation procedure was validated for the purpose and did not affect the level of oxidized lipids (Ahotupa et al., 1998). Lipids were extracted from isolated LDL by chloroform-methanol (2:1), dried under nitrogen and re-dissolved in cyclohexane. The amount of peroxidized lipids in LDL was assessed spectrophotometrically at 234 nm. Validation studies for the assay have ruled out interference by nonspecific substances, and shown that diene conjugation is a measure of oxidative LDL modification found in all LDL lipid classes. The coefficient of variation (CV) for within-assay precision for the determination of ox-LDL lipids was 4.4 %, and the CV for between-assay precision was 4.5 %.

4.4.2. Serum lipid (HDL cholesterol, LDL cholesterol, triglycerides, total cholesterol) assays

Serum total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were analysed with a Konelab 20 XTi clinical chemistry analyzer (Thermo Electron Corp., Vantaa, Finland) by means of enzymatic photometric assay. The precisions of within-assay/between-assay CV were 0.5 % / 1.4 % for total cholesterol, 3.4 % / 3.9 % for LDL cholesterol, 3.4 % / 2.0 % for HDL cholesterol and 2.5 % / 2.5 % for triglycerides.

4.4.3. Fasting plasma glucose analysis

Fasting plasma glucose was measured using a Nova Biomedical pHOx Plus L analyser (Nova Biomedical, Waltham, Massachusetts, USA), and the precisions of within-assay/between-assay CV for fasting plasma glucose were 3.0 % / 5.0 %.

4.4.4. Markers of inflammation - tumour necrosis factor alpha and interleukin-6

Plasma concentrations of both TNFα and il-6 were measured using commercial high sensitivity ELISA kits according to the manufacturer's instructions (Quantikine HS, R&D Systems, Minneapolis, USA). Assay specifications were as follows: for il-6 sensitivity the limit was 0.11 pg/mL, maximum intra- and inter-assay CV % were 5.9 % and 9.8 %; for TNF-α the sensitivity limit was 0.19 pg/mL, maximum intra- and inter-assay CV % were 6.1 % and 7.7 %.

4.4.5. Testosterone and free testosterone

The concentration of TES was measured from serum with Immulite 1000 immunochemical analyzer (Siemens Healthcare Diagnostics Products Ltd., Gwynedd, UK). The sensitivity for the assay was 0.5 nmol/l while the within- and inter-assay coefficients of variance were 5.7 % and 2.4 %. The concentration of free testosterone (FTES) was determined statistically using the Ly & Handelsman model 2 empirical equation (Ly & Handelsman, 2005).

4.5. STATISTICAL ANALYSIS

Values of ox-LDL, ox-LDL/HDL-cholesterol, ox-LDL/LDL-cholesterol and triglycerides were $\log_e$ transformed in order to correct skewness. The statistical analysis was performed by statistical softwares SPSS (IBM Corporation, Armonk, NY, USA) versions 15.0, 18.0.1 and PASW 18.0.1 (IBM Corporation, Armonk, NY, USA) with a statistical significance inferred as $P \leq 0.05$.

Study I

Normality of the variables was tested, and logarithm transformations were applied in the analysis as needed. Presented means, standard deviations were calculated with untransformed variables. In order to investigate the association between VO_2max/MFI/LTPA and serum lipids, the lipid results were presented and analysed using both fitness (VO_2max and MFI groups) and LTPA groups. For this, an analysis of variance followed by Bonferroni pairwise comparisons was used. Age and age + waist circumference were used as covariates.

Study II

Means and standard deviations were used for results and calculated with standard procedures. The means of lipid variables in BMI groups were examined with analysis of variance where age and smoking were used as covariates. Further, subjects were divided into six subgroups based on BMI (normal vs. overweight) and cardiorespiratory / muscular fitness (unfit, average and fit) tertiles. Analysis of covariance was applied to determine the differences in lipids, where age and smoking were used as covariates. Bonferroni's correction was used in *post-hoc* tests. The subjects who did not take part either in the cardiorespiratory fitness test or in the muscle fitness tests did not differ in age, weight, height and BMI from those subjects who took part.

Study III

The normality of the variables was tested in each lipid analysed, and logarithm transformations were applied in the analysis of ox-LDL, ox-LDL/HDL-cholesterol, ox-LDL/LDL-cholesterol and triglycerides in order to standardize the deviated values into a normal distribution. Pearson correlations were used to determine interrelations between oxidative stress, inflammation and fitness tests in subjects with and without MetS. Stepwise multivariate logistic regression analysis was used to calculate whether elevated ox-LDL, poor physical fitness and high systemic inflammation was associated with the prevalence of MetS in otherwise healthy young men. Differences between the MetS and non-MetS groups were detected by using age, smoking and LTPA as covariates (ANCOVA).

Study IV

We divided the participants to fitness tertiles (unfit, average fit, fit) according to their test results on each fitness test (VO_2max, MFI, MaxUB, MaxLE). Furthermore, these fitness tertiles were separated into low and high androgen variable (TES, FTES) groups in order to examine the influence of fitness (unfit/average fit/fit) x androgens (low/high) on serum lipids and ox-LDL lipid markers (ox-LDL, ox-LDL/HDL-cholesterol, ox-LDL/LDL-cholesterol). We used medians as cut-off points for low or high testosterone / free testosterone. The analysis of variance (ANOVA) was conducted between these six groups followed by covariate analysis (ANCOVA). Age,

smoking and waist circumference were used as covariates (ANCOVA) whilst Least Statistical Difference was used as a *post-hoc* test.

4.6. APPROVAL OF THE ETHICAL COMMITTEE

The present study was approved by the ethical committees of the University of Jyväskylä and the Central Finland Health Care District, as well as the Headquarters of the Finnish Defence Forces. Written informed consent was obtained from each participant after detailed information of the study procedure was given. In this informed consent, the participant was given information of the possible benefits of the study as well as possible risk factors involved in execution of the physical fitness tests and laboratory analysis. Participants were able to terminate their participation in the study at any time or for any reason.

5. RESULTS

5.1. CHARACTERISTICS OF THE STUDY POPULATION (STUDY I)

The basic characteristics of the entire study cohort (n=846) were as follows (mean; SD): age (25.1; ±4.6 year), height (180.1; ±6.3 cm), body mass (80.6; ±13.4 kg), BMI (24.8; ±3.8 kg/m^2), waist circumference (86.3; ±10.4 cm).

The lipoprotein and physical fitness (VO_2max and MFI) characteristics of the whole cohort and divided subgroups according to BMI, MetS and smoking status are presented in Table 2. The different lipid (ox-LDL, ox-LDL/HDL-cholesterol, ox-LDL/LDL-cholesterol, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) and physical fitness (VO_2max and MFI) variables were compared between subgroups: normal weight vs. overweight / obese; non-MetS vs. MetS; smokers vs. non-smokers. Normal weight had lower concentrations of ox-LDL, LDL cholesterol, total cholesterol, triglycerides and higher concentrations of HDL cholesterol and higher ratios of ox-LDL to HDL cholesterol and LDL cholesterol. Normal weight also had a significantly higher VO_2max and MFI. Subsequently, participants who met the criteria of IDF for MetS had higher concentrations ox-LDL, LDL cholesterol, total cholesterol, triglycerides and lower concentrations of HDL cholesterol and higher ratios of ox-LDL/HDL-cholesterol and LDL cholesterol together with poorer VO_2max and MFI. The analysis was conducted using age + smoking (ANCOVA1) and age + smoking + LTPA (ANCOVA2) as covariates, respectively (Table 3).

Table 3. Physical fitness and lipoprotein profile of the study participants. Mean (±SD). Number of participants, n=846.

Variable	Cohort (n=846)	Normal weight (n=486)	Overweight (n=269)	Obese (n=76)	ANCOVA 1	non-MetS (n=790)	MetS (n=54)	ANCOVA 2
VO_2max	41.6 (8.1)	44.4 (7.6)	39.1 (6.6)[a]	31.9 (5.6)[a]	<0.0001	42.2 (7.9)	33.2 (6.5)[b]	<0.0001
MFI	12.4 (3.8)	13.1 (3.6)	11.9 (3.8)[a]	9.7 (3.7)[a]	<0.0001	12.6 (3.8)	10.0 (3.1)[b]	<0.0001
ox-LDL	24.6 (8.3)	22.9 (6.4)	26.2 (8.9)[a]	30.3(12.5)[a]	<0.0001	23.8 (7.3)	36.3 (12.1)[b]	<0.0001
ox-LDL/HDL	17.8 (8.9)	15.3 (5.4)	20.2 (10.1)[a]	25.7(14.1)[a]	<0.0001	16.6 (7.0)	34.6 (14.3)[b]	<0.0001
ox-LDL/LDL	10.6 (3.0)	10.3 (2.8)	10.4 (3.2)	10.6 (2.8)	0.162	10.2 (2.9)	12.1 (3.2)[b]	<0.0001
total cholesterol	4.54 (0.87)	4.39 (0.80)	4.67 (0.85)[a]	5.01(1.03)[a]	<0.0001	4.50 (0.84)	5.12 (0.86)[b]	<0.0001
LDL cholesterol	2.43 (0.63)	2.29 (0.57)	2.58 (0.63)[a]	2.82(0.72)[a]	<0.0001	2.39 (0.60)	3.01 (0.69)[b]	<0.0001
HDL cholesterol	1.49 (0.36)	1.57 (0.35)	1.40 (0.34)[a]	1.29(0.35)[a]	<0.0001	1.52 (0.35)	1.10 (0.24)[b]	<0.0001
Triglycerides	1.03 (0.53)	0.89 (0.34)	1.13 (0.60)[a]	1.54(0.83)[a]	<0.0001	0.95 (0.41)	2.11 (0.84)[b]	<0.0001

Asterisks indicate a difference between subgroups as follows (used analysis / post-hoc test): normal weight vs. overweight / obese: a) $P < 0.05$ (Bonferroni).
non-MetS vs. MetS: b) $P < 0.05$ (paired t-test).
Analysis of covariance used: ANCOVA1= age + smoking; ANCOVA2 = age + smoking + LTPA.

5.2. ASSOCIATIONS OF CARDIORESPIRATORY, MUSCLE FITNESS AND LTPA WITH CONCENTRATION OF OXIDIZED LDL IN YOUNG MEN (STUDY I)

VO_2max was associated with ox-LDL, ox-LDL/HDL-cholesterol ratio and ox-LDL/LDL-cholesterol ratio while MFI was associated with ox-LDL and the ratio of ox-LDL/HDL-cholesterol after adjusted covariates. The group with the lowest VO_2max and MFI had a higher concentration of ox-LDL and ratio of ox-LDL/HDL-cholesterol than the highest VO_2max and MFI groups, respectively (Figure 1). From all individual muscle fitness tests, only excellent sit-up test results (≥41 repetitions/min) compared to poor sit-up test results (≤25 repetitions/min) was significantly associated with a low concentration of ox-LDL (Study I).

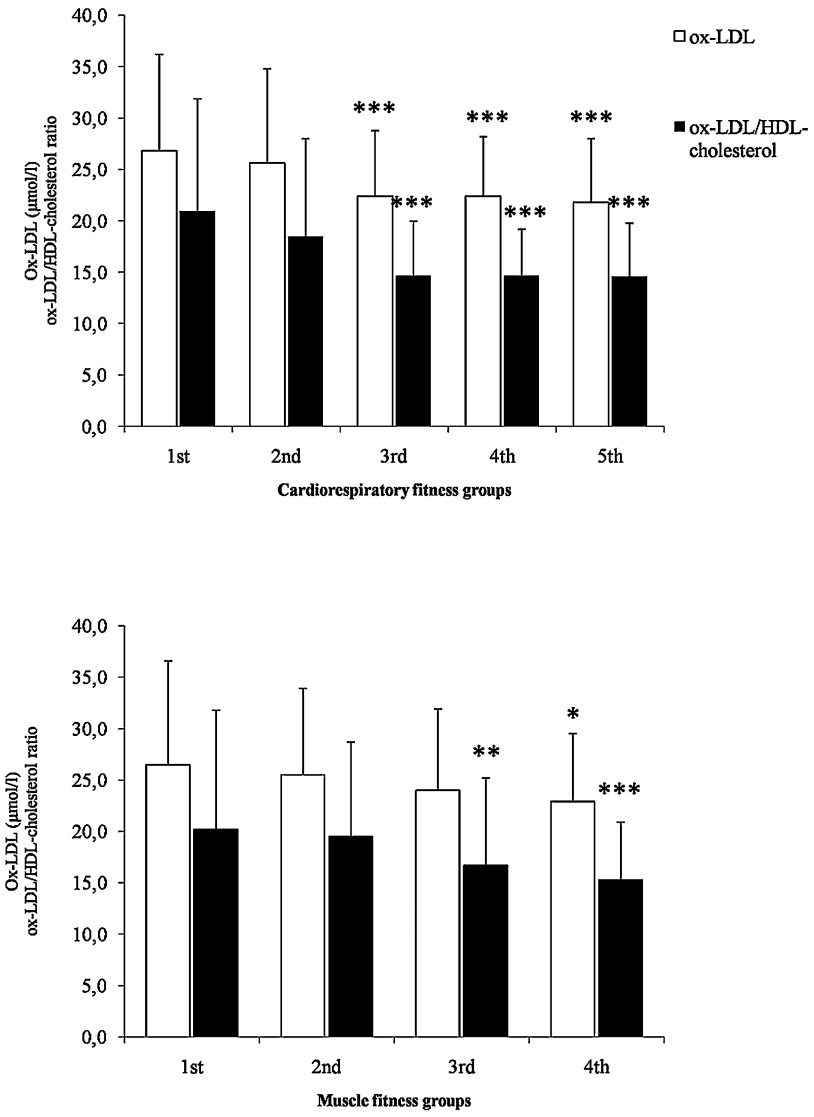

Figure 1. Concentration of oxidized LDL lipids (ox-LDL) and ox-LDL/HDL-cholesterol ratio in cardiorespiratory and muscle fitness groups (means with SD). Cardiorespiratory groups: 1) <38.0 ml/kg/min 2) 38.0 – 41.9 ml/kg/min 3) 42.0 – 45.9 ml/kg/min 4) 46.0 – 49.9 ml/kg/min and 5) >50.0 ml/kg/min. MFI groups: 1) poor 2) average 3) good 4) excellent (Malmberg, 2011). Age and waist circumference were used as covariates. Asterisks indicate a statistical difference between 1st vs. other fitness groups, respectively: * $0.01 \leq P < 0.05$; ** $0.001 \leq P < 0.01$; *** $P < 0.001$.

When actual physical fitness tests showed lower concentrations of ox-LDL and lower levels of ox-LDL/HDL-cholesterol, LTPA was associated with the levels of ox-LDL and ox-LDL/HDL-cholesterol. Accordingly, the lowest LTPA group had a higher concentration of ox-LDL and a higher ratio of ox-LDL/HDL-cholesterol, when compared to the highest LTPA group (Figure 2).

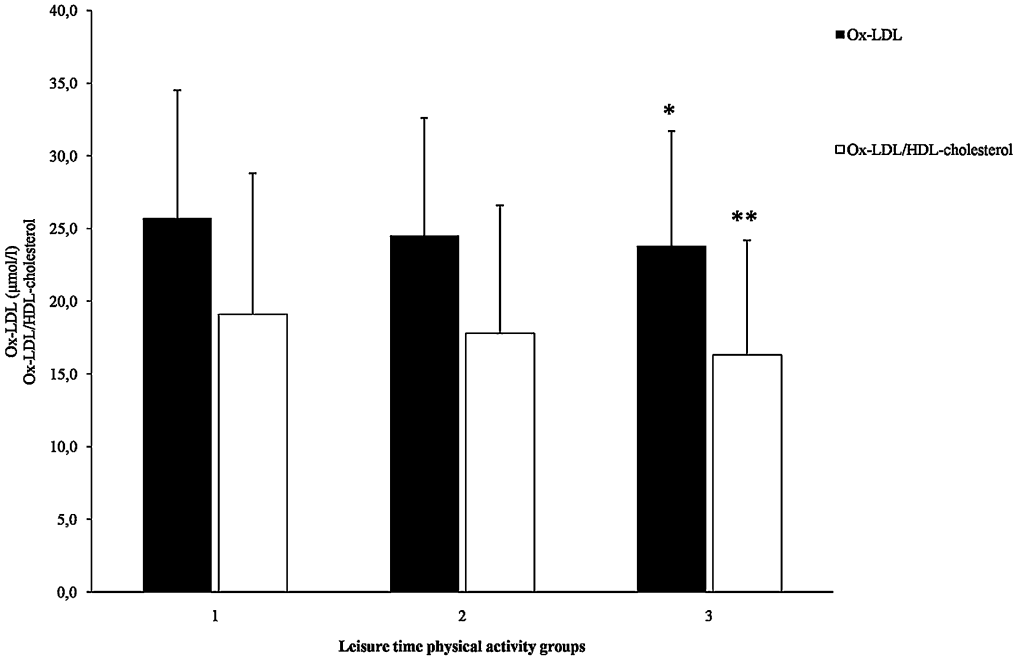

Figure 2. Concentration of oxidized LDL lipids and the ratio of ox-LDL/HDL-cholesterol in Leisure time physical activity groups (means with SD): 1) none or one time per week activity of less than moderate intensity, 2) one to two times per week of moderate to vigorous activity, 3) at least three times per week of moderate to vigorous activity (Fogelholm et al., 2006b). Age was used as covariate. Asterisks indicate a statistical difference between the groups:
** Ox-LDL concentration between group 1 and 3 (P=0.039).*
***Ox-LDL/HDL-cholesterol between group 1 and 3 (P=0.001).*

5.3. DIFFERENCES IN THE CONCENTRATIONS OF OXIDIZED LDL BETWEEN SMOKERS AND NON-SMOKERS IN PHYSICAL FITNESS GROUPS (STUDY I)

In the lowest non-smoking VO_2max and MFI groups, the concentrations of ox-LDL and the ratios of ox-LDL/HDL-cholesterol were higher compared to the non-smoking higher fitness groups (Figure 3).

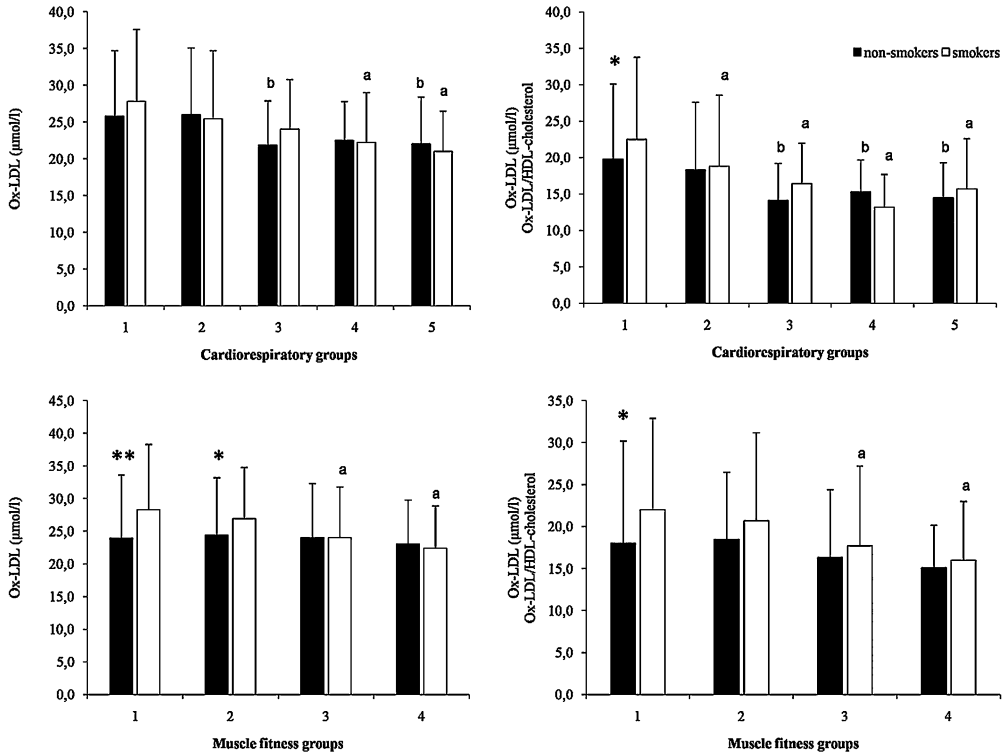

Figure 3. The concentrations of ox-LDL and the ratios of ox-LDL/HDL-cholesterol in smokers and non-smokers in cardiorespiratory and in muscle fitness groups (means with SD). Cardiorespiratory groups: 1) <38.0 ml/kg/min 2) 38.0 – 41.9 ml/kg/min 3) 42.0 – 45.9 ml/kg/min 4) 46.0 – 49.9 ml/kg/min and 5) >50.0 ml/kg/min. MFI groups: 1) poor 2) average 3) good 4) excellent (Malmberg, 2011). Age was used as covariate. Asterisks indicate the statistical difference between the groups.
*Between smokers and non-smokers group:*0.01 < P < 0.05; ** 0.001 < P < 0.01; *** P < 0.0001.*
Differences in smoker and non-smoker groups between the lowest fitness and other fitness groups:
a) P < 0.05 (smokers); b) P < 0.05 (non-smokers).

This same trend for higher rates of ox-LDL and ox-LDL/HDL-cholesterol ratio was also seen in smoking VO_2max and MFI groups. As for comparisons between smokers and non-smokers; the smoking low MFI group had a higher ox-LDL and higher ratio of ox-LDL/HDL-cholesterol compared to the non-smoker group, however, in the muscle fitness groups, the smoking low and average VO_2max group had independently higher ox-LDL, when compared to the non-smoker low VO_2max group. The comparisons mentioned above were adjusted for age – for a more detailed covariates analysis see Study I.

5.4. ASSOCIATION OF PHYSICAL FITNESS WITH CONCENTRATIONS OF OXIDIZED LDL IN OVERWEIGHT AND OBESE YOUNG MEN USING BODY MASS INDEX (STUDY II)

The lipid concentrations were evaluated in both VO_2max and MFI fitness tertiles between normal weight and overweight subgroups and within overweight subgroups (Table 4). The overall trend of ox-LDL and the ox-LDL/HDL-cholesterol ratio was heightened levels in overweight participants when using age and smoking as covariates.

Table 2 shows significantly higher levels in concentrations of ox-LDL and ratios of ox-LDL/HDL-cholesterol in both unfit VO_2max and MFI subgroups between normal weight and overweight when the conventional Bonferroni test was used and analysis was adjusted with age and smoking. In addition, the ratio of ox-LDL/HDL-cholesterol was significantly higher in the average fit subgroup in both unfit VO_2max and MFI, whilst between the fittest subgroups, the ratio of ox-LDL/HDL-cholesterol was higher solely in MFI. Although the cross-analysis of normal weight and overweight showed significant results in ox-LDL and ox-LDL/HDL-cholesterol ratio, within the overweight subgroups no significant differences in the concentration of ox-LDL were seen in overweight MFI subgroups. Overall, in the VO_2max subgroups, unfit overweight participants had a higher concentration of ox-LDL than fit overweight participants, and furthermore, the ratio of ox-LDL/HDL-cholesterol showed similar results within the overweight groups as the cross-analysis showed within both VO_2max and MFI subgroups.

Table 4. Serum lipids in Body Mass Index (BMI) and cardiorespiratory, muscle fitness subgroups.

			Normal weight (BMI < 24.99)	Overweight (BMI ≥ 25.00)	*P*
Cardiorespiratory fitness	**fit (3rd tertile)** >44.87 ml/kg/min		**(n=215)**	**(n=45)**	
		ox-LDL (µmol/L)	22.2 (±6.1)	22.7 (±5.7)*	**NS**
		ox-LDL/HDL-cholesterol	14.5 (±4.9)	16.0 (±4.6)*	**NS**
		total cholesterol (mmol/L)	4.32 (±0.76)	4.56 (±0.89)	**NS**
		HDL cholesterol (mmol/L)	1.59 (±0.35)	1.48 (±0.32)	**NS**
		LDL cholesterol (mmol/L)	2.27 (±0.55)	2.47 (±0.61)	**NS**
		triglycerides (mmol/L)	0.84 (v0.32)	0.87 (±0.28)*	**NS**
	average fit (2nd tertile) 37.87 - 44.87 ml/kg/min		**(n=156)**	**(n=110)**	
		ox-LDL (µmol/L)	23.4 (±6.8)	25.9 (±10.1)	**NS**
		ox-LDL/HDL-cholesterol	15.4 (±5.7)	19.3 (±10.9)*	**0.004**
		total cholesterol (mmol/L)	4.44 (±0.83)	4.61 (±0.88)*	**NS**
		HDL cholesterol (mmol/L)	1.59 (±0.33)	1.46 (±0.38)*	**0.032**
		LDL cholesterol (mmol/L)	2.31 (±0.56)	2.50 (±0.67)*	**NS**
		triglycerides (mmol/L)	0.90 (±0.33)	1.12 (±0.67)*	**0.042**
	unfit (1st tertile) <37.87 ml/kg/min		**(n=96)**	**(n=163)**	
		ox-LDL (µmol/L)	23.5 (±6.6)	28.7 (±10.2)	**<0.0001**
		ox-LDL/HDL-cholesterol	16.5 (±5.9)	24.0 (±12.2)	**<0.0001**
		total cholesterol (mmol/L)	4.49 (±0.87)	4.88 (±0.90)	**0.010**
		HDL cholesterol (mmol/L)	1.49 (±0.35)	1.29 (±0.30)	**<0.0001**
		LDL cholesterol (mmol/L)	2.37 (±0.62)	2.75 (±0.63)	**<0.0001**
		triglycerides (mmol/L)	0.98 (±0.37)	1.38 (±0.72)	**<0.0001**
Muscle fitness	**fit (3rd tertile)**		**(n=179)**	**(n=74)**	
		ox-LDL (µmol/L)	22.9 (±6.5)	24.9 (±7.2)	**NS**
		ox-LDL/HDL-cholesterol	14.8 (±5.2)	18.2 (±6.8)	**0.005**
		total cholesterol (mmol/L)	4.41 (±0.81)	4.67 (±0.92)	**NS**
		HDL cholesterol (mmol/L)	1.61 (±0.34)	1.45 (±0.36)	**0.012**
		LDL cholesterol (mmol/L)	2.29 (±0.57)	2.55 (±0.70)	**NS**
		triglycerides (mmol/L)	0.87 (±0.36)	1.03 (±0.46)*	**NS**
	average fit (2nd tertile)		**(n=163)**	**(n=92)**	
		ox-LDL (µmol/L)	22.6 (±5.8)	27.0 (±11.3)	**0.011**
		ox-LDL/HDL-cholesterol	15.1 (±4.7)	21.5 (±13.7)	**<0.0001**
		total cholesterol (mmol/L)	4.44 (±0.85)	4.72 (±0.88)	**NS**
		HDL cholesterol (mmol/L)	1.56 (±0.31)	1.39 (±0.34)	**0.003**
		LDL cholesterol (mmol/L)	2.33 (±0.59)	2.64 (±0.66)	**NS**
		triglycerides (mmol/L)	0.90 (±0.32)	1.29 (±0.85)	**<0.0001**
	unfit (1st tertile)		**(n=107)**	**(n=133)**	
		ox-LDL (µmol/L)	23.0 (±7.0)	28.5 (9.8)	**<0.0001**
		ox-LDL/HDL-cholesterol	15.5 (±6.3)	23.5 (11.3)	**<0.0001**
		total cholesterol (mmol/L)	4.37 (±0.75)	4.82 (0.93)	**0.035**
		HDL cholesterol (mmol/L)	1.56 (±0.37)	1.30 (0.31)	**<0.0001**
		LDL cholesterol (mmol/L)	2.30 (±0.55)	2.69 (0.66)	**0.001**
		triglycerides (mmol/L)	0.91 (±0.34)	1.29 (0.63)	**<0.0001**

*Age and smoking was used as covariate (ANCOVA-2). Data presented as Mean ± SD. Differences within the respective BMI group between the fitness tertiles (compared to the unfit subgroup): * P ≤ 0.05*

5.5. INTERRELATIONSHIPS OF OXIDIZED LDL, SYSTEMIC INFLAMMATION AND PHYSICAL FITNESS IN NON-MetS AND MetS YOUNG MEN (STUDY III)

In comparison between the non-MetS and MetS participants, MetS had a higher mean concentration of ox-LDL and ratios of ox-LDL/HDL-cholesterol and ox-LDL/LDL-cholesterol, respectively. Accordingly, the concentrations of TNFα and il-6 were higher in the MetS group, when compared to the non-MetS group (Figure 4).

In order to assess the associations between MetS and non-MetS participants, correlations were calculated between markers of oxidative stress (ox-LDL, ratios of ox-LDL for HDL-/LDL-cholesterol), systemic inflammation (TNFα, il-6) and physical fitness components (cardiorespiratory and muscular fitness). Pearson correlation showed relationships between ox-LDL/HDL and TNFα and il-6 in the non-MetS participants, while ox-LDL/LDL-cholesterol showed significant correlation with TNFα in the MetS participants (Table 5).

In stepwise multivariate logistic regression analysis, elevated ox-LDL together with poor results in sit-ups and VO_2max, significantly associated with the prevalence of MetS, while the other fitness tests, il-6 and TNFα did not associate with MetS prevalence (Table 6).

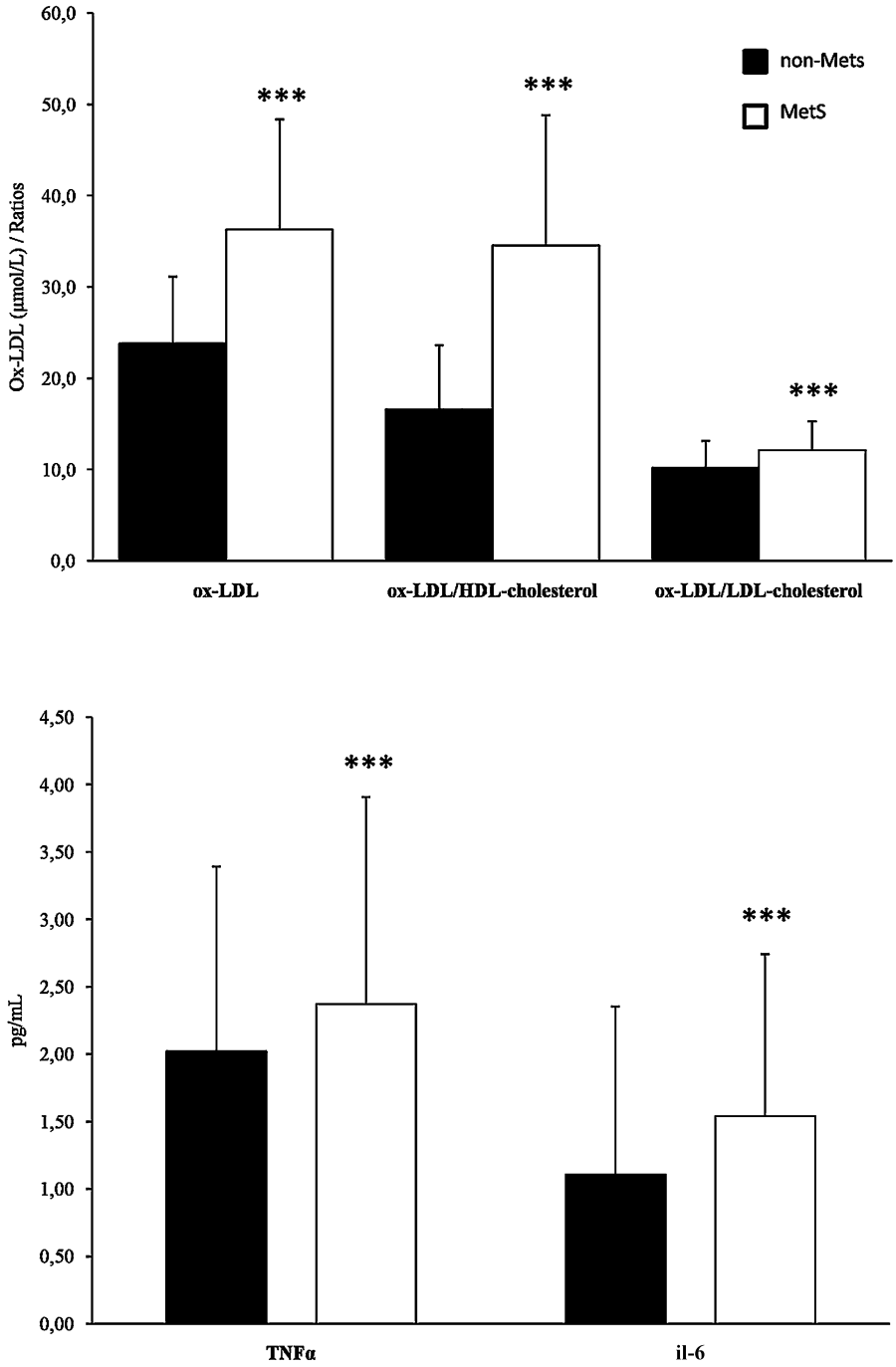

Figure 4. The concentration of oxidized LDL (ox-LDL), tumour necrosis factor α (TNFα) and interleukin-6 (il-6) and ratios of ox-LDL/HDL-cholesterol and ox-LDL/LDL-cholesterol in metabolic syndrome (MetS) and non-MetS groups. Age, smoking and leisure-time physical activity (LTPA) were used as covariates (ANCOVA2). Statistical differences between MetS and non-MetS: ***P < 0.0001.

Table 5. Correlations between oxidative stress, systemic inflammation and physical fitness in metabolic syndrome (MetS) and non-MetS.

non-MetS participants	**TNFα**	**il-6**	**MetS participants**	**TNFα**	**il-6**
ox-LDL	0.057	0.039	ox-LDL	0.264	0.051
ox-LDL/HDL-cholesterol	0.114**	0.079*	ox-LDL/HDL-cholesterol	0.256	0.170
ox-LDL/LDL-cholesterol	0.036	0.007	ox-LDL/LDL-cholesterol	0.276*	0.235

non-MetS participants	**VO_2max**	**Sit-ups**	**Push-ups**	**Repeated squats**	**Grip-strength**	**Muscle Fitness Index**
ox-LDL	-0.180***	-0.080*	-0.070	-0.075*	0.047	-0.073
ox-LDL/HDL-cholesterol	-0.236***	-0.116**	-0.155***	-0.105*	0.056	-0.141***
ox-LDL/LDL-cholesterol	-0.024	0.049	0.061	0.049	-0.054	-0.012
TNFα	-0.012	-0.026	-0.007	0.020	-0.019	0.012
il-6	-0.251***	-0.189***	-0.225***	-0.216***	-0.017	-0.231***
MetS participants						
ox-LDL	-0.006	0.181	0.084	0.264	-0.004	0.049
ox-LDL/HDL-cholesterol	-0.198	0.100	0.018	0.104	-0.072	-0.070
ox-LDL/LDL-cholesterol	-0.083	0.281*	0.062	0.310*	-0.041	0.053
TNFα	0.110	0.093	-0.134	0.033	-0.091	-0.154
il-6	-0.365**	-0.121	-0.003	-0.252	-0.075	-0.172

*The significances of Pearson correlations: *0.01< p ≤0.05, **0.0001 ≤ p ≤ 0 .01, ***p < 0.0001. Oxidized LDL (ox-LDL), tumor necrosis factor α (TNFα), interleukin-6 (il-6).*

Table 6. Stepwise multivariate logistic regression analysis for the determination of associations with metabolic syndrome (MetS) prevalence using oxidized LDL lipids (ox-LDL), systemic inflammation (interleukin-6 [il-6], tumor necrosis factor α [TNFα]), and physical fitness (individual muscle group fitness tests, muscle fitness index [MFI], maximal oxygen uptake [VO_2max]). Odds ratios (OR) and corresponding 95% confidence intervals (C.I.) indicate the risk of having MetS according to the IDF 2007 criteria for MetS*.

		P-value	OR	95% C.I.	Wald	Standard Error
Step 1	**ox-LDL**	**<0.0001**	**1.141**	**1.102-1.181**	**56.06**	**0.018**
ox-LDL, il-6 and TNFα entered	il-6	0.249				
	TNFα	0.860				
Step 2	**ox-LDL**	**<0.0001**	**1.132**	**1.092-1.174**	**45.12**	**0.018**
muscle fitness variables entered	**sit-ups**	**<0.0001**	**0.910**	**0.878-0.944**	**25.88**	**0.018**
	push-ups	0.373				
	repeated squats	0.382				
	grip strenght	0.232				
	MFI	0.672				
Step 3	**ox-LDL**	**<0.0001**	**1.118**	**1.078-1.160**	**35.52**	**0.019**
maximal oxygen uptake entered	**sit-ups**	**0.002**	**0.938**	**0.901-0.977**	**9.37**	**0.021**
	VO2max	**0.001**	**0.898**	**0.844-0.956**	**11.36**	**0.032**

Muscle Fitness Index is calculated from different individual muscle fitness tests (sit-ups, push-ups, repeated squats and grip strength).
**IDF 2007 criteria for MetS (IDF, 2006). Waist circumference ≥ 0.94 m or BMI > 30 kg / m^2 plus 2 of the following four factors: Triglycerides (≥1.7 mmol/L) or lipid abnormality treatment, HDL-cholesterol (<1.03 mmol/L) or lipid abnormality treatment, Systolic BP≥130 mmHg or diastolic BP≥85 mmHg or treatment for diagnosed hypertension, Fasting glucose ≥5.6mmol/L.*

5.6. ASSOCIATIONS OF OXIDIZED LDL AND HIGH OR LOW LEVELS OF TESTOSTERONE OR FREE TESTOSTERONE IN YOUNG MEN (STUDY IV)

Based on the findings, cardiorespiratory fitness is associated with testosterone combined with the concentration of ox-LDL and the ratio of ox-LDL/HDL-cholesterol. However, muscular fitness or strength did not show significant differences between low or high TES subgroups, respectively (Study IV).

In more detail, the low TES subgroup with the lowest cardiorespiratory fitness with low level of TES had the highest concentration of ox-LDL and ratio of ox-LDL/HDL-cholesterol compared to the other five subgroups (Table 7). However, within the low TES subgroups, the unfit subgroup had a significantly higher concentration compared

to the fit subgroup when age, smoking and waist circumference were adjusted. In addition to high levels of ox-LDL, the unfit low TES subgroup had a higher ox-LDL/HDL-cholesterol ratio compared to other five subgroups. In the evaluation of lipids between low and high TES subgroups, ox-LDL did not have any significant difference between unfit, average fit or fit subgroups; moreover, the ratio of ox-LDL/HDL-cholesterol was higher in the unfit low TES subgroup than in the unfit high TES subgroup. The comparisons of FTES groups showed similar results. No significant differences were seen between the fittest subgroup in either the TES or FTES groups (Study IV).

Table 7. The concentrations of oxidized LDL (ox-LDL) and other serum lipids in testosterone and free testosterone subgroups according to cardiorespiratory status (unfit/average fit/fit). Mean (SD). Age, smoking and waist circumference were used as covariates.

Cardiorespiratory fitness						
	Low testosterone (TES < 16.9 nmol/L, n = 381)			**High testosterone (TES ≥ 16.9 nmol/L, n = 391)**		
	unfit	**average fit**	**fit**	**unfit**	**average fit**	**fit**
	(n=151)	(n=127)	(n=102)	(n=104)	(n=131)	(n=153)
ox-LDL (µmol/L)	28.2 (±10.0)	24.9 (±9.1)	21.9 (±5.9)*	24.7 (±8.1)	24.0 (±7.8)	22.4 (±6.1)
ox-LDL/HDL-cholesterol	23.3 (±11.8)	17.6 (±9.4)*	14.9 (±4.7)*	18.3 (±8.7)*	16.5 (±6.6)*	14.6 (±5.0)*
ox-LDL/LDL-cholesterol	10.6 (±2.7)	10.6 (±3.1)	9.6 (±2.5)	10.2 (±3.2)	10.3 (±2.8)	10.2 (±3.0)
total cholesterol (mmol/L)	4.82 (±0.91)	4.55 (±0.85)	4.39 (±0.77)	4.61 (±0.90)	4.47 (±0.86)	4.35 (±0.80)
HDL cholesterol (mmol/L)	1.31 (±0.32)	1.52 (±0.37)*	1.52 (±0.28)	1.44 (±0.34)	1.54 (±0.35)*	1.61 (±0.38)*
LDL cholesterol (mmol/L)	2.69 (±0.67)	2.40 (±0.62)	2.34 (±0.54)	2.49 (±0.61)	2.38 (±0.61)	2.28 (±0.58)
triglycerides (mmol/L)	1.32 (±0.74)	1.01 (±0.57)*	0.79 (±0.26)*	1.10 (±0.44)	0.97 (±0.44)	0.88 (±0.34)*
	Low free testosterone (FTES < 280.4 pmol/L)			**High free testosterone (FTES ≥ 280.4 pmol/L)**		
	unfit	**average fit**	**fit**	**unfit**	**average fit**	**fit**
	(n=146)	(n=130)	(n=107)	(n=108)	(n=128)	(n=147)
ox-LDL (µmol/L)	27.7 (±10.0)	24.00 (±7.7)	21.9 (±5.6)	25.7 (±8.4)	24.9 (±9.1)	22.5 (±6.3)
ox-LDL/HDL-cholesterol	22.8 (±11.9)	16.4 (±7.9)*	14.8 (±4.6)*	19.2 (±9.1)	17.7 (±9.1)	14.7 (±5.0)*
ox-LDL/LDL-cholesterol	22.8 (±2.6)	10.2 (±2.9)	9.6 (±2.4)	10.6 (±3.4)	10.6 (±3.1)	10.3 (±3.3)
total cholesterol (mmol/L)	4.83 (±0.9)	4.57 (±0.80)	4.41 (±0.77)	4.61 (±0.89)	4.44 (±0.91)	4.34 (±0.80)
HDL cholesterol (mmol/L)	1.32 (±0.32)	1.57 (±0.37)	1.54 (±0.30)	1.43 (±0.34)	1.50 (±0.35)	1.60 (±0.37)
LDL cholesterol (mmol/L)	2.70 (±0.67)	2.40 (±0.57)	2.35 (±0.55)	2.49 (±0.60)	2.38 (±0.66)	2.27 (±0.58)
triglycerides (mmol/L)	4.66 (±0.74)	0.94 (±0.45)	0.77 (±0.25)	1.16 (±0.47)	1.04 (±0.56)	0.90 (±0.34)

*Differences between the low testosterone or free testosterone and unfit cardiorespiratory subgroup and other five subgroups in question within the testosterone or free testosterone groups using Least Significant Differences as a post-hoc test: * $P < 0.05$.*

6. DISCUSSION

The present thesis shows how ox-LDL is associated with physical fitness in young men. Higher levels of ox-LDL were seen in participants with poor physical fitness together with higher BMI, smoking status and lower levels of TES. Also higher levels of ox-LDL were seen in participants with low levels of LTPA and MetS. These results indicate significant beneficial attributes of good physical fitness towards levels of ox-LDL in young men.

6.1. DIFFERENCES IN THE LEVELS OF OX-LDL AND IN THE RATIO OF OX-LDL/HDL-CHOLESTEROL BETWEEN MEN WITH POOR OR GOOD PHYSICAL FITNESS (STUDY I)

Study I demonstrated significant differences in concentrations of atherogenic ox-LDL between young men of poor and good cardiorespiratory fitness. We found the VO_2max cut-off point for lower levels of ox-LDL with participants who had >42 mL/kg/min or higher VO_2max. Our results seem consistent when compared to the intervention of Vasankari et al. (1998), where 34 middle aged men finished a 10-month exercise period where ox-LDL was decreased by 23 % and meanwhile the VO_2max rose by 19%, respectively. Between the un-fittest and fittest cardiorespiratory subgroups in the present study, the difference was interestingly 23% which shows that having higher levels of cardiorespiratory fitness protects young men from harmful levels of ox-LDL and is in line with the results of Vasankari et al. By enhancing VO_2max, young men could decrease high levels of ox-LDL and might affect lower rates of mortality (Linna et al., 2012).

The ratio of ox-LDL/HDL-cholesterol is a considered marker for an atherogenic lipid profile combining two atherosclerotic risk factors and therefore is suggested to be a more sensitive indicator of subclinical atherosclerosis than individual lipid measurements (Johnston et al., 2006). The three fittest cardiorespiratory groups had substantially lower levels of this sensitive marker of atherosclerosis when compared to both poor and average cardiorespiratory fitness groups, underlining again the importance of good VO_2max even in young men. Added to the fact that the percentile difference of ox-LDL/HDL-cholesterol ratio was superior when compared to the individual differences of ox-LDL and HDL cholesterol between these same cardiorespiratory groups, these results lend presumption that young men possessing high levels of cardiorespiratory fitness have an enhanced capability to inverse cholesterol transportation as well as a higher affection of controlling their atherogenic peroxidation of LDL lipids (Mertens & Holvoet, 2001).

In addition to the ratio of ox-LDL and HDL cholesterol, the ratio of ox-LDL and LDL cholesterol was determined. This ratio has been associated with patients with clinical

multi-vessel disease caused by atherosclerosis (Vasankari et al., 2001a & 2001b). However, in Study I no significant differences in the ratios of ox-LDL/LDL-cholesterol were detected between cardiorespiratory or muscle fitness in young men, suggesting that further evidence should be gathered in order to draw conclusions from the affections of LDL cholesterol metabolism, oxidation and physical fitness.

According to Study I, young men who have poor muscular fitness possess a higher concentration of ox-LDL. Comparing the poorest muscular fitness subgroup to the fittest, the difference was linear whereas the fittest muscle fitness group had the lowest concentration of ox-LDL. These results show novel findings of the advantages of having a better muscle fitness status even at a young age and furthermore, the possible benefits to be gained from resistance training which increases muscle fitness (Bemben et al., 2000).

Ox-LDL and muscular fitness have not been thoroughly examined in the past. A major focus in the history of related research has been towards cardiorespiratory fitness, while muscular fitness has not been recognised even though good muscular fitness has been negatively correlated to triglycerides and the ratio of total cholesterol and HDL cholesterol in adolescence and independently from cardiorespiratory fitness to form a cluster of metabolic risk factors (Steene-Johannessen et al., 2009; Artero et al., 2011). Furthermore, Katmarzyk & Craig (2002) found poor muscular fitness to be in association with mortality in a large population based study in Canada. The independent relation of mortality and muscular fitness, especially abdominal fitness, could be related to heightened levels of oxidative stress and an increased concentration of ox-LDL based on the results of Study I. The findings of the current study reveal some relationships between ox-LDL and muscular fitness, however, unfortunately the cross-section study method does not yield a sufficient amount of data concerning ox-LDL and muscular fitness for direct causality to be drawn, thus more research ought to be conducted. In addition to the novel results of the ratio of ox-LDL/HDL-cholesterol in cardiorespiratory groups, the significant difference of 32 % was discovered between the ratios of ox-LDL/HDL-cholesterol in the poorest and fittest muscle fitness groups. Compared to differences between the concentrations of ox-LDL (16 %) and HDL cholesterol (10%) in these same muscle fitness groups, the ratio of ox-LDL/HDL-cholesterol seems to be more demonstrative for an atherosclerotic lipid profile, compared to individual lipid markers when considering physical fitness as a measurement method.

6.2. LEISURE-TIME PHYSICAL ACTIVITY ASSOCIATIONS WITH OX-LDL AND THE RATIO OF OX-LDL / HDL-CHOLESTEROL IN YOUNG MEN (STUDY I)

LTPA has been recognized widely for manipulating an unhealthy lipid profile into a more antiatherogenic one (Jeppensen et al., 1998; Stefanick et al., 1998; Mora et al., 2006). A recent multifaceted guideline suggests physical activity for adults (over 20 years old) ≥ 150 min/week of moderate intensity in order to promote individual "ideal

cardiovascular health" (Lloyd-Jones et al., 2010). The findings of the present study reinforce the presented guideline as young men who conducted moderate to vigorous activity at least three times per week (with a minimum of 20 min activity) had lower levels of ox-LDL, a lower ox-LDL/HDL-cholesterol ratio and higher concentrations of HDL cholesterol. Previous studies have indicated similar results, however these have been of low cohort quantity and involved older participants (Park et al., 2011). Although the ox-LDL and ox-LDL/HDL-cholesterol ratio differences were more modest compared to the differences of cardiorespiratory or muscle fitness groups, conducting LTPA three or more times per week has a significant positive impact on ox-LDL when compared to inactivity. Based on the present study results and acknowledging the results of past decades, agreement can be reached that regular exercising could lower the levels of ox-LDL and the ratio ox-LDL/HDL-cholesterol.

6.3. OX-LDL LEVELS IN NON-SMOKERS AND SMOKERS – COMPARISONS BETWEEN UNFIT AND FIT (STUDY I)

A similar difference between unfit and fit groups was seen when participants were divided into smokers and non-smokers. Smokers had higher levels of ox-LDL compared to non-smokers, which is in line with a previous study where the level of ox-LDL was found to be elevated in middle-aged smokers (Linna et al., 2008). However, our results only showed a significant difference in the levels of atherogenic ox-LDL between unfit smokers and unfit non-smokers. This highlights that having poor fitness together with a habit of smoking is associated with a higher concentration of ox-LDL and therefore an increased risk of cardiovascular diseases and mortality (Lakier, 1992). In contrast, the cessation of smoking reduces the progression of atherosclerosis (McPride, 1992). Especially noteworthy were the results seen between non-smokers and smokers in the ratio of ox-LDL/HDL-cholesterol, as both the unfittest cardiorespiratory and muscle fitness smoker groups had higher ratios of ox-LDL/HDL-cholesterol when compared to non-smoker groups. Moreover, smoking has been suggested to have even a causal role behind the low concentrations of HDL cholesterol (Willet et al., 1983), which contributes to the novel findings for smoking and poor fitness being the hallmark of atherogenicity due to high levels of ox-LDL and low levels of HDL cholesterol in young men.

6.4. DIFFERENCES IN OX-LDL AND OX-LDL/HDL-CHOLESTEROL BETWEEN UNFIT TO FIT NORMAL AND OVERWEIGHT MEN (STUDY II)

High levels of ox-LDL and poor cardiorespiratory fitness are independently associated with increased waist circumference and BMI (Weinbrenner et al., 2006; Njajou et al., 2009; Fogelholm et al., 2006a). The present study demonstrated a linear fashion of

increase in the levels of ox-LDL through normal weight to obese in between cardiorespiratory groups. These results are in line with a previous study using the diene conjugation method for the determination of ox-LDL and BMI as an indicator for overweight and obesity (Vasankari et al., 2001a). Furthermore, in order to evaluate possible associations between ox-LDL, cardiorespiratory fitness and BMI, we divided participants into smaller subgroups according VO_2max using a BMI cut-off point of 25 kg/m^2. After distributions to the fitness subgroup, unfit normal weight participants had a lower concentration of ox-LDL, compared to unfit overweight participants. However, no significant differences in the concentration of ox-LDL were seen between fit normal weight and fit overweight subgroups.

Comparing this insignificance between the two highest VO_2max subgroups and whilst significant differences were seen between to the two lowest VO_2max subgroups, being fit seems to protect against an atherogenic ox-LDL lipid profile altogether. Based on the past research data of Hellsten et al. (1996) and Elosua et al. (2003) concerning the increased capability of scavenger receptor activity and LDL resistance to oxidation in cardio-trained subjects, Study II results show that individuals with a higher cardiorespiratory fitness status could attenuate harmful oxidative stress and ox-LDL through compensatory reactions. Adding the fact that the unfit subgroup had higher levels of ox-LDL compared to the fit subgroup within the overweight group, overweight individuals could attenuate their harmful heightened ox-LDL lipid profile by increasing their VO_2max. This has previously been shown in middle aged men (mean age 43.6, mean BMI 29.6) by Vasankari et al. (1998), where a decrease of ox-LDL was noted after a 10-month exercise intervention.

When dividing the cohort into normal weight and overweight subgroups, the concentration of ox-LDL in overweight young men with poor cardiorespiratory fitness seemed to be even higher compared to the concentration of ox-LDL in overweight men without weight dividing. Also, the difference in ratio of ox-LDL/HDL-cholesterol (50 %) was even higher compared to separate differences in ox-LDL (26 %) and HDL cholesterol (15 %) concentrations. This might indicate that the levels of ox-LDL are dire in overweight unfit young men. However, in overweight fit young men, the reverse transportation of lipids could be made more efficient by having a higher HDL particle count in addition to a lower ox-LDL concentration. Yet individuals with high physical fitness (i.e. athletes) seem to compensate the oxidation of LDL via an increased high quantity of free radical scavenger enzymes which are produced during aerobic exercise (Marzatico et al. 1997; Hellsten et al. 1996). This mechanism could protect athletes from atherosclerosis despite the fact that exercise enhances the oxidation of LDL (Pincemail et al, 2000).

In Study II, muscular fitness in the overweight (BMI $\geq$ 25.0kg/m^2) was clearly poorer when compared to normal weight participants. As presumed, the levels of ox-LDL were also highest in poor and average fitness overweight participants after muscular subgroups were formed. A novel finding of Study II was the similarity of

cardiorespiratory fitness results between the two poorest muscular fitness groups, whilst no significant results were found in the two fittest subgroups. However, the results show how muscular fitness differs between overweight groups as was also the case in cardiorespiratory groups. No studies have been published focusing on the overweight, which determines a relationship between muscular fitness and ox-LDL. While good muscular fitness is associated with better insulin sensitivity (NCEP, 2002), weight management and improved body composition (Treuth et al., 1994), clear consensus for the role of muscular fitness is yet to be found. On the other hand, guidelines which exist for high cholesterol related diseases include resistance training which leads to better muscle fitness (NCEP, 2002). From a muscular fitness point of view, Study II concludes that overweight young men with poor muscular fitness have higher levels of atherogenic ox-LDL, when compared to normal weight individuals with same fitness status.

The ratio of ox-LDL/HDL-cholesterol showed similar results of difference between unfit overweight and fit overweight subgroups as did these same subgroups in cardiorespiratory fitness evaluation. Curiously though, between the two fittest muscular fitness subgroups, the ox-LDL/HDL-cholesterol ratio was significantly higher in the overweight subgroup compared to the normal weight subgroup. The fittest subgroup of the cardiorespiratory group had no significant difference between normal weight and overweight participants in the ox-LDL/HDL-cholesterol ratio. This could be explained by the findings of Banz et al., (2003) where only their group undergoing aerobic training (affecting cardiorespiratory fitness) increased their HDL cholesterol (by 13 % in a 10 week period) while the group undergoing resistance training (affecting muscular fitness) had insignificant changes in blood lipids, including HDL and LDL cholesterol. However, the fittest subgroups in cardiorespiratory fitness had lower overall ratios of ox-LDL/HDL-cholesterol when compared to the fit muscular fitness subgroup of normal weight. This highlights the role of cardiorespiratory fitness as an eminent way for modifying the levels of atherogenic ox-LDL and antiatherogenic HDL cholesterol (LeMura et al., 2000).

6.5. INTERRELATIONS BETWEEN PHYSICAL FITNESS, OX-LDL SYSTEMIC INFLAMMATIONS IN YOUNG MEN WITH AND WITHOUT METS (STUDY III)

In previous studies metabolic syndrome has been independently associated with poor physical fitness, ox-LDL and low-grade systemic inflammation (Holvoet et al., 2008; Wisse, 2004). The present study demonstrated novel interrelationships between these individual components. The study revealed several key correlations between young men with and without MetS: I) MetS participants had higher levels of ox-LDL, TNFα and il-6 than participants without MetS. II) MetS participants had lower amounts of repetitions in sit-ups, push-ups and repeated squats as well as VO_2max than non-MetS

men. III) VO_2max, sit-ups and ox-LDL associated with MetS. These results underline an overall poorer level of health in MetS subjects. In more detail, the results of section I are well published as Holvoet et al. (2008) showed, by using ELISA assay, that MetS subjects have a higher concentration of ox-LDL than non-MetS subjects. Wisse (2004) reviewed the substantial roles of different cytokines and chronic inflammation markers in abdominal obesity and components of MetS. As for the results of section II, a poorer overall physical fitness and cardiorespiratory fitness of MetS has been discovered in the past, but not however fully understood (Steele et al,. 2008; LaMonte et al., 2005). Even so, guidelines are formed advising regular physical activity in order to improve physical fitness, and by way of this increased physical fitness, the risk of MetS could be decreased by better weight regulation (IDF, 2006). The present study's results from sections I and II are therefore in line with earlier mentioned studies which emphasize the key positions of ox-LDL and physical fitness in MetS. However, studies using an interrelationship perspective and measuring correlations between ox-LDL, inflammation, physical fitness with MetS young men, have not yet been conducted. While ox-LDL did not have correlations with either physical fitness or systemic inflammation in MetS, the ratio of ox-LDL to LDL had significant correlation with TNFα. This ox-LDL/LDL ratio can be used to test for atherogenicity in lipoprotein. As ox-LDL/LDL-cholesterol is related to atherosclerosis associated TNFα, the present study concurs with Vasankari et al (2001a) that LDL lipids carried to the endothelium have more potential to be highly oxidized into ox-LDL in MetS. Furthermore, MetS could have a higher risk of increased intima media thickness due to atherosclerosis, leading to intraluminal plaque formation and clinical cardiovascular disease.

Previously, high muscular fitness and strength and cardiorespiratory fitness are independently associated with MetS (Radim et al., 2005; Kim et al., 2011; Stewart et al., 2005). Laaksonen et al. (2011) showed that higher cardiorespiratory fitness protected middle-aged men from MetS. The present study section IV findings suggest that while poor results from the sit-ups test (OR=0.938, p=0.002) and low VO_2max (OR=0.898, p=0.001) were associated with MetS, high ox-LDL (OR=1.118, $p<0.0001$) had an even higher odds ratio, compared to both of these poor cardiorespiratory or muscle fitness variables when entered into a the model of stepwise multivariate logistic regression analysis. This proves a higher plausibility of the ox-LDL role in MetS pathophysiology, although not forgetting the important aspect of good physical fitness in the deflection of MetS. Furthermore, these results raise a question as to whether low-grade inflammation is more a cause than a reason of MetS, compared to Holvoet's (2008) causality suggestion between ox-LDL and MetS.

6.6. CONCENTRATIONS OF OX-LDL AND TESTOSTERONE IN YOUNG HEALTHY MEN AND COMPARISONS BETWEEN DIFFERENT LEVELS OF PHYSICAL FITNESS (STUDY IV)

According to Study IV, young men with low levels of TES with poor cardiorespiratory fitness have significantly higher concentrations of ox-LDL compared to high cardiorespiratory young men with low levels of TES. Moreover, unfit high TES young men have lower ratio of ox-LDL/HDL-cholesterol compared to these unfit low TES individuals. Fortunately, having VO_2max uptake seems to eliminate the differences of ox-LDL levels between high and low TES individuals. In the past, high levels of ox-LDL and low levels of TES have been demonstrated using the diene conjugation method (Linna et al., 2008). Low levels of TES have been associated with numerous atherosclerotic related conditions and moreover, increased mortality independent from MetS, diabetes and cardiovascular diseases (Svartberg et al., 2006; Lauhglin et al., 2008). No study has been conducted in order to examine the interrelationships between testosterone levels and ox-LDL via physical fitness. By examination of ox-LDL levels with HDL cholesterol (which has been strongly negatively associated with TES in the past), the results of the ox-LDL/HDL-cholesterol ratio shows a highly atherogenic lipid profile in unfit cardiorespiratory young men who have low levels of TES (Semmens et al., 1983). These results sum together the relations of TES and ox-LDL, together with strong interrelations of VO_2max as cardiorespiratory exercising could increase the resistance of LDL towards oxidation (Wetztein et al., 1998).

While evidence has been published that maximal muscular strength is inversely and independently associated to all-cause mortality (Ruiz, 2008), the present study did not find any evidence for the associations between ox-LDL, TES/FTES and muscular strength.

6.7. METHODOLOGICAL CONSIDERATIONS

Participants were gathered from a nationwide request from Finnish Defence Force compulsory refreshment courses during the year of 2008. Altogether 1150 were initially invited, from which 846 (74 %) actually volunteered and participated in the present study. From the different exclusion criteria of each study segment (I-IV), the number of participants varied. However, the lowest sub-cohort used was 831 (98.2 %) from the original 846 keeping the study segment's participant count representative. The collected cohort of young Finnish men can be considered as an adequate national sample of young men as it gathered participants from the South, West and East of Finland.

Both cardiorespiratory and muscular fitness tests have age-specific reference values which are based on the data of 3635 civilians (Pihlainen et al., 2009). These fitness tests have been used in the Finnish Defence Forces since the year 2000 to determine

fitness levels and their changes among professional soldiers, reservists and civilians, which make the tests an appropriate method of determining physical fitness in young men. The LTPA of the participants was ascertained with several questions, also containing a single-item question of the weekly frequency of vigorous LTPA, which has earlier been shown to have a positive association in the fitness categories in young healthy men (Fogelholm et al. 2006b).

In order to analyse ox-LDL lipids with high sensitivity and specificity, we used the diene conjugation method for determination of ox-LDL lipid concentration. In the used diene conjugation method of measuring ox-LDL, time-consuming ultracentifugation is not included which protects the ex vivo oxidation of LDL. This attenuates the amount of damaged LDL lipoproteins. It is also highly adequate for use in large scale epidemiological studies (Ahotupa et al., 1996; Nyyssonen et al., 2012). Considering atheroma formation, the used measurement of LDL conjugated dienes has proven to be clinically related to carotid and brachial intima-thickness as signs of atherosclerosis (Raitakari et al., 2001; Vasankari et al, 2001a). Moreover, earlier studies have found associations between ox-LDL and exercise, weight and physical activity interventions using the same measurement of LDL conjugated dienes which is also used in the present study (Linna et al., 2007; Vasankari et al., 2001b; Vasankari et al., 1998).

6.8. STRENGTHS AND LIMITATIONS

The present study strengths lay in the significant amount of homogenous participants, the comprehensive physical fitness tests and the sensitive analysis of ox-LDL. Its limitations were the cross-sectional design of the study, a lack of previous ox-LDL data for the comparison of muscle fitness results and the low numbers of MetS participants.

Due to the cross sectional design of the study, we had to collect a large number of healthy participants with the same gender, age group and nationality, whilst having different physical fitness status. By recruiting the participants from the Finnish Defence Force refreshment courses, we managed to obtain almost 850 volunteer participants which all were more or less capable of conducting the required physical fitness tests. The actual physical fitness tests are well recognized and widely used for the determination of physical fitness status in young men (Fogelholm et al., 2006a & 2006b). VO_2max levels are highly associated with cardiovascular disease morbidity and mortality. On the other hand, muscular fitness seems to also have associations with cardiovascular diseases and the muscle fitness tests employed were selected for muscle group coverage and reference values (Katzmarzyk & Craig, 2002). With a combination of cardiorespiratory and muscular fitness assessments we were able to obtain sufficient and comparable data from the cohort.

We used a direct analysis of ox-LDL from serum to diminish possible bias often existing in immunological measurements. Immunological analysis of ox-LDL can be

troublesome due to the variable nature of oxidized LDL particles, which can be at different levels of oxidation. The ox-LDL concentration measured by an immunoassay depend on the antibody used in assay. We used a sensitive measurement of diene conjugation method in the determination of ox-LDL which shows especially the levels of circulating ox-LDL. This diene conjugation method is widely used in clinical studies among the Finnish and European population and associates with many cardiovascular diseases, so making it highly appropriate for the purpose of the present study (Ahotupa & Vasankari, 1999; Nyyssonen et al., 2012).

Considering the limitations of the study, the greatest potential limitation would be the cross-sectional study design. The results of present thesis are evaluated only at a single time point which makes the clinical implications more or less in need of a longitudinal study design. By use of the cross-sectional study design however, we were able to evaluate participants across a range of different aspects including the different physical fitness tests. Also with different cross-sectional data, we were able to determine age and gender related reference values for ox-LDL for future purposes. The present thesis highlights novel ideas for the future research of the role of physical fitness in the prevention of high concentrations of LDL, atherosclerosis and cardiovascular diseases. Levels of ox-LDL are shown to decrease over a 10-month exercise intervention when VO_2max is used as a variable. In the present thesis, similar levels of ox-LDL were obtained between VO_2max groups. While we could compare VO_2max data from previous studies, no previous muscular fitness and ox-LDL research has been published which could have provided sufficient data for comparison. Therefore, we only could evaluate the differences between our muscle fitness subgroups to assess significant trends of ox-LDL concentration. Comparing previous studies with MetS and ox-LDL, the quantity of MetS participants is in no doubt vast compared to Holvoet et al. (2008) had 1889 MetS participants in their study. However, in the present thesis, our 54 MetS participants were able to execute all the cardiorespiratory and muscular fitness tests which provides a sufficient amount of data by which to consider the relationships between physical fitness, atherosclerosis and levels of lipoproteins. To our knowledge, no previous studies have been reported which collect physical fitness data from young healthy MetS men with ox-LDL levels.

7. SUMMARY

1. Ox-LDL, together with the ratios of HDL and LDL cholesterol, is significantly associated with physical fitness levels in young men, and a higher VO_2max or muscular fitness are associated with higher levels of atherogenic ox-LDL, respectively. The levels of ox-LDL are also higher in unfit smokers compared to unfit non-smokers. This indicates increased atherosclerosis risk in participants with poor fitness. These results suggest that both good cardiorespiratory and muscle fitness might help to prevent atherosclerosis by decreasing the concentration of serum conventional lipids and ox-LDL lipids.

2. The levels of ox-LDL are higher in overweight and obese men compared to normal weight men. In addition, the unfit overweight young men seem to possess more harmful profile of lipoproteins and oxidative modified lipids than fit overweight young men. These results indicate that, being more fit seems to compensate atherogenic ox-LDL levels in young men who are overweight.

3. Individuals with MetS have increased levels of ox-LDL and systemic inflammation combined with poorer physical fitness status. Having a high concentration of ox-LDL, low VO_2max and poor abdominal muscular fitness indicates an increased risk of MetS.

4. The concentration of ox-LDL is increased in young men who have low levels of TES together with poor cardiorespiratory fitness. Being fit seems to attenuate this atherosclerotic lipid profile, thus the concentrations between fit young with lower or higher concentrations of TES werevirtually equal.

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11. APPENDIX

Table 1. Finnish Classification Tables for Professional Male and Female Soldiers, as well as Male and Female Civilian Personnel. The conscripts and reservists are evaluated according to the civilian classification (age group 20-24 years). Male soldiers (age-groups 20-64).

Push-Ups 60 sec, repetitions

		20-24	25-29y	30-34y	35-39y	40-44y	45-49y	50-54y	55-59y	60-64y
Excellent	5	48	46	44	42	40	38	36	34	32
	4.75	47	45	43	41	39	37	35	33	31
	4.5	46	44	42	40	38	36	34	32	30
Very good	4.25	45	43	41	39	37	35	33	31	29
	4	44	42	40	38	36	34	32	30	28
	3.75	43	41	39	37	35	33	31	29	27
	3.5	42	40	38	36	34	32	30	28	26
Good	3.25	41	39	37	35	33	31	29	27	25
	3	40	38	36	34	32	30	28	26	24
	2.75	39	37	35	33	31	29	27	25	23
	2.5	38	36	34	32	30	28	26	24	22
Satisfactory	2.25	37	35	33	31	29	27	25	23	21
	2	36	34	32	30	28	26	24	22	20
	1.75	35	33	31	29	27	25	23	21	19
	1.5	34	32	30	28	26	24	22	20	18
Fair	1.25	29	27	25	23	21	19	17	15	13
	1	24	22	20	18	16	14	12	10	8
Poor	0.75	19	17	15	13	11	9	7	5	4
	0.5	14	12	10	8	6	4	2	1	1
	0.25	9	7	5	3	1	1	1	1	1

Sit-Ups 60 sec, repetitions

		20-24y	25-29y	30-34y	35-39y	40-44y	45-49y	50-54y	55-59y	60-64y
Excellent	5	53	50	47	44	41	38	35	32	29
	4.75	52	49	46	43	40	37	34	31	28
	4.5	51	48	45	42	39	36	33	30	27
Very good	4.25	50	47	44	41	38	35	32	29	26
	4	49	46	43	40	37	34	31	28	25
	3.75	48	45	42	39	36	33	30	27	24
	3.5	47	44	41	38	35	32	29	26	23
Good	3.25	46	43	40	37	34	31	28	25	22
	3	45	42	39	36	33	30	27	24	21
	2.75	44	41	38	35	32	29	26	23	20
	2.5	43	40	37	34	31	28	25	22	19
Satisfactory	2.25	42	39	36	33	30	27	24	21	18
	2	41	38	35	32	29	26	23	20	17
	1.75	40	37	34	31	28	25	22	19	16
	1.5	39	36	33	30	27	24	21	18	15
Fair	1.25	34	31	28	25	22	19	16	13	10
	1	29	26	23	20	17	14	11	8	5
Poor	0.75	24	21	18	15	12	9	6	3	1
	0.5	19	16	13	10	7	4	1	1	1
	0.25	14	11	8	5	2	1	1	1	1

Repeated squats 60 sec, repetitions

		20-24y	25-29y	30-34y	35-39y	40-44y	45-49y	50-54y	55-59y	60-64y
Excellent	5	64	62	60	58	56	54	52	50	48
	4.75	62	60	58	56	54	52	50	48	46
	4.5	60	58	56	54	52	50	48	46	44
Very good	4.25	58	56	54	52	50	48	46	44	42
	4	56	54	52	50	48	46	44	42	40
	3.75	54	52	50	48	46	44	42	40	38
	3.5	52	50	48	46	44	42	40	38	36
Good	3.25	50	48	46	44	42	40	38	36	34
	3	48	46	44	42	40	38	36	34	32
	2.75	46	44	42	40	38	36	34	32	30
	2.5	44	42	40	38	36	34	32	30	28
Satisfactory	2.25	42	40	38	36	34	32	30	28	26
	2	40	38	36	34	32	30	28	26	24
	1.75	38	36	34	32	30	28	26	24	22
	1.5	36	34	32	30	28	26	24	22	20
Fair	1.25	34	32	30	28	26	24	22	20	18
	1	32	30	28	26	24	22	20	18	16
Poor	0.75	30	28	26	24	22	20	18	16	14
	0.5	28	26	24	22	20	18	16	14	12
	0.25	26	24	22	20	18	16	14	12	10

Grip strength, power

		20-24y	25-29y	30-34y	35-39y	40-44y	45-49y	50-54y	55-59y	60-64y
Excellent	5	72	71	70	69	68	67	66	65	64
	4.75	70	69	68	67	66	65	64	63	62
	4.5	68	67	66	65	64	63	62	61	60
Very good	4.25	66	65	64	63	62	61	60	59	58
	4	64	63	62	61	60	59	58	57	56
	3.75	62	61	60	59	58	57	56	55	54
	3.5	60	59	58	57	56	55	54	53	52
Good	3.25	58	57	56	55	54	53	52	51	50
	3	56	55	54	53	52	51	50	49	48
	2.75	54	53	52	51	50	49	48	47	46
	2.5	52	51	50	49	48	47	46	45	44
Satisfactory	2.25	50	49	48	47	46	45	44	43	42
	2	48	47	46	45	44	43	42	41	40
	1.75	46	45	44	43	42	41	40	39	38
	1.5	44	43	42	41	40	39	38	37	36
Fair	1.25	42	41	40	39	38	37	36	35	34
	1	40	39	38	37	36	35	34	33	32
Poor	0.75	38	37	36	35	34	33	32	31	30
	0.5	36	35	34	33	32	31	30	29	28
	0.25	34	33	32	31	30	29	28	27	26