UNIVERSITY OF TURKU

THE EFFECT OF TRAINING BACKGROUND ON OXIDIZED LIPOPROTEIN LIPIDS AND ANTIOXIDANT CAPACITY IN ATHLETES AND KEEP-FIT RUNNERS

Iiro Välimäki

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ABSTRACT

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THE EFFECT OF TRAINING BACKGROUND ON OXIDIZED LIPOPROTEIN LIPIDS AND ANTIOXIDANT CAPACITY IN ATHLETES AND KEEP-FIT RUNNERS
University of Turku, Faculty of Medicine, Department of Physical Activity and Health, Department of Physiology, Doctoral Programme in Clinical Research, Paavo Nurmi Center. Turku. Annales Universitatis Turkuensis. Medica – Odontologica, Turku, Finland, 2018.

Background: High-intensity acute exercise is known to induce oxidative stress and may lead to harmful oxidation of lipids, and protein and DNA damage. On the other hand, exercise is known to positively influence risk factors of atherosclerosis, such as LDL and HDL cholesterol. The role of oxidized LDL lipids as a major risk factor in atherosclerosis is well-established; however, the significance of oxidized HDL lipids has not yet been determined.

Aim: The aim of this study is to investigate the acute and training effects of various types of physical exercise and training background on oxidation of LDL and HDL lipids and antioxidant capacity. The subject of this study consists of three different sets of data. A total of 223 individuals participated in the study, 188 of which were endurance runners, and 35 were ice hockey players. Participants ranged in age from 20–69 years old.

Results: Regular low-intensity training and better maximal oxygen uptake leads to decreased oxidative stress levels compared with high-intensity training. Lactobacillus GG (*LGG*) probiotics do not protect endurance runners from oxidation of LDL lipids. Low levels of exercise combined with increased carbohydrate intake induces oxidation of LDL lipids within as little as 6-days. Acute physical exercise increases the concentration of oxHDLlipids in serum. Oxidative stress and the removal of lipid oxidation products by HDL are greater during athletes' acute physical activity, and less during normal training.

Conclusions: Low-intensity aerobic exercise training protects our bodies by decreasing the levels of oxidative stress compared with anaerobic and high-intensity exercises. Aerobic exercise especially decreases oxLDLlipids levels in the human body and, at the same time, increases the removal of lipid peroxides through transport mechanisms of HDL. The results presented in this thesis strengthen the role of aerobic exercise in preventing atherosclerosis and give a new aspect to the clearing protective role of HDL.

Keywords: Aerobic exercise, anaerobic exercise, physical training, oxidized HDL lipids, oxidized LDL lipids, atherosclerosis

TIIVISTELMÄ

Iiro Välimäki

HARJOITUSTAUSTAN VAIKUTUS HAPETTUNEISIIN LIPOPROTEINIINIEN LIPIDEIHIN JA ANTIOKSIDANTTIKAPASITEETTIIN URHEILIJOILLA JA KUNTOJUOKSIJOILLA

Turun yliopisto, Lääketieteellinen tiedekunta, Terveysliikunta ja fysiologia, Paavo Nurmi – keskus, Turku. Annales Universitatis Turkuensis. Medica – Odontologica, Turku, Finland, 2018.

Tausta: Intensiteetiltään voimakkaan liikunnan tiedetään aiheuttava oksidatiivista stressiä aiheuttaen kudoksiin haitallista rasvojen, proteiinien ja DNA:n hapettumista. Toisaalta on todistettu liikunnan vaikuttavan positiivisesti valtimonkovettumataudin riskitekijöihin, kuten LDL –ja HDL-kolesteroliin. Hapettuneen LDL-kolesterolin rooli yhtenä merkittävämpänä riskitekijänä valtimonkovettumataudissa tiedetään, mutta hapettuneen HDL-kolesterolin merkitys on vielä epäselvä.

Tavoitteet: Väitöskirjatyön tavoitteena on ollut tutkia fyysisen harjoittelun ja aerobisen kunnon vaikutusta pitkäaikaisessa ja akuutissa rasituksessa LDL ja HDL-lipidien hapettumiseen ja antioksidanttikapasiteettiin. Tutkimuksen koehenkilöt muodostuivat kolmen eri aineiston pohjalta. Tutkimukseen osallistui yhteensä 223 mieshenkilöä, joista kestävyysjuoksijoita oli 188 ja jääkiekkoilijoita 35. Ikähaitari tutkituilla oli 20-69 vuotta.

Tulokset: Pitkäaikainen matala intensiteettinen harjoittelu ja parempi kestävyyskunto aiheuttavat vähemmän lipidien hapettumista kuin voimakas intensiteettinen harjoittelu. Lactobacillus GG (LGG) probiootit eivät suojaa kestävyysjuoksijoita LDL-lipidien hapettumiselta. Vähäinen aerobinen liikunta yhdistettynä hiilihydraattipitoiseen ruokavalioon lisää oxLDL -pitoisuutta merkittävästi jo lyhyessä ajassa. Akuutti fyysinen liikuntasuoritus nostaa oxHDL -pitoisuuksia verenkierrossa. OxHDL –pitoisuus nousee aikaisemmin, jos harjoitus vastaa urheilijan harjoitushistoriaa.

Johtopäätökset: Aerobinen matala intensiteettinen liikunta suojaa elimistöä vähentämällä oksidatiivisen stressin pitoisuuksia anaerobiseen korkean intensiteetin liikuntaan verrattuna. Erityisesti aerobinen liikunta vähentää oxLDL –pitoisuuksia elimistössä ja samalla lisää hapettuneiden lipidien poiskuljetusta lisäämällä oxHDL –pitoisuuksia verenkierrossa. Tässä väitöskirjassa esitetyt tulokset vahvistavat aerobisen liikunnan roolia valtimonkovettumataudin ennaltaehkäisyssä ja toisaalta tuovat uuden näkökulman HDL:n suojaavasta roolista hapettuneiden lipidien poiskuljettajana.

Avainsanat: aerobinen liikunta, anaerobinen liikunta, hapettunut HDL, hapettunut LDL, valtimonkovettumatauti

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals (I-IV).

I Välimäki I, Vasankari T, Vuorimaa T, Ahotupa M. Low intensity training and good maximal oxygen uptake associates with decreased oxidative stress in endurance runners. *Gazzetta Medica Italiana* 2010;169(6):303-309.

II Välimäki I, Vasankari T, Vuorimaa T, Ahotupa M, Kekkonen R, Korpela R. Decreased training volume increases oxidized LDL levels. *Int J Sport Med* 2012; 33(4): 291-296.

III Välimäki IA, Vuorimaa T, Ahotupa M, Vasankari TJ. Strenuous physical exercise accelerates the lipid peroxide clearing transport by HDL. *Eur J Appl Physiol* 2016; 116(9): 1683-1691.

IV Välimäki I, Vuorimaa T, Ahotupa M, Vasankari T. Effect of continuous and intermittent exercises on oxidized HDL and LDL lipids in runners. *Int J Sport Med* 2016; 37(14):1103-1109.

ABBREVIATIONS

ABAB	2,2′azobis(2-amidinopropane)HCl
AHA	the american heart association
ATP	adenosine-5 triphosphate
BMI	body mass index
CD	cardiovascular disease
CV	coefficient variant
CHD	coronary heart disease
DC	diene conjugation
DNA	deoxyribonucleid acid
EDTA	ethylenediaminetetraacetic acid
HDL	high density lipoprotein
HPLC	high-performance liquid chromatography
LDL	low density lipoprotein
LGG	*lactobacillus rhamnosus* GG
MAR	marathon runners
MDA	malondialdehyde
MID	middle-distance runners
MetS	metabolic syndrome
NIH	national institute of health
oxHDLlipids	oxidized HDL lipids
oxLDLlipids	oxidized LDL lipids
RONS	reactive oxygen and nitrogen species
ROS	reactive oxygen species
TRAP	total radical peroxyl trapping antioxidant potential
VO_{2max}	maximal oxygen uptake
VO_2	oxygen uptake
WHO	world health organization

1. INTRODUCTION

The normal process(es) of the human body produce(s) unstable molecules. These molecules are mostly free radicals, which may lead to lipid peroxidation and damage cells, creating more free radicals (Sies 1985). On the other hand, we have antioxidant defences to control free radical (oxidative) damage. Exogenous (consumed from diet and other sources) and endogenous (produced inside the body) are the two main kinds of antioxidants. When oxidative stress induced by free radicals overwhelm these antioxidant systems, lipid peroxidation and cell damage can occur, contributing to atherosclerosis and heart disease (Gray et al. 2011; Radak et al. 2013).

Since Harman (1956) first discovered free radicals, there has been debate over whether they are necessary for our well-being or if they cause aging and disease. We now know that oxidative stress caused by free radicals, in small amounts, improves health. Furthermore, it is essential because it promotes antioxidant production in the body via molecule signalling (Dröge et al. 2002; Radak et al. 2008).

All forms of exercise produce free radicals, which is one reason that exercising is beneficial to health (Fischer-Wellman 2009). If oxidative stress is not excessive and chronic, the human body recovers from free radicals and becomes more resistant to oxidative stress in time. This adaptation mechanism is one way that regular moderate exercise can protect our bodies and improve health (Gomez-Cabrera 2008; Wagner et al. 2011; Steinbacher & Eckl 2015). However, the human body only has a limited capacity to protect against radical damage by the up-regulation of antioxidant functions. Some researchers believe that endurance training generates a large amount of free radical outstripping of the antioxidant system, which leads to harmful effects of oxidative stress (Radak et al. 2008; Patil et al. 2012). The main source of free radicals is mitochondria, and during endurance exercise, large quantities of oxygen are inhaled into the body, increasing the metabolism in mitochondria. In theory, this can produce more free radicals (Radak et al. 2013; Fischer-Wellman 2009). Moreover, it has been shown that anaerobic training can produce an increase in oxidative stress similar to that of aerobic training, despite the much higher oxygen intake (Alessio et al. 2000). Also theoretically, endurance exercise, both anaerobic and aerobic, can run out our body's antioxidants, leads to rise of free radicals and increases the risk of atherosclerosis (O'keeefe et al. 2012).

According to the World Health Organization (WHO), cardiovascular disease (CD) is the most common cause of death globally, and physical inactivity is one of the major risk factors for developing CD. As mentioned above, in theory, physical exercise may lead to lipid peroxidation, and oxidation of lipid products in low density lipoproteins (LDL) especially increases the risk for atherosclerosis (Parthasarathy et al. 1998; Steinberg & Witzum 2002). However, physical activity and exercise have been recognised to promote beneficial effects against risk factors of cardiovascular disease (Mora et al. 2007; Monda et al. 2009; Waggner et al. 2015). Exercise improves the lipid profile of plasma, reducing the levels of triglycerides and oxidized LDL lipids (oxLDLlipids) and increasing the levels of high density lipoproteins (HDL) and apolipoprotein A-I (Leon & Sanchez 2001; Vuorimaa et al. 2005). Ahotupa et al. (2010) suggest that HDL may also have role in reverse transport of lipid peroxidation products, and show that acute strenuous exercise increases oxidized HDL lipids (oxHDLlipids) levels.

The information on the transport of lipid oxidation products by lipoproteins is still very limited. The main objective of this thesis is to identify how acute and chronic physical exercise, performed with different intensities, affects lipid peroxidation levels of LDL and the reverse transport of oxidation products of lipids by HDL in athletes and keep-fit runners.

2. REVIEW OF LITERATURE

2.1. ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASE

2.1.1. Atherosclerosis relation to ischemic heart disease

Atherosclerosis of coronary arteries is the most common cause of morbidity and premature death of non-communicable diseases globally (GBD 2015). Atherosclerosis begins in early childhood and progresses slowly with age, usually symptoms does not occur until middle or older age. Exactly how atherosclerosis begins or what causes it is not know, but few theories have been proposed. However, we know that certain habits or conditions can elevate the risk for this disease, these conditions are known as risk factors. The most common risk factors for coronary artery disease are high LDL cholesterol, low HDL cholesterol, high blood pressure, lack of exercise, family history, diabetes, smoking, for women being post-menopausal, and for men being older than 45 (AHA 2015). Other associated risk factors include obesity, a sedentary lifestyle, low socio-economic status and low birth weight. Atherosclerosis can occur in any artery in human body, including arteries in the brain, heart, limbs and kidneys. Atherosclerosis of coronary arteries, also called coronary heart disease (CHD), develops when plaque builds up in artery wall. Atheroma or plaque formation due to atherosclerosis is one the most common causes of a reduced coronary blood flow. Symptoms of angina pectoris develop when there is imbalance between myocardial oxygen supply and demand. In other words, if the artery is too narrow to allow an adequate blood flow in the face of increased oxygen consumption, it causes myocardial ischemia. Acute coronary syndromes or even death can occur when the atherosclerotic plaque develops a fissure or rupture.

Prevention of atherosclerosis in normal subjects is largely a matter of lifestyle. Control of blood pressure, weight reduction, smoking cessation, dietary modifications and regular exercise are all of benefit and therefore widely promoted. There is good evidence from many different studies that treatment with cholesterol-lowering drugs decreases the rates of cardiac events both in patient with a history of coronary heart disease and in asymptomatic subjects with hypercholesterolemia (Feingold & Grunfeld 2016). Statins are still the most widely used cholesterol-lowering compounds.

2.1.2. Lipid theory of atherosclerosis

Serum lipoproteins are the transporters of lipids and lipid soluble materials in the bloodstream and from the liver. LDL delivers cholesterol to peripheral tissues and HDL mediates the reverse transport mechanisms (Barter et al. 2007). Elevated plasma cholesterol has been linked to a higher incidence of atherosclerosis as long as over four decades ago (Ross 1976). High levels of plasma LDL especially accelerate the formation of atherogenic plaque (Pyörälä et al. 1994). According to the National Institute of Health (NIH 2002 & 2004), the incidence rises over a total plasma cholesterol value of 5.17 mmol/L, an LDL cholesterol value of 2.59 mmol/L, a triglycerides value of 1.69 mmol/L, and under an HDL cholesterol value of 1.03 mmol/L. This lipid theory has been the main reason for statin drug usage. Furthermore, plasma lipoproteins contain lipophilic materials including lipid oxidation products, which are known to have negative effects on normal physiological functions and stimulate atherosclerosis processes (Ahotupa et al. 2017). The transport of lipid oxidation products by lipoproteins is poorly recognized and has gained little attention. The is an alternative that the transport of atherogenic lipid oxidation products will turn out to be a crucial element in development of atherosclerosis.

2.1.3. Oxidation theory of atherosclerosis

Atherosclerosis takes place when the walls of medium size arteries are thickening, because of fibrous and lipid tissue deposition. This process narrows the artery lumen and decreases the blood flow. However, the reasons behind this plaque development have not yet fully been discovered. The theory of chronic inflammation and lipid oxidation has been suggested to be one of the major reasons of early onset of atherogenesis. According to this theory, the development of atheroma is initiated by lipid oxidation and vascular production of reactive oxygen species (ROS). ROS are linked to endothelial dysfunction/injury due to a rapid decrease in anti-atherogenic and anti-inflammatory activities of endothelium-derived nitric oxide (Ellulu et al. 2016). The oxidation of LDL is known to be involved in coronary heart disease due to atherosclerosis. Oxidized LDL injures the arterial wall, and the retention of apoB containing lipoproteins in the artery wall leads to an inflammatory response that activates the atherogenic pathway, which promotes foam cell formation (Li D 2005). Nevertheless, atherosclerotic lesion formation can be separated from the manifestation of the lipid peroxidation in the arterial wall. Oxidative events and atherosclerosis are not causally linked, suggesting that the oxidation of lipids is not essential for foam cell formation (Stocker & Keaney 2004).

2.1.4. Oxidative response to inflammation hypothesis of atherosclerosis

The hypothesis that atherosclerosis is an inflammatory disease has been discussed for over 20 years in the field of cardiology. The fact is that inflammation promotes all phases of atherosclerosis, from its inception (endothelial dysfunction, recruitment of immune cells, modifications of LDL, foam cell formation, and apoptosis) to the acute thrombotic complications induced by plaque rupture or erosion that ultimately follow (Ramji & Davies 2015). In the early 1970s, Ross discovered that atherogenic diets generated inflammatory cell adhesion to the arterial wall. Many studies following this observation have established that circulating markers of inflammation (e.g. sensitive C-reactive protein, myeloperoxidase, adhesion molecule-1) are predictive of both atherosclerosis and clinical events of atherosclerosis (Hwang et al. 1997; Malik et al. 2001; Libby 2002; Pradhan et al. 2002). In addition, studies have shown that hypercholesterolemia induces atherogenic plaque formation by accelerating macrophage innate immune signalling pathways, which connect elevated serum lipids to a pro-inflammatory signalling cascade (Björkbacka et al. 2004). It is well-addressed that oxidative stress is a by-product of inflammation (Shishehbor et al. 2003). The inflammatory process produces ROS and reactive oxygen and nitrogen species (RONS), but their role in the inflammation of vascular diseases is not yet clear. However, there is evidence that ROS promote vascular injury, yet are also responsible for promoting tissue reorganisation and regenerative responses to injury (Chenz et al. 2004; Stocker & Keaney 2004). According to this oxidative response to the inflammation hypothesis, the injurious response to cardiovascular risk factors is due to the inflammatory reaction in the vascular wall, which is the resultant of lipoprotein retention and vascular injury (Stocker & Keaney 2004).

2.2. LIPOPROTEINS, OXIDATIVE STRESS AND ATHEROGENIC PLAQUE FORMATION

2.2.1. Oxidative stress and oxidized LDL

Oxidative stress is caused by an imbalance between the production of ROS and the anti-oxidant defence system (Sies 1985) and may lead to the oxidation of lipids, proteins and nucleic acids. Moreover, the attack of ROS against LDL alters it into a more reactive lipoprotein called oxidized LDL (oxLDL). This modified oxLDL is exposed by scavenger receptors of

macrophages and is toxic to endothelial cells (Henriksen1981; Hessler 1983). Furthermore, several studies have indicated that the oxidation of LDL is the primary event inducing the initiation and progression of atherosclerosis (Steinberg et al., 1989; Witztum, 1994, Parthasarathy et al. 1998; Steinberg & Witzum 2002). As mentioned previously, oxidized LDL particles cause endothelial dysfunction and initiates the inflammatory cascade, which eventually leads to atherosclerosis. It is very important to understand that oxidized LDL particles are a very heterogenous group, and the oxidation of LDL by lipid peroxidation is a progressive progress that includes three phases: minimally oxidized LDLs, mildly oxidized LDLs and extensively oxidized LDLs (Jessup & Kritharides 2000; Miller et al., 2003). Minimally oxidized LDL particles (carrier of apoB lipoprotein) are not able to create foam cells (Jessup & Kritharides 2000; Miller et al. 2003; Berniler et al. 1990), but they can induce the migration of monocytes into the arterial wall by inflammation and chemotaxis (Berniler et al. 1990). Inside the arterial wall, monocytes turn into macrophages, which are able to take up the extensively oxidized LDLs (oxLDL) and eventually convert to foam cells. Therefore, the oxLDLs have the greatest potential to create atherogenic plaque (Vasankari et al. 2001; Toikka et al. 2000; Raitakari et al. 2001; Toikka et al. 1999).

2.2.2. HDL relation to atherosclerosis and the reverse transport mechanism

The function of high density lipoprotein (HDL) particles is to collect and carry lipid-soluble material, such as cholesterol, from peripheral tissue to the liver (Figure 1). Because of the reverse transport mechanism, HDL cholesterol is referred to as “good cholesterol” (Rye 2009). A large scale of evidence has accumulated pointing that lipoprotein lipid oxidation products transport is linked to to the risk of atherosclerosis. High level of lipid oxidation products transported by HDL occurs to associate with lower risk of atherosclerosis, while high level of lipid oxidation products in LDL are indicative of elevated risk (Ahotupa 2017). According to the American Heart Association, a low HDL cholesterol concentration is an independent risk factor for coronary heart disease, and levels of below 1.04 mmol/L have been linked 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. 1989). A low concentration of HDL cholesterol can be caused by many factors, including smoking, dietary intake and physical inactivity.

Chronic inflammation, dysfunction of endothelia and oxidative stress have been established as the major initiators of atherosclerosis. The protective role of HDL particles in atherogenesis seems to be opposite to that of LDL particles (e.g. Lewis & Rader 2005; Stein & Stein 1999). HDL particles consist of subpopulations of HDLs of various sizes (Rye et al. 2009), and have many anti-atherogenic functions, such as reverse cholesterol transport, anti-inflammatory, anti-oxidative and anti-thrombotic properties (Kontush 2009; Navab 2011; Wan Ahmad 2015). For example, several studies have shown that HDL particles can be anti-thrombotic in that they enhance the endothelium function and repair injuries, but the capability of these particles to inhibit monocytes binding into the endothelium by down-regulating the expression of endothelial cell adhesion molecules also plays an important role (Cockerill 1995; Patel 2010; Tso et al. 2006; Mineo et al. 2006; Murphy et al. 2008; Rye et al. 2009; Nofer et al. 2002). The antioxidant and anti-inflammatory features of HDL cholesterol have been reported in various studies. Ansell et al. found a negative correlation between the concentration of HDL cholesterol and high-sensitivity C-reactive Protein, indicating anti-inflammatory effects of HDL (Ansell et al. 2003). Despite these several studies pointing the anti-atherogenic properties of HDL, some doubts have risen in recent studies (Boden et al. 2011; Feig et al. 2014; Schwarz et al. 2012;). It is obvious that more research is needed to understand the basic mechanisms of HDL function and how it relates to the antiatherogenic properties.

Serum lipoproteins are known to carry variable amounts of lipid peroxides. A recent study suggests that HDL particles may transport lipid oxidation products during physiological conditions (Ahotupa et al. 2010). Furthermore, there is some evidence indicating that HDL does not only remove excess cholesterol, but also actively removes lipid oxidation products (Shao & Heinecke 2009; Ahotupa et al. 2010). Regarding this transport, Christison et al. have shown that cholesteryl transfer protein can mediate the transport of LDL-associated oxidized lipids to HDL (Christison et al. 1995). Our studies have also found that under physiological oxidative stress, induced by acute physical exercise, the concentration of oxidized HDL lipids (oxHDLlipids) increases, indicating accelerated transport of noxious lipid peroxides by HDL (Figure 1). Furthermore, one recent study done with elderly women showed that a 6 months' supervised physical activity program elevated the concentration of oxHDLlipids and the ratio of oxHDLlipids : HDL cholesterol (Tiainen et al. 2016). However, there is very little data regarding to improve the transport function of lipid oxidation products by HDL. The components determining the lipid oxidation products transportation capacity of HDL needs better and deeper understanding before it is possible to discover ways to adjust this function.

Raitakari and Ahotupa (unpublished data) have taken a step to this direction by showing that oxHDLlipids concentration was highest in the very large and large HDL, and was strongly involved to phospholipid contents of HDL particles. These findings would be in line with two other studies, which have reported that phospholipid component of HDL serve as a significant modulator of reverse cholesterol transport (Cho et al. 2010; Yancey et al. 2000).

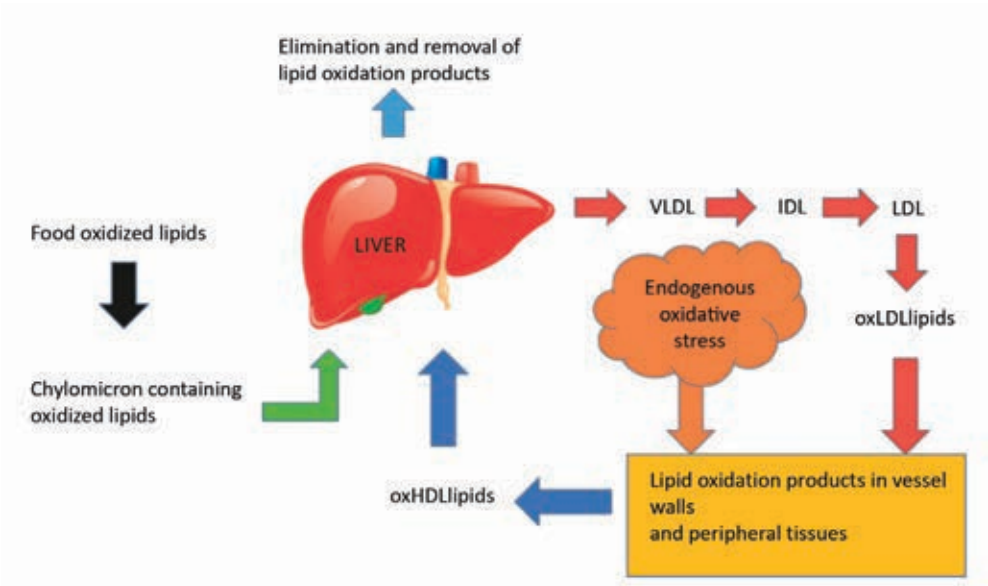

Figure 1. Graphical abstract of proposed transport of lipid oxidation products by plasma lipoproteins

2.2.3. Antioxidant system

Metabolic reactions in the body are constantly producing free radicals and non-radicals such as reactive oxygen/reactive nitrogen species, creating a pro-oxidant state. Pro-oxidants may cause lipid peroxidation, protein oxidation and oxidative deoxyribonucleid acid (DNA) damage, which can lead to cellular/tissue damage. Because of the potential of pro-oxidants to attack macromolecules, the human body creates antioxidants to balance the oxidative state. The key role of antioxidants is to inhibit the oxidation of other molecules. Antioxidant protection in the human body consists of several antioxidant mechanisms: nonenzymatic antioxidants (such as

vitamins E and C, glutathione, uric acid, albumin, bilirubin, ceruloplasmin, transferrin) and antioxidant enzymes (such as superoxide dismutases, catalase, glutathioneperoxidases, gluthathionereductase) (Halliwell 1991).

Since the antioxidant protection system of the human body consists of several mechanisms, the antioxidant capacity as a whole cannot be determined by measuring only one or a few individual antioxidants. Therefore, several methods have been generated to estimate the antioxidant capacity. The serum total peroxyl radical trapping method (TRAP) is one of the most employed methods that evaluates antioxidant capacity. The TRAP analysis involves the trapping of peroxyl radicals from serum samples *ex vivo* (Wayner et al. 1985; Lissi et al. 1992). However, serum concentrations of certain antioxidant compounds may significantly affect the serum TRAP value. In particular, the elevated serum concentration of vitamin E (α-tocopherol), which is known to be one the most powerful antioxidants in the lipid-aqueous system in vitro, raises the serum TRAP value (Vasankari et al. 1997). In addition to vitamin E, uric acid and vitamin C are also known to significantly raise TRAP values (Kaur & Halliwell 1990; Kanter et al. 1993). On the other hand, we know that acute physical exercise increases the concentration of vitamin E (Pincemail et al. 1988) and uric acid in plasma (Svensson et al. 2002). At this point, it is imperative to understand that our bodies are constantly producing ROS products that fight against pathogens, and these pro-oxidants serve as stimuli for antioxidant production (Radak et al. 2007). For example, physical exercise is a well-recognised model of oxidative stress, which increases the cellular pro-oxidant states (Sies 1997; Bloomer 2008). Exercise is also known to create a stimulus to up-regulate the endogenous antioxidant systems (Fisher-Wellman & Bloomer 2009; Radak et al. 2013).

Paraoxonases are a group of three enzymes called PON1, PON2 and PON3. These enzymes function in various biochemical pathways such as protection against lipid peroxidation and oxidative damage. Furthermore, they are known to have role in contribution of innate immunity system and detoxification of reactive molecules (Martinelli et al. 2013). The most studied enzyme of paraoxonase family is PON1 (Camps et al. 2009; Costa et al. 2005; Furlong et al. 2010; Costa et al. 2011). The synthesis of PON1 takes primarily place in the liver and is associated with HDL in serum (Mackness et al. 1991). Furthermore, small amount of PON1 is bound to VLDL and postprandial chylomicrons. PON3 also occurs in serum bounding to HDL and PON2 is located intracellularly. Because of the protective role from poisoning by

organophosphate derivates, the focus is gathered around PON1 in most of the studies. The antioxidant activity of HDL against oxidation of LDL is suggested to be due PON1 enzyme activity, therefore it has been discussed whether low serum PON1 activity is associated with coronary heart disease (Durrington et al. 2001).

2.2.3 Probiotics

Probiotics are defined as living bacteria with proven beneficial effects to human health. According to their ability to modulate metabolic and immune functions, they are presented for therapeutic, prophylactic and nutritional purposes (Scarpellini et al. 2008). These widespread beneficial effects of probiotics on human health are associated with enhancing immune response (Kawashima et al. 2011), lowering serum cholesterol (Lee et al. 2009), reducing gut inflammation (Armit-Romach et al. 2010), decreasing intestinal dysfunction and improving the immunological barrier functions of the intestine (Ohland et al. 2010). In addition, antioxidative effects have been reported in several studies (Kaizu et al. 1993; Lin & Yen, 1999; Lin & Chang, 2000; Kullisaar et al. 2002). Due to the strong evidence that chronic inflammation and oxidative stress are associated with atherosclerosis, researchers have suggested that probiotics may have potential in the prevention of atherosclerosis. Furthermore, Kullisaar et al. show that *Lactobacillus fermentum* decreases the amount of oxidized LDL particles in serum (Kullisaar et al. 2003). However, the use of probiotics together with exercise training among athletes is not well known.

2.3. EXERCISE AND OXIDATIVE STRESS

It is widely accepted that physical exercise is beneficial to health and that being physically active on a regular basis prevents and delays many diseases, especially cardiovascular disease. Many studies have indicated that physical exercise positively affects risk factors of cardiovascular disease (Jenkins 1988; Mora et al. 2007; Swift et al. 2013). However, during exhaustive physical exercise, a large amount of oxygen is inhaled into the body, and thus creates oxidative stress by increasing the cellular pro-oxidant states (Davies et al. 1982; Jenkins 1988), which in turn creates free radicals. If the cells in the antioxidant system are outstripped by free radicals, oxidative stress increases above healthy levels, causing lipid peroxidation, and protein and DNA damage. In theory, this is possible in intense and extreme exercise, even in highly

trained individuals (Radak et al. 2008; Patil et al. 2012). Radak et. al (2008) suggest that intense endurance exercise creates more free radicals than our cells can protect. However, free radical generation induced by exercise, whether it is moderate or intense, is needed to promote a beneficial effect of physical activity. Increased cellular pro-oxidant levels trigger endogenous production of antioxidants, leading to better protection against oxidative stress in the future (Knetz et al. 2006; Vollaard et al. 2005; Wagner et al. 2011).

Indeed, acute exercise challenges the body with increased reactive oxygen and nitrogen species (RONS) production, while regular exercise induces antioxidant and anti-inflammatory enhancements. In the human body, RONS are eliminated by the non-enzymatic (e.g. glutathione peroxidase, catalase, superoxide dismutase) and enzymatic (e.g. uric acid, vitamin A, vitamin C, vitamin E) antioxidant systems (Finaud et al. 2006a). Immediately after exercise, free radical formation is induced mainly by increased activity of nicotine adenine disphosphonucleotide and xanthine oxidase as well as electron leakage from mitochondria (Konig et al. 2001). During the recovery period of 2 to 72 hours after exercise, neutrophils are infiltrated into injured muscle tissue generating free radical production as part of an overall inflammatory response to exercise (Fatouros & Jamurtas 2016). Furthermore, chronically inactive people have lower levels of endogenous antioxidants (Nyberg et al. 2014). Moderate exercise training modifies the oxidative balance, leading to a better endogenous antioxidant state by decreasing basal levels of oxidative damage and by increasing resistance to oxidative stress (Urso & Clarkson 2003).

Exercise can be defined as aerobic or anaerobic exercise depending on how metabolic process occurs. Both types of exercise produce energy through glycolysis, but the substance used to break glucose to pyruvate is different. During aerobic training oxygen is catalyst used for breaking down glucose, while during anaerobic exercise body utilize a molecule, which is stored in the muscle tissue, called phosphocreatine to break down glucose. During aerobic exercise heart rate and respiration level increases. Respiration transports oxygen from the outside air to cellular tissue and carbon dioxide in the opposite direction. The energy required to perform aerobic exercise comes from carbohydrates and fats. However, during anaerobic exercise the energy sources are derived from adenosine-5 triphosphate (ATP) and creatinine phosphate.

Aerobic exercise training enhances cardiorespiratory functions. Teixeira-Lemos et al. (2011) show that aerobic exercise is capable of decreasing levels of hypoxanthine, which is the

substrate for xanthine oxidase, leading to less RONS production. On the other hand, intense and anaerobic exercise is shown to increase the levels of xanthine oxidase (Radak et al. 1995). Chronic exercise is demonstrated to optimise antioxidant environment by inhibiting the ROS formation and improving the cells' capacity to neutralise ROS.

There is evidence that endurance athletes live longer than the average life expectancy (Sanchis-Gomar et al. 2011; Teramoto et al. 2010), and studies carried out with animals have shown that exhaustive exercise enhances the antioxidant system (Goto & Radak et al. 2009; Oztasan et al. 2004). One study points to an increase of oxidative stress markers during rest in well-trained endurance athletes, but a closer look at this study reveals that participants were tested two days after a hard race, meaning that their bodies may not have had enough time to recover from this extreme oxidative stress-inducing exercise (Pittaluga et al. 2006).

Exercise and oxidative stress are strongly linked. Regular moderate exercise is needed to get the beneficial effects on health. If one only exercises sporadically, it is possible that it leaves the body susceptible to oxidative stress because of the lack of adaptation mechanism induced by regular exercise (Wagner et al. 2011). Intense and extreme exercises can overwhelm the antioxidant protection system against free radicals, but one's exercise history and fitness levels would be determinant of cellular capacity to decrease oxidative damage. The big question is: how much exercise is too much? Eijsvogels et al. have investigated this question in their study (Eijsvogels et al. 2016).

3. AIMS OF THE STUDY

High-intensity acute exercise is a well-known producer of oxidative stress (Finaud 2006b) and may lead to lipid peroxidation and the damage of proteins and nucleic acids (Sies 1985; Vasankari 1995). On the other hand, we recognise the beneficial effects of exercise to health, especially affecting the risk factors of cardiovascular disease (Kontush 2003: Vasankari 1998 & 2000). Furthermore, higher levels of oxidized LDL particles are related to a higher risk for atherosclerosis. HDL particles seem to have a protective role, but the role of oxidized HDL is not yet fully understood.

The aim of this study is to investigate how acute and longitudinal exercises affect cardiovascular risk factors and antioxidant capacity.

The specific aims were:

I. To investigate the long-term effects of anaerobic and aerobic type of exercise training (ice hockey vs. marathon runners) on lipid peroxidation (diene conjugation) and antioxidant function;

II. To study 1) the response of a 3-month training period, a 6-day preparation period and marathon run on the concentration of oxidized LDL lipids and antioxidant capacity, and 2) how LGG-treatment may alter the above-mentioned physiological response;

III. To study the link between endogenous oxidative stress, induced by acute exhaustive physical exercise, and oxidized HDL lipids.

IV. To investigate how the physiological responses of various types of acute exercise in athletes, who are familiar with different type of training, affect oxidative stress and the removal of lipid peroxides by HDL particles.

4. MATERIALS AND METHODS

4.1. SUBJECTS AND STUDY DESIGN

Subjects were gathered from three different sets of data. The total number of subjects was 223, of which 188 were endurance runners and 35 were ice hockey players. The participants ranged in age from 20–61 years old.

4.1.1. Longitudinal study of endurance runners and ice hockey players

Twenty-three male national top level endurance runners and 35 male ice hockey players of the Finnish men's national under-20 team were analysed for this study. Athletes' basic characteristics are summarised in Table 1 below.

Data was collected from participants at three different time points during one season. The three data collection points were chosen based on the training intensity of each sport. Endurance runners were tested in autumn–winter (low-intensity training season), in spring (preparation period before competition season, combination of low-intensity and interval training) and in summer (competition season with high-intensity training). Correspondingly, ice hockey players were tested in spring (training season with mainly low-intensity training), in summer (training season with both low and high-intensity training) and in winter (competition season with high-intensity). The diets of the participants were standardised at the training camp for the two days preceding a test day. On the test day, fasting (12h) blood samples were taken from an antecubital vein at 7 o'clock. At the start of this study, all participants completed a physical performance test to determine maximal oxygen uptake; this test is characterised later in the text (see 4.2, treadmill tests). The following indicators were analysed from the subjects' blood samples at each time point: serum diene conjugation (DC), serum antioxidant capacity (TRAP) and serum vitamins (α-tocopherol, γ-tocopherol, retinol, β-carotene and ubiquinone-10).

Table 1. Basic characteristics of the subjects. Mean (±SD).

	Runners	Ice hockey players
Age	24.6 ± 4.6	< 20
Height (cm)	179.1 ± 5.7	184.0 ± 4.9
Weight (kg)	65.7 ± 6.2	85.7 ± 8.2
VO_{2max} (mL/kg/min)	73.6 ± 5.7	53.0 ± 3.4
BMI (kg/m^2)	18.8 ± 1.8	25.3 ± 2.1

From the original publication number I.

4.1.2. Three months' study of marathon runners including training period, preparation period and marathon run

In this project, we examined keep-fit runners among those who planned to participate in the Helsinki City Marathon. Subjects were recruited through an advertisement placed in a national runners' magazine and using a recruitment letter sent to previous Helsinki City Marathon participants. If subjects were healthy, were not participating in any other study, and their personal best marathon time was less than 3 hours and 45 minutes for women and less than 3 hours and 30 minutes for men, they were suitable for this study. Potential participants were excluded if they had taken antibiotics within the two months before the study, if they had had an acute gastrointestinal disorder within the two months prior to the study, if they had any gastrointestinal diseases or took any related medication, or if they were pregnant or lactating. A total of 144 subjects were enlisted in the study, of whom 141 (16 females, 125 males) were randomized. The Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the study protocol. The baseline characteristics of the subjects are shown in Table 2.

For this parallel group intervention study, the subjects were randomised, double-blinded and placebo-controlled. Prior to the intervention period, there was a four-week run-in period in April, after which the participants were randomly assigned to take either *Lactobacillus rhamnosus* GG (LGG) or a placebo. The subjects received LGG or the placebo for the duration of the training period (three months), from beginning of May until the day of Helsinki City Marathon (2nd August). Fasting venous blood samples were taken from an antecubital vein at four different time points during the project from both of the groups: 96 days before the marathon run, six days preparative period before the marathon run, on the marathon day two to four hours before the run, and immediately after the run. Subjects were allowed to ingest food and fluids freely during the marathon run. The following indicators were analysed from the

subjects' blood samples from each time point: ox-LDL, S-TRAP, S-α-tocopherol, S-γ-tocopherol, S-retinol, S-β-carotene and S-ubiquinone-10.

Table 2. Basic characteristics of the subjects. Number or mean (range).

	Placebo		LGG
Age	40 (23–69)		40 (22–58)
Female	8		8
Male	63		62
Weight (kg)	69 (51-88)		71 (49-98)
Height (cm)	178	(158-190)	177 (160-200)
BMI (kg/m^2)	22 (19-26)		22 (18-26)
Use of nutritional supplements	34		41
Exercise habits			
Years of marathon training	7 (0-35)		8 (0-30)
Marathons participated in	11 (0-60)		13 (0-71)
Best marathon time (h:min)	3:11	(2:23-3:40)	3:10 (2:35-3:42)

From the original publication number II.

4.1.3. Effect of acute strenuous physical exercise on oxHDL and oxLDLlipids in endurance runners

In this study, we investigated 24 male endurance runners at the top level nationally (12 marathon and 12 middle-distance runners). The basic characteristics of the participants are presented in Table 3.

The athletes performed a velocity-incremented tread mill run (Telineyhtymä, Kotka, Finland), at a slope of one, until exhaustion. The duration of each stage of the test was two minutes; the initial 2-minute stage was run at a velocity of 10 km · h^{-1}, and thereafter the velocity was increased by 1 km · h^{-1} for each consecutive stage until exhaustion. The whole duration of the treadmill run was 20–22 minutes, of which the last six to eight minutes were performed in anaerobic level. Venous blood samples were taken at four different time points during the trial:

before, immediately after and 15 minutes and 90 minutes after the run. Participants consumed a standardised light breakfast three hours before the exercise, on the test day. From the blood samples. we analysed concentrations of oxHDLlipids and oxLDLlipids, serum LDL and HDL cholesterol, serum antioxidant potential (TRAP), paraoxonase activity and malondialdehyde (MDA). The study protocol was approved by a local ethics committee (Hospital District of Southwest Finland: 91/1801/2015) and was carried out in accordance to the Declaration of Helsinki on the use of human subjects.

Table 3. The basic characteristics of the participants. Mean (±SD)

	Middle-distance runners	**Marathon runners**
Age	21.3 (2.3)	27.3 (4.4)
BMI (kg/m^2)	21.2 (1.8)	20.5 (1.5)
Training (km/year)	4189 (1097)	6333 (854)
VO_{2max} (mL/kg/min)	72.3 (6.9)	76.6 (4.7)
V_{max} (km/h)	19.0 (1.4)	20.2 (0.8)
Time to exhaustion (min)	20 (2.8)	22.3 (1.7)
Season best 800m (min:s)	01:53 (00:03)	
Season best 42.2 km (h:min:s)		2:21:17 (0:04:58)

From the original publication number III

4.1.4. The acute effect of different intensity of exercises on oxHDLlipids and oxLDLlipids

Twenty-three voluntarily participating male endurance runners at the top level, nationally, (12 middle-distance and 11 marathon runners) were recruited for this study. The basic characteristics of the subjects are presented in Table 3.

Two different non-exhaustive treadmill runs: a 40-minute intermittent run (two minutes of running and two minutes of rest, during which slow walking beside the treadmill was allowed) at a velocity corresponding to 100% maximal oxygen uptake (VO_{2max}) and a 40-minute continuous run at a velocity corresponding to 80% VO_{2max}, were completed by all athletes. The order of the running tests was randomly assigned.

Determination of the maximal oxygen uptake took place two weeks prior to the first performance. Venous blood samples were taken at four different time points: before and after 20 and 40 minutes of exercising and 15 minutes and 90 minutes after exercising had ended. A light standardised breakfast was consumed three hours before the exercise. From the blood samples, we analysed concentrations of oxHDLlipids and oxLDLlipids, serum LDL and HDL cholesterol, serum antioxidant potential (TRAP), paraoxonase activity and malondialdehyde (MDA). The study protocol was approved by a local ethical committee (Hospital District of Southwest Finland: 91/1801/2015) and was performed in accordance to the Declaration of Helsinki on the use of human subjects.

4.2. DETERMINATION OF MAXIMAL OXYGEN UPTAKE

The maximal oxygen uptake was defined during the incremental VO_{2max} test by an automated Oxygon Sigma gas analyser (Mijnhard, the Netherlands), which was calibrated before each test in line with the manufacturer's instructions. The calibration was regularly controlled after the tests. Breath-by-breath metabolic data were averaged to 30-second intervals. VO_{2max} was defined as the highest 60-second oxygen uptake (VO_2)during the test. V_{max} was the treadmill velocity at which the subject first attained VO_{2max} (Billat et al. 1995). This was either the velocity of the last 2-minute stage or the previous 2-minute velocity, if VO_2 was higher at this stage. If the value of VO_2 at the two last stages was the same, the mean of these two velocities was approved as V_{max} (Lindsay et al. 1996). Billat et al. (1994 & 1996) has been confirmed the authenticity of this protocol.

4.3. LABORATORY ANALYSIS

4.3.1. Determination of oxidized lipoprotein lipids

The analysis of lipoprotein oxidized lipids was derived from determining the baseline level of conjugated dienes in lipoprotein lipids (Ahotupa et al. 1998). The appearance of conjugated dienes has been commonly used as the index of oxidation in *in vitro* and *ex vivo* studies on LDL oxidation. Serum LDL was isolated by precipitation with buffered heparin (Ahotupa et al. 1998). Isolation of the HDL fraction from serum samples was based on phosphotungstic acid precipitation (Väisänen et al. 1992). Serum samples (to which 1 mg/mL of EDTA were added)

and precipitation reagents were allowed to equilibrate to room temperature, before the isolation of serum lipoproteins. The isolation procedures were validated for the purpose, and did not have an effect on the level of oxidized lipids (Ahotupa et al. 1998). Lipids were extracted from isolated lipoprotein samples (100 μL) using chloroform-methanol (2:1), dried under nitrogen, then redissolved in cyclohexane (1 mL), and measured spectrophotometrically at 234 nm. Absorbance units (difference A234–A300) were transformed to molar units utilising the molar extinction coefficient 2.95 x 10^4 M^{-1} cm^{-1}. 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. In addition to the specific absorption spectra at 234 nm, the presence of conjugated dienes has been verified by nuclear magnetic resonance studies (Vasankari et al. 2001). The coefficient of variation (CV) for within-assay precision for determination of oxidized lipoprotein lipids was 4.4%, and the CV for the between-assay precision was 4.5%.

A model based on Cu^{++}-induced oxidation of HDL and LDL *in vitro* was used to investigate the oxidation resistance of lipoproteins. Lipoproteins were isolated, as mentioned above. Lipoprotein concentrations in the incubations corresponded to 0.1 mL of serum/mL of the incubation volume. Oxidation of lipoprotein lipids was monitored by the formation of conjugated dienes, as described by Esterbauer et al. (1992).

4.3.2. Other analytical methods

The serum total peroxyl radical trapping antioxidant potential (TRAP) was estimated *ex vivo* by the potency of serum samples to resist ABAP-induced peroxidation (Ahotupa et al. 1996). Peroxyl radical trapping potential was assayed briefly as follows: 0.45 mL of 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9% NaCl, 0.02 mL of 120 mM linoleic acid, 0.05 mL of luminol (0.5 mg/mL), and the serum samples were compound in a cuvette. The assay was initiated by 0.05 mL of ABAP (83 mg/mL). Chemiluminescence in triplicate cuvettes at 37° was measured until a peak value for each sample was detected. The half-peak time point was used to define peroxyl radical trapping capacity. Trolox served as a standard radical scavenger. Paraoxonase activity was determined using paraoxon (O,O-diethyl-O-p nitrophenylphosphate) as the substrate and measuring the increase in absorbance, due to the formation of 4-nitrophenol, at 412 nm (Harangi et al. 2004). Measurements of serum LDL cholesterol and HDL cholesterol were based on standard enzymatic methods and completed with commercial

analytical kits (Roche Diagnostics, Mannhein, Germany). The concentration of HDL cholesterol was measured after phosphotungstic acid precipitation. The malondialdehyde concentration was measured as serum total (free and protein-bound) malondialdehyde as the 2,4-dinitrophenylhydrazine derivative by high-performance liquid chromatography (HPLC) with 1,1,3,3-tetraethoxypropane as the standard (Pilz et al. 2000). The HPLC analyses were performed with a Shimadzu 10ADVP. A Luna 3 μm reversed-phase column, 150 x 4.6 mm, was used, and the detection was based on a UV detector operating at 307 nm. The eluent consisted of acetonitrile (55%), H_2O (44.8%) and acetic acid (0.2%), and the flow rate was 1.2 mL/min. Serum α-tocopherol, γ -tocopherol, β-carotene, retinol and ubiquinol-10 concentrations were analysed using standard HPLC procedures with UV-detection (Milne et al. 1986; Takada et al. 1985).

4.4. STATISTICAL ANALYSES

All statistical analyses were performed with SPSS for Windows Statistical Software (version 15.0 and 18.0). The normality of the distribution was calculated by using the normality tests of SPSS software. If the data was not normally distributed, we used ln-transformation at each time point (see detailed data from original publications). General linear model analysis of variance (ANOVA) with repeated measurements was used in each study for all measured variables to analyse changes over time. Only if the time effect was significant, the paired t-test was used as a post hog test, applying a Bonferroni correction. In the cases of significant group effect differences between the groups, analyses were carried out using independent samples' t-tests between the groups using the corresponding time points of each study. In studies III-IV, if no interaction for time x group was detected between the marathon and middle-distance runners, the groups were combined and time effect was tested for all runners together. The Pearson correlation coefficient was used to define the relationships between variables. An a priori *P*-value for statistical significance of 0.05 was used. The concentrations are expressed as means ± SD.

5. RESULTS

5.1. LONGITUDINAL STUDY OF ENDURANCE RUNNERS AND ICE HOCKEY PLAYERS (STUDY I)

5.1.1. Endurance runners

During the season, S-γ-tocopherol was the only variable that changed statistically. It increased from training season to competition season by 28% ($p < 0.02$). A similar increase was found from preparation period to competition season ($p < 0.05$), because S-γ-tocopherol remained unchanged from training season to preparation period. Other measured valuables did not change during the season. All results are shown in Table 4.

Table 4. Blood serum values at three different time points during the season for runners and ice hockey players (mean ± SD)

	T1	T2	T3
DC (µmol/L)			
Runners	43.9 ± 12.8[a]	44.9 ± 13.4[a]	39.9 ± 8.4[a]
Ice hockey players	63.9 ± 9.0	67.1 ± 10.8	61.1 ± 8.1
TRAP (µmol/L)			
Runners	1001 ± 176	961 ± 132[a]	1005 ± 213
Ice hockey players	994 ± 111	1048 ± 128[b]	1086 ± 134[b]
α-tocopherol (µmol/L)			
Runners	36.0 ± 5.7[a]	36.8 ± 9.8	35.1 ± 8.6[a]
Ice hockey players	32.1 ± 7.5	33.0 ± 7.7	30.0 ± 6.8[c]
γ-tocopherol (µmol/L)			
Runners	0.49 ± 0.29	0.49 ± 0.29[a]	0.63 ± 0.39[b, c]
Ice hockey players	0.64 ± 0.33	0.70 ± 0.30	0.64 ± 0.21
Retinol (µmol/L)			
Runners	4.88 ± 1.03	4.44 ± 0.89[a]	5.17 ± 1.47
Ice hockey players	4.77 ± 0.84	4.89 ± 0.71	4.66 ± 0.67
β-carotene (µmol/L)			
Runners	28.7 ± 14.1	30.7 ± 15.3[a]	40.2 ± 21.0[a]
Ice hockey players	22.7 ± 11.5	22.0 ± 10.4	27.5 ± 12.8 [b, c]
Ubiquinone-10 (µmol/L)			
Runners	1.60 ± 0.74	1.32 ± 0.69	1.32 ± 0.72
Ice hockey players	1.32 ± 0.30	1.29 ± 0.24	1.18 ± 0.29 [b, c]

[a] Statistical difference ($P < 0.05$) between runners and ice hockey players.
[b] Statistical difference ($P < 0.05$) from T1.
[c] Statistical difference ($P < 0.05$) from T2.
T1=low-intensity training period. T2=medium intensity training period. T3=high-intensity training period.
From original publication number I

5.1.2. Ice hockey players

In each ice hockey group, there were significant changes from low-intensity training season to competition season in S-TRAP (+9%, $p < 0.001$), S-β-carotene (+21%, $p < 0.05$) and S-ubiquinone-10 (-11%, $p < 0.001$). When comparing preparation season (low and high-intensity training) to competition season S-α-tocopherol decreased by 9% ($p < 0.05$), S-β-carotene increased by 25% ($p < 0.02$) and S-ubiquinone-10 decreased by 9% ($p < 0.05$). All results are show in Table 4.

5.1.3. Runners vs. ice hockey players

Considering serum diene conjugation levels (S-DC), both groups behaved similarly during the season, but at each time point from the training season to the competition season the serum concentration of DC was 45–53% higher in the ice hockey players (Table 4).

5.2. THREE MONTHS' STUDY OF MARATHON RUNNERS INCLUDING TRAINING PERIOD, PREPARATION PERIOD AND MARATHON RUN (STUDY II)

5.2.1. Aerobic training and oxidized LDL lipids levels

In this study, we examined the effects of longitudinal aerobic training and LGG treatment on antioxidant functions and oxidized LDL lipids. The LGG treatment had no effect on ox-LDL levels, concentrations of serum TRAP or vitamins, compared with the placebo group during the whole study. More interestingly, during the 6-day preparative period, the concentrations of ox-LDL increased by 28% in the placebo group and by 33% in the LGG group (both $p < 0.001$). During the marathon run, ox-LDL levels decreased by 16% and 19% in the placebo group and the LGG group, respectively (both $p < 0.001$). The marathon run also affected serum concentration of TRAP; there was a 16% increase in both the placebo and the LGG group ($p < 0.0001$ both). Another interesting result was the decrease of β-carotene levels during the 6-day preparative period. In the placebo group, the decrease was 42%, and in the LGG group, 43% (both $p < 0.001$). All results are shown in Figures 2 and 3 and in Table 5.

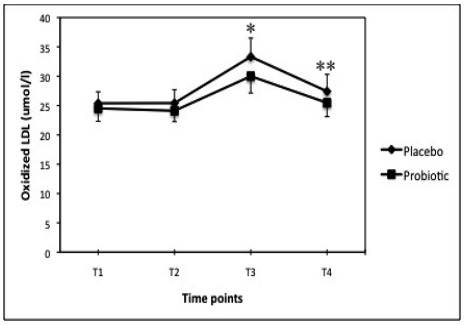

Figure 2. Concentration of serum oxidized LDL in probiotic and placebo groups (mean ± SD).

* Statistical difference within placebo/probiotic group from T2 ($p < 0.001$)
** Statistical difference within placebo/probiotic group from T3 ($p < 0.001$)
From original publication number II.

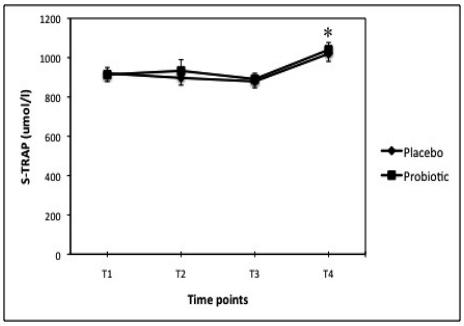

Figure 3. Concentration of serum TRAP in probiotic and placebo groups (mean ± SD).

* Statistical difference within placebo/probiotic group from T3 ($p < 0.0001$)
From original publication number II.

Table 5. Laboratory values before 90-day training period (T1) and after 90-day training period (T2), before marathon run (T3) and after marathon run (T4) in the placebo and probiotic (*Lactobacillus rhamnosus* GG) groups (mean ± SD).

	T1	T2	T3	T4
Serum γ-tocopherol (µmol/L)				
Placebo	0.48 ± 0.35	0.30 ± 0.28[a]	0.38 ± 0.31	0.47 ± 0.30[b]
Probiotic	0.45 ± 0.30	0.28 ± 0.23[a]	0.36 ± 0.36	0.36 ± 0.27
Serum α-tocopherol (µmol/L)				
Placebo	23.6 ± 6.2	24.9 ± 6.6	21.5 ± 4.1[b]	22.2 ± 4.8[b]
Probiotic	22.2 ± 5.9	23.8 ± 6.4	20.6 ± 4.7[b]	20.9 ± 4.6[b]
Serum β-carotene (µmol/L)				
Placebo	34.9 ± 23.7	38.7 ± 28.9	22.8 ± 18.9[b]	16.7 ± 12.1[b]
Probiotic	30.6 ± 20.3	35.6 ± 23.0	20.4 ± 16.5[b]	15.6 ± 10.4[b]
Serum ubiquinone-10 (µmol/L)				
Placebo	1.85 ± 0.73	2.13 ± 0.77	1.75 ± 0.55[b]	2.26 ± 0.70[a, c]
Probiotic	1.97 ± 0.83	2.33 ± 0.85[a]	1.73 ± 0.55[b]	2.14 ± 0.66[c]
Serum retinol (µmol/L)				
Placebo	5.34 ± 1.35	5.32 ± 1.34	5.30 ± 1.28	5.68 ± 1.46
Probiotic	5.34 ± 1.41	5.60 ± 1.57	5.55 ± 1.28	5.71 ± 1.47

[a] Statistical difference within placebo/probiotic group from T1 ($P < 0.05$)
[b] Statistical difference within placebo/probiotic group from T2 ($P < 0.05$)
[c] Statistical difference within placebo/probiotic group from T3 ($P < 0.05$)
From original publication number III.

5.3. EFFECT OF ACUTE STRENUOUS PHYSICAL EXERCISE ON oxHDL AND oxLDLlipids IN ENDURANCE RUNNERS (STUDY III)

5.3.1. Treadmill run accelerates transport of lipid peroxides by HDL

Marathon runners and middle-distance runners behaved similarly during this study, and there were no significant time x group interactions in any measured variables. However, regarding time many interesting results were found, especially relating to how the concentrations of oxidized lipoprotein lipids behaved. Immediately after the treadmill run, oxHDLlipids levels increased by 24% ($p < 0.01$) and remained elevated during 15-minute recovery follow-up (Figure 4). Contrarily, during the treadmill run, the concentration of oxLDLlipids decreased by

19% ($p < 0.001$) and remained decreased during the follow-up period (Figure 5). Most interestingly, the ratio of oxHDLlipids to oxLDLlipids elevated by 55% immediately after the treadmill run and 71% after the 15minute recovery period, compared to pre-exercise levels (both $p < 0.001$) (Table 6).

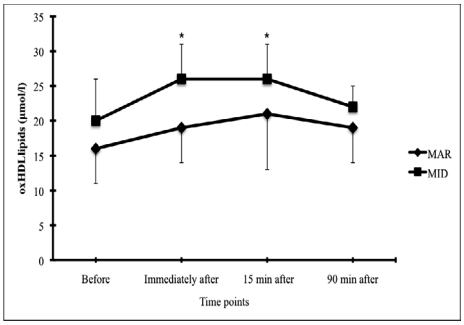

Figure 4. Serum concentration of oxidized HDL lipids before, immediately, 15 minutes and 90 minutes after the treadmill run. The results are mean ± SD.

The asterisks (*) indicate a significant difference ($p < 0.01$) compared to pre-exercise levels when data groups are combined. Between group effect, the *P*-value is 0.055.
From original publication number III.

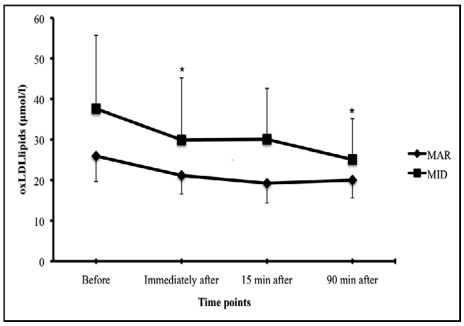

Figure 5. Serum concentration of oxidized LDL lipids before, immediately, 15 minutes and 90 minutes after the treadmill run. The results are mean ± SD.

The asterisks (*) indicate a significant difference ($p < 0.001$) compared to pre-exercise levels when data groups are combined. Between group effect, *P*-value is 0.004.
From original publication number III.

Table 6. Laboratory values from middle-distance runners and marathon runners separately and combined for each time point (mean ± SD)

	Before	Immediately after	15 min after	90 min after
LDL cholesterol (mmol/L)	2.28 (0.6)	2.41 (0.6)***	2.40 (0.7)	2.24 (0.6)^{bb}
Mar	2.20 (0.6)	2.26 (0.6)	2.19 (0.6)	2.15 (0.6)
Mid	2.42 (0.6)	2.59 (0.6)	2.69 (0.7)	2.34 (0.7)
HDL cholesterol (mmol/L)	1.31 (0.3)	1.48 (0.3)**	1.44 (0.3)*	1.42 (0.3)^{bb}
Mar	1.38 (0.3)	1.48 (0.3)	1.50 (0.3)	1.45 (0.3)
Mid	1.24 (0.3)	1.45 (0.3)	1.33 (0.2)	1.44 (0.3)
Malondialdehyde (μmol/L)	1.80 (0.9)	2.82 (1.7)**	2.75 (1.8)**	1.90 (0.9)$^{b, aa}$
Mar	1.96 (1.1)	3.17 (2.0)	3.18 (2.1)	1.90 (0.8)
Mid	1.54 (0.5)	2.23 (1.1)	2.25 (1.2)	1.76 (1.0)
Paraoxonase (U/L)	122 (59)	126 (65)	137 (64)**	121 (60)^{a}
Mar	127 (59)	125 (59)	135 (63)	123 (57)
Mid	124 (61)	135 (70)	140 (64)	130 (75)
TRAP (μmol/L)	956 (160)	1235 (230)*	1251 (220)*	1335 (250)$^{*, b, aa}$
Mar	877 (104)	1116 (184)	1128 (156)	1205 (183)
Mid	1092 (137)	1440 (137)	1461 (138)	1558 (191)
OxLDLlipids/LDL cholesterol	14.1 (6.0)	10.4 (2.9)**	10.1 (3.4)*	10.2 (2.6)**
Mar	12.1 (2.5)	9.5 (1.9)	9.0 (2.1)	9.5 (1.3)
Mid	16.0 (7.4)	11.2 (3.4)	11.4 (4.1)	10.8 (3.1)
OxHDLlipids/HDL cholesterol	14.4 (5.4)	15.5 (4.7)	17.4 (5.9)	16.4 (5.3)
Mar	12.2 (4.0)	13.6 (4.0)	14.5 (5.2)	14.0 (4.4)
Mid	18.1 (5.8)	18.1 (4.3)	19.8 (5.2)	18.2 (4.9)
OxHDLlipids/ OxLDLlipids	0.63 (0.3)	1.00 (0.4)*	1.08 (0.5)*	1.00 (0.3)*
Mar	0.66 (0.3)	1.00 (0.4)	1.20 (0.5)	1.00 (0.4)
Mid	0.59 (0.2)	1.00 (0.4)	1.00 (0.4)	1.00 (0.3)

Significantly different from before run: * $p < 0.001$; ** $0.001 < p < 0.01$;
*** $0.01 < p < 0.05$
Significantly different from immediately after run: ^{b} $0.001 < p < 0.01$; ^{bb} $0.01 < p < 0.05$
Significantly different from 15 minutes after run: ^{a} $p < 0.001$; ^{aa} $0.01 < p < 0 < 0.05$
For some further information, see original publication number III.

5.3.2. Antioxidant vs oxidant status

The serum concentration of MDA by 54% ($p < 0.001$) during the treadmill run, while no affect was observed in paraoxonase activity. After the 15-minute follow-up period, the paraoxonase activity increased slightly, by 12%, compared to pre-exercise levels, but decreased back to pre-exercise levels after the 90minute recovery period. Also, the serum concentration of MDA decreased back to pre-exercise levels without further increase during the 15-minute recovery. Antioxidant parameter serum TRAP concentration elevated immediately after the treadmill run by 29% ($p < 0.01$) and kept the elevated levels during the 90-minute follow-up period ($p < 0.01$). All results of measured variables are shown in Table 6.

5.3.3. Correlations

The change in oxLDLlipids (from pre-exercise value to immediately after value) correlated positively with VO_{2max} ($r = 0.67$, $p < 0.001$) and negatively with the change in paraoxonase activity ($r = -0.47$, $p < 0.05$). At baseline, the concentration of serum TRAP correlated positively with BMI ($r = 0.56$, $p < 0.05$). Furthermore, before exercise serum levels of oxLDLlipids correlated negatively with VO_{2max} ($r = -0.50$, $p < 0.05$) and HDL cholesterol ($r = -0.44$, $p < 0.05$).

5.4. THE ACUTE EFFECT OF DIFFERENT INTENSITIES OF EXERCISES ON oxHDL AND OXLDLlipids (STUDY IV)

5.4.1. Time x group interaction

The concentration of oxLDLlipids decreased by 17% in the middle-distance runners and by 4% in the marathon runners, from pre-exercise to 90 minutes after exercise levels (difference of change, $P < 0.01$) during the continuous run. Among the middle-distance runners, the ratio of oxLDLlipids to LDL cholesterol decreased by 9%, while among the marathon runners, it increased by 4% after 40 minutes of exercising compared to before exercise levels ($P < 0.05$). There was a 15% increase in the serum concentration of TRAP from pre-exercise levels to after 20 minutes of exercising among the middle-distance runners, while it increased 3% among marathon runners ($P < 0.01$) (Figure 7-8).

During the intermittent run, the concentration of oxHDLlipids increased by 12% among the marathon runners and by 25% among the middle-distance runners from pre-exercise levels to after 20 minutes of exercising (difference of change, P <0.05). All results are shown in Figures 6–9.

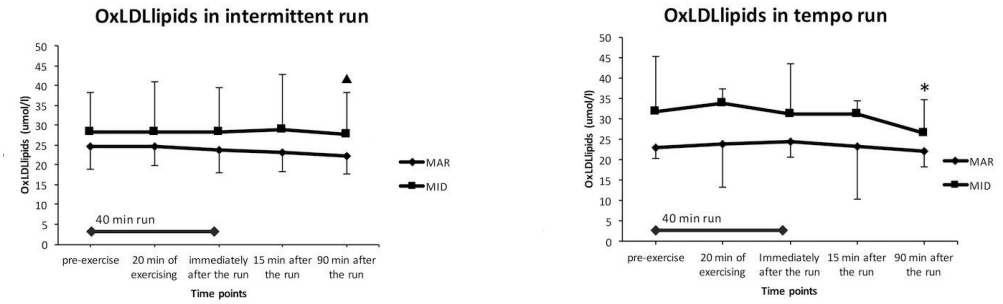

Figure 6. Serum concentration of oxidized LDL. The results are mean ± SD.

The asterisk (*) indicates a significant difference (P <0.01) between groups from pre-exercise levels. The asterisk ▲ indicates significant difference (P < 0.05) compared to levels after 40 minutes of exercising when groups are combined.
Modified from original publication number IV.

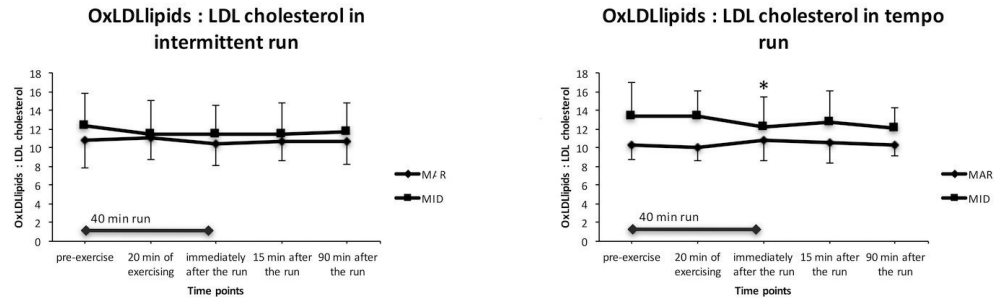

Figure 7. Serum concentration of the ratio of oxidized LDL lipids to LDL cholesterol. The results are mean ± SD.

The asterisk (*) indicates a significant difference (P <0.05) between groups from pre-exercise levels.
Modified from original publication number IV.

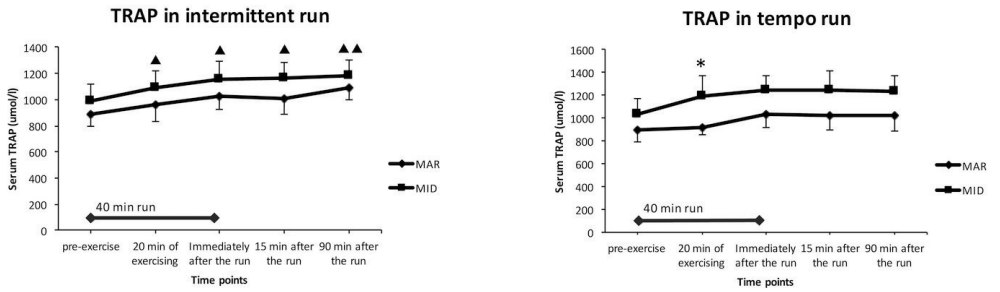

Figure 8. Serum concentration of TRAP.The results are mean ± SD.

The asterisk (*) indicates a significant difference (P <0.01) between groups from pre-exercise levels. The asterisk ▲ indicate significant difference (P <0.001) compared to pre-exercise levels, and the asterisk ▲▲ indicates significant difference (P <0.05) compared to levels after 40 minutes of exercising when groups are combined.
Modified from original publication number IV.

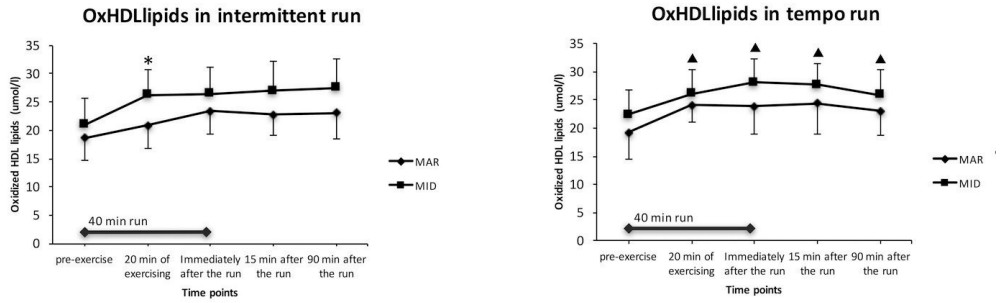

Figure 9. Serum concentration of oxidized HDL lipids. The results are mean ± SD.

The asterisk (*) indicates significant difference of change (P <0.05) between groups from before exercise levels. The asterisks ▲ indicate a significant difference (P < 0.001) compared to pre-exercise levels when groups are combined.
Modified from original publication number IV.

5.4.2. Time effect (marathon and middle-distance runner groups combined)

During the continuous run, a 25% increase was observed in the levels of oxHDLlipids, and these levels remained elevated during the 90-minute recovery ($p < 0.001$) (Figure 6). From pre-exercise levels to 15 minutes after the end of the exercise, a slight increase of 6% took place in HDL cholesterol levels ($p < 0.05$), the ratio of oxHDLlipids to HDL cholesterol rose by 20%

($p < 0.001$), and the ratio of oxLDLlipids to oxHDLlipids decreased by 25% ($p < 0.001$) during the intermittent run. At the same time as the continuous run, HDL cholesterol levels increased by 8% ($p < 0.001$) and during the 90-minute recovery follow-up, the concentration decreased back to pre-exercise levels ($P < 0.01$), the ratio of oxHDLlipids to HDL cholesterol increased by 17% ($p < 0.001$), and the ratio of oxLDLlipids to oxHDLlipids decreased by 20% ($p < 0.001$) (Table 7). The concentration of oxLDLlipids did not change during the intermittent run, but during the 90-minute recovery period, there was slight ($p < 0.05$) decrease of 4%, compared to after the 40-minute trial levels (Figure 3). All other measured variables are also shown in Table 7.

Table 7. Laboratory values in every time point (mean ± SD)

	Before	After 20 min of exercising	After 40 min of exercising	15 min after the end of exercising	90 min after the end of exercising
HDL cholesterol (mmol/L)					
Continuous run	1.37 (0.3)	1.45 (0.4)	1.48 (0.4)*	1.43 (0.4)	1.39 (0.3)^{bb}
Intermittent run	1.37 (0.4)	1.42 (0.4)*	1.41 (0.4)	1.45 (0.4)***	1.41 (0.4)
Paraoxonase (U/L)					
Continuous run	126 (61)	133 (56)	136 (67)*	126 (62)	122 (57)^{b}
Intermittent run	122 (63)	131 (65)	129 (60)	128 (63)	130 (60)
Malondialdehyde (µmol/L)					
Continuous run	1.69 (0.8)	2.11 (1.0)**	2.24 (1.1)**	2.14 (1.1)**	1.91 (1.0)
Intermittent run	1.94 (1.1)	2.33 (1.2)	2.53 (1.4)***	2.39 (1.2)***	2.03 (0.9)
OxHDLlipids:HDL cholesterol					
Continuous run	15.6 (3.6)	23.5 (5.0)*	18.2 (4.4)*,^{aa}	18.9 (4.3)*, ^{aa}	18.3 (4.4)**,^{aa}
Intermittent run	14.9 (3.2)	22.4 (4.8)*	18.7 (4.8)*	18.1 (4.8)*, ^{aa}	18.7 (4.4)*, ^{aa}
OxLDLlipids:OxHDLlipids					
Continuous run	1.36 (0.4)	1.16(0.3)***	1.09 (0.3)*	1.05 (0.3)*	1.01 (0.2)*, ^{aa}
Intermittent run	1.41 (0.5)	1.15(0.4)***	1.06 (0.3)**	1.05 (0.3)*	1.02 (0.3)*, ^{aa}

Significantly different from before run: * $p < 0.001$; ** $0.001 < p < 0.01$;
*** $0.01 < p < 0.05$
Significantly different from halfway run: ^{a} $p < 0.001$; ^{aa} $0.001 < p < 0 < 0.05$
Significantly different from immediately after run: ^{b} $0.001 < p < 0.01$; ^{bb} $0.01 < p$ 0.05
From original publication number IV

5.4.3. Correlations

The change in MDA (from the pre-exercise value to the value after 20 minutes of exercising) correlated negatively with the change in TRAP (r = -0.447, $P < 0.05$) and positively with VO_{2max} (r = 0.462, $P < 0.05$) in the intermittent run. On the other hand, during the continuous run, the change in MDA (from the value in the middle of the run to the value after 40 minutes of exercising) correlated positively with the change in paraoxonase (r = 0.537 $P < 0.02$), and the change in TRAP (from the pre-exercise value to the value after 20 minutes of exercising) correlated negatively with the training volume (r = -0.448, $P < 0.05$). Furthermore, the change in TRAP (from the pre-exercise value to the value after 20 minutes of exercising) correlated negatively with VO_{2max} (r = -0.494, $P < 0.02$).

6. DISCUSSION

This thesis gives further evidence that aerobic exercise training protects LDL from oxidation and potentially lowers the risk of atherosclerosis. Higher levels of serum DC was found in athletes who preferred anaerobic training in their sports. On the other hand, even a 6-day period without this protective aerobic training remarkably increased oxLDLlipis levels. The two latter studies shed more light on the role of HDL in preventing atherosclerosis. HDL particles seem to have the capacity to remove oxidation products from the periphery to the liver. Even more interestingly, this clearing reverse transport mechanism of HDL was induced and increased by different types of acute exercises, giving more proof of the beneficial health influence of exercise.

6.1. LONGITUDINAL STUDY OF ENDURANCE RUNNERS AND ICE HOCKEY PLAYERS (STUDY I)

Oxidative stress induced by reactive oxygen species (ROS) occurs during intense physical activity, perhaps as a result of increased consumption of oxygen. Previous studies have shown that both exhaustive sprint training and long-duration strenuous exercising may overcome an individual's natural defence system, leading to oxidative stress (Finaud et al. 2006b; Marzatico et al. 1997; Mishchenko et al. 1985; Vasankari et al. 1997). Even so, longitudinal effects of various types of training have not been fully investigated. The aim of Study I was to show how different types of physical exercise among ice hockey players and endurance runners effect lipid peroxidation and antioxidant status.

Study I demonstrated that athletes who perform more continuous and low-intensity types of training have lower levels of lipid peroxidation measured by diene conjugation than athletes who perform high-intensity types of training. Long-distance runners had 46-54% higher levels throughout the whole study, compared to ice hockey players, while no differences were found in antioxidant capacity.

During any type of exercise, increased oxygen utilization and oxidative stress increases leading to production of ROS. The body's natural antioxidant system should detoxify reactive oxygen species, but if this protection system is overwhelmed, exercise may lead to lipid peroxidation, and damage to DNA and cells (González-Flecha et al. 1993; Reid 1996). In this study, we

examined the levels of lipid peroxidation during the season by using diene conjugation measurements; our previous study showed that serum diene conjugation (S-DC) is a good indicator of exercise-induced oxidative stress level (Vasankari et al. 1995).

Limitations of this study should be taken into account when drawing conclusions. First, all the subjects were top-level athletes, nationally, and it is uncertain if less trained individuals would produce similar findings. Second, the number of subjects is low. Third, serum diene conjugation measures lipid peroxidation levels in serum. Since we know both LDL and HDL carry lipid soluble materials, it would have been interesting to investigate oxidized lipid levels in lipoproteins and also other parameters of lipid peroxidation than diene conjugation.

High-intensity exercise induces ROS production, releasing free radicals *in vivo*, and in this study, the S-DC values of ice hockey players were twice as high as runners during the season. One explanation for why intense training causes oxidative stress is that exercise is associated with transient tissue hypoxia in several organs because active skeletal muscles and skin demands more blood supply. In addition, training represented at intensities above VO_{2max} may lead to relative hypoxia of muscle fibres because of the imbalance between energy requirements and oxygen supply (Koyama et al. 1999). Also, an individual's training history alters the responses of lipid peroxidation to exercise (Alessio & Goldfarb 1988; Kretzscmar et al. 1991). It is known that runners perform continuous and low-intensity types of exercise, while ice hockey players prefer fast intermittent exercise. The study showed that long-distance runners had lower lipid peroxidation levels, and VO_{2max} correlated negatively with S-DC. These facts suggest that better aerobic capacity and chronic low-intensity exercise decreases the lipid peroxidation caused by oxidative stress. If lipid peroxidation occurs in lipoprotein lipids, it increases the risk of atherosclerosis by causing foam cell formation in vessel walls. Kujala et al. (1996) showed low LDL oxidation in veteran endurance athletes, and the results of a 2-day walk exercise study support previous speculations (Vuorimaa et al. 2005).

6.2. THREE MONTHS' STUDY OF MARATHON RUNNERS INCLUDING TRAINING PERIOD, PREPARATION PERIOD AND MARATHON RUN (STUDY II)

Oxidized LDL accumulates into vessel walls, leading to atherosclerosis. One way to prevent this episode is through physical exercise. An explanation for this could be the ability of HDL to protect LDL from oxidation (Kontush et al. 2003). Brites has shown that chronic aerobic exercise increased the HDL-associated antioxidant enzymes that improves the antioxidant functions of HDL for LDL (Brites et al. 2006). Furthermore, we know that both aerobic and anaerobic exercises increase the levels of circulating HDL, and the studies included in this thesis have indicated that not only the serum HDL levels are improved, but the reverse transport mechanism of oxidized lipid products by HDL also increases during physical exercise.

In Study II we wanted to investigate how chronic aerobic physical exercise affects oxLDLlipids levels and whether probiotics (*LGG)* have an influence on this process. Probiotics are defined to be living bacteria or yeasts that are good for human health. Although in this study we did not find any effect of *LGG* treatment on measured variables, *LGG* has been found to be antioxidative *in vitro* (Ahotupa et al. 1996b).

During the 3-month training period, the levels of oxLDLlipids did not change, but during the short 6-day preparative period, there was a significant increase in oxLDLlipids levels. Furthermore, after the 6-day preparative period, during the following marathon run the oxLDLlevels decreased back to training period levels. Comparing this to our previous study (Linna et al. 2007), we can say that oxLDLlipids levels were low during the training period, and therefore these results give us evidence that chronic low-intensity aerobic physical exercise protects LDL from oxidation. The above-mentioned mechanisms can partly explain how an adequate amount of aerobic training protects LDL from oxidation, or at least does not increase it. Earlier studies done with veteran endurance athletes and adolescent female gymnasts are in line with our results (Kujala et al. 1996; Vasankari et al. 2000).

Another key result was that during the 6-day preparative period, the oxLDLlipids levels increased significantly, by 33% in the probiotic group and 22% in the placebo group. During this period, the amount of aerobic training was reduced by half and caloric intake (mostly carbohydrates) was increased. As mentioned before, one explanation for the increase of

oxLDLlipids could be the reduced amount of protective aerobic exercise during this short period. However, the increased caloric intake also plays an important role in this remarkable increase.

The balance between energy consumption and caloric intake shifts into a state of over-nutrition when marathon runners start preparing for a marathon, an important occurrence that ensures the load of muscles with carbohydrates. There is evidence that a 7-day over-nutrition period brought on mostly with carbohydrates is associated with an acute increase of oxidative stress markers (Boden et al. 2015). This is in line with the study that indicated that there is a postprandial increase of serum oxLDLlipids for 6 hours after a hamburger meal with a high content of carbohydrates and fat (Ahotupa et al. 2010). Furthermore, a clinical study carried out by Stanhope and Havel (2008) showed that the metabolic effects of fructose promote atherogenic lipid profile development, but this type of effect was not found with glucose. On the other hand, earlier studies show that restricting caloric intake lowers oxLDLlevels (Linna et al. 2007).

Considering these facts, it is evident that the increase of oxLDLlipids during a 6-day preparative period is caused by the combination of the lack of an adequate amount of low-intensity aerobic training combined with carbohydrate-rich food. The exact mechanism behind the oxidation of LDL lipids remains unsolved. However, as our recent studies have shown, one explanation could be the impaired reverse transport activity of oxidative products by HDL due to a reduced amount of aerobic exercise.

Some limitations are important to disclose, when reviewing the results of this study. First, the subjects were advised to follow their traditional dietary habits during the trial, but no food diary requirements were given. We point out that carbohydrate-rich food is at least a partial explanation for the quick increase in the response of oxLDLlipids during the preparative period; therefore, it would be interesting to know the exact dietary habits of the subjects. Second, nutritional supplements were allowed during the trial, but we only informed the participants about this before the trial and encourage them to continue usage.

Both aerobic and anaerobic training promote pro-oxidant states that lead to oxidative stress. This process triggers the antioxidant system. An enhanced antioxidant system, when not overwhelmed, leads to beneficial health effects on our bodies. In this study, we also measured serum antioxidant capacity by using the TRAP method. During the training period and

preparative period, the levels of serum TRAP did not change, but in comparing the before and after marathon run levels, we found raised levels in both groups. In the probiotic group, levels increased by 16% and in the placebo group by 14%. This strengthens the validity of our previous findings (Vasankari et al. 1997). Furthermore, we analysed several vitamin levels during the study, but the increase we observed could not be explained by the simultaneous increase of investigated vitamins, as only the level of ubiquinone-10 increased. Svensson et al. (2002) demonstrate that acute exercise increases some water-soluble antioxidants, such as uric acid. Because of the presence of uric acid and numerous other antioxidants in serum, we suggest that the increase of serum TRAP is at least a partial cause of the increase of any other antioxidants.

6.3. EFFECT OF ACUTE STRENUOUS PHYSICAL EXERCISE ON oxHDL AND oxLDLlipids IN ENDURANCE RUNNERS (STUDY III)

Regular exercise training enhances the serum lipid profile by reducing the serum oxLDLlipids levels and increasing serum HDL levels (Powell et al. 1987; Durstine and Haskell 1994; Swift et al. 2013; Vasankari et al. 1998). There are several mechanisms and hypotheses for how exercise training may alter the above-mentioned risk factors of atherosclerosis. One explanation could be that HDL is able to protect LDL lipid products from oxidation (Kontush et al. 2003). Several studies have shown many other anti-atherogenic functions of HDL, for example, the reverse transport mechanism, and anti-inflammatory, anti-oxidative and anti-thrombotic properties (Kontush 2009; Navab 2011; Wan Ahmad 2015).

In this research, we studied the lipid peroxide transport function of serum lipoproteins during physiological oxidative stress stimulated by an acute exhaustive run. Acute physical exercise substantially raised the concentration of oxHDLlipids and the opposite effect was found in the concentration of oxLDLlipids. This exercise-induced lipoprotein distribution is not comparable to the exogenous lipid peroxides that enters the body via food ingestion. Ahotupa (2010) showed, in his hamburger meal study, a rapid increase in the concentration of serum oxLDLlipids, and only a late moderate increase of oxHDLlipids postprandial. Furthermore, Shao and Heinecke (2009) concluded that it is unlikely that either LDL or HDL would be oxidized in plasma. Our findings strengthen the validity of this assumption because, during the exercise test, the concentration oxHDLlipids increased and oxLDLlipids levels remained

unchanged. The explanation that oxidation products would be transferred directly from LDL to HDL in serum is unlikely because this process seems to too slow to have effect on the distribution in lipoproteins (Bowry et al. 1992).

The most glaring finding was that exhaustive exercise significantly influenced the oxHDLlipids/oxLDLlipids ratio (Table 6). This suggests a clear shift in the direction of the transportation of lipid oxidation products in the blood stream, endorsing the idea of the lipid peroxide clearing and protective transport functions by HDL (Shao & Heinecke 2009, Ahotupa et al. 2010).

During sports training, endurance runners are exposed to various kind of stress, which leads to adaption mechanisms at different levels: from adaptation on the subcellular, cellular and tissue levels, to adaption of organs and the athlete as a whole organism (Mahoney & Tarnopolsky 2005, Coffey & Hawkey 2007). Acute exhaustive physical exercise is known to raise the pro-oxidant cellular levels, which can lead to inflammation and cell damage induced by oxidative stress (Sies 1997, Bloomer 2008). However, this increased state of pro-oxidant is also necessary to trigger favourable effects of exercise that lead to a better antioxidant status and protection against oxidative stress. In this study, acute intense exercise promoted the increase of serum malonadialdehyde concentration. One explanation behind this finding could be that the production of reactive oxygen/reactive nitrogen species serve as a stimulus to upregulate the endogenous antioxidant mechanisms (Radak et al. 2008). It is noticeable that our subjects were well-trained, were adjusted to regular training, and had a long training history. Athletes with this background have the potential of having a well-functioning antioxidant mechanism, and their LDL lipids products protected from oxidation (Brites et al. 2006).

As we pointed out earlier, both marathon and middle-distance runners had comparable responses immediately after the exhaustive treadmill run, despite the fact that the training forms and duration of these runner groups differed. Furthermore, if the pre-exercise levels of serum oxHDL and oxLDLlipids and TRAP are compared between the groups, we find that middle-distance runners have somewhat higher pre-exercise levels in all variables (Figure 3, 4; Table 6). The higher level of TRAP (as a measure of antioxidant capacity) in middle-distance runners may suggest better antioxidant protection against oxidative stress. The reason for this could be the fact that the training form of middle-distance runners exposes their bodies to more oxidative stress, which upregulates better antioxidant states.

In this study, there are three limitations that should be considered. First, this study only involves participants who were top-level athletes, nationally, and we do not know whether the results are similar in the general public. Second, as in Study IV, the participants in this study are all male; it would be interesting to know if females would have similar responses to this running test. Third, there was no longer follow-up period after the trial, so data on potential chronic effects on the investigated variables are lacking.

Overall, in this study we indicated that immediately after acute physical exercise, in the form of a treadmill run, the serum concentrations of oxHDLlipids increased and that of oxLDLlipids decreased. Even more interestingly, the ratio of oxHDLlipids to oxLDLlipids increased dramatically. These results may give evidence that physiological oxidative stress triggered by exhaustive physical exercise stimulates the transport of lipid oxidation products by HDL. These findings deepen the view of the of HDL in the clearance of noxious lipid peroxides, and the adaptive capacity of protective HDL functions. Furthermore, the group differences between the runners in pre-exercise levels show us how different training histories can affect endogenous antioxidant and lipid transportation mechanisms in the long-term, though the acute responses to exercise are similar.

6.4. THE ACUTE EFFECT OF DIFFERENT INTENSITIES OF EXERCISES ON oxHDL AND OXLDLlipids (STUDY IV)

Several studies have shown the well-recognised association between high levels of aerobic training and the beneficial effects on oxidative stress and the plasma lipid profile, preventing atherogenesis in both animal and human profiles (Pellegrin et al. 2009, Ramachandran et al. 2005, Swift et al. 2013). Despite the broad studies done in this field, the mechanisms behind these beneficial effects are not yet fully understood. The association between exercise and cholesterol transport functions of HDL and LDL has been shown in many studies (Powell et al. 1987, Durstine & Haskell 1994, Vasankari et al. 1998, Vasankari et al. 2001). However, there are only a few studies that address the effect of exercise intensity and training history in this study field. Study IV points out that two different intensities of running exercises (continuous and intermittent runs) substantially increased the levels of oxHDLlipids. Moreover, we found

that training history could modulate the acute effect of running exercise on the serum concentration of oxidized lipids.

In this investigation, both running test resulted in a similar increase in the serum concentration of oxHDLlipids. However, interestingly, during the intermittent run, the increase from pre-exercise levels to after 20 minutes of exercise was 2.3 times greater among the middle-distance runners than among the marathon runners. The levels of oxHDLlipids did not increase further after the first 20 minutes of exercise among the middle-distance runners, and similar findings were observed among the marathon runners during the continuous run. These findings indicate that the exercise, which was strongly linked to participants' training history, could not induce any further stimulus after the first 20 minutes of the run, an observation that suggests training adaption to that type of exercise. These results demonstrate that genetic background and/or training history can have a strong impact on endogenous transporting mechanisms of oxHDLlipids (Ahotupa et al. 2010).

It is well-established that a high concentration of oxidized LDL lipids is a key pathogenic factor behind underlying cardiovascular disease (Shao & Heinecke 2009, Maiolino et al. 2013). Furthermore, regular physical activity seems to have an effective role in preventing this process (Powell et al. 1987, Kujala et al. 1997). One explanation behind this favourable effect of regular physical exercise on cardiovascular risk factors is that exercise decreases oxLDLlipids levels (Ahotupa et al. 1996a, Vasankari et al. 1997, Vuorimaa et al. 2005, Välimäki et al. 2012). Middle-distance runners perform fast and intermittent types of training, whereas marathon runners prefer more continuous types of running exercises. With this adaption to different types of training, combined with genetic differences, marathon runners have the potential to develop better VO_{2max}, compared to middle-distance runners, who have a higher anaerobic capacity (Vuorimaa et al. 1996). In this study, we found different degrees of a decrease in the levels of oxLDLlipids, from pre-exercise levels to levels immediately after the continuous run, when we compared the change among the middle-distance runners and change among the marathon runners. Furthermore, the baseline levels of oxLDLlipids were lower for marathon runners than for middle-distance runners. One hypothesis behind these findings is that the training history of marathon runners had resulted in well-functioning antioxidant protection and had built up a mechanism of defence for LDL oxidation (Brites et al. 2006). This is line with the results shown in Study III.

The effect of acute exercise inducing the production of the reactive oxygen species was

discovered in the early 1980s by Davies et al. (1982), and recent data has confirmed this evidence (Morales-Alamo & Calbet 2014). However, on the basis of present knowledge we have detected that reactive oxygen species are not only harmful to the body but also up-regulate adaption mechanisms, including antioxidant protection (Fisher-Wellman & Bloomer 2009, Radak et al. 2013). Furthermore, several studies have indicated that exercise intensity has a major role in the production of reactive oxygen species by adapting the level of exercise-induced oxidative stress (Wang et al. 2006, Sureda et al. 2009, Bouzid et al. 2015). Moreover, if the intensity of exercise is more than 60–70% of VO_{2max}, negative effects of exercise will arise (Lamprecth et al. 2008). In this study, the immediate effect of exercise induced an increase of malondialdehyde levels by 34% during the intermittent running test (100% of VO_{2max}) and by 24% during the continuous running test (80% of VO_{2max}). In accordance with these results, we assert that anaerobic types of intermittent running could have a greater influence on the production of reactive oxygen species/reactive nitrogen species, which could explain the higher increase of malondialdehyde.

Antioxidant mechanisms are improved after each single period of exercise via stimulating adaptive changes in signalling pathways. Antioxidant paraoxonase is one endogenous free radical scavenger in the body and is associated with HDL; it protects HDL against LDL oxidation (Mackness et al. 1993). Paraoxonase activity is known to be influenced by physical activity and genetic background (Cakmak et al. 2010, Costa et al. 2011). Furthermore, the effects of acute exercise-induced oxidative stress can be modulated by aerobic training, so that regularly trained individuals adapt more efficiently to oxidative stress via paraoxonase activity (Otocka-Kmiecik et al. 2014). In this study, the paraoxonase activity levels increased by 8% during the aerobic continuous running test, but this effect was not seen during the intermittent running test. Accordance to these findings, we suggest that different types of training intensities affect the antioxidant system differently. We also used the TRAP method to evaluate the the immediate effect of exercise in both running tests and the recovery of total antioxidant capacity. During both trials the serum concentration of TRAP increased similarly as a result of acute exercise. This is line with our earlier findings involving prolonged acute exercise (Vasankari et al. 1997, Välimäki et al. 2012).

There are some limitations that should be take into account when interpreting these results. First, these results only give us information regarding how athletes react acutely to different types of running tests, longitudinal data is lacking. Second, as in Study III, there is the participants of

this study were all male. Third, we did not measure plasma volume in this study, but we did not expect it to change in a running test during which water was allowed to be drunk ad libitum.

In conclusion, this study demonstrates that both continuous and intermittent intensive running tests increase oxHDLlipids significantly. Furthermore, the faster intermittent type of running elevated oxHDLlipids levels more for middle-distance runners than for marathon runners, and no further increase was observed among middle-distance runners after the first 20 minutes of exercise. Likewise, no further elevations were observed in oxHDLlipids among marathon runners after the first 20 minutes of exercise during the continuous run. These different oxHDLlipids reactions to running tests seem to be associated with varying anaerobic-aerobic training histories.

7. SUMMARY

1. The levels of diene conjugation in serum is higher in athletes who perform more anaerobic types of training over a whole season, compared with athletes who prefer aerobic types of training.

2. Low-intensity aerobic training seems to protect LDL lipids from oxidation during the three months' training period before a marathon run among keep-fit runners. More interestingly, only a six-day preparation period, when training amounts were reduced by half and carbohydrate intake was increased, was enough to elevate the serum oxLDLlipids levels remarkably.

3. The treadmill run until exhaustion performed by endurance runners significantly increased oxHDLlipids and the ratio of oxHDLlipids to oxLDLlipids levels in serum. These findings seem to indicate that HDL has a role in the reverse transport of lipid oxidation products induced by exercise.

4. Both intermittent and continuous types of exercise increased oxHDLlipids levels in serum significantly. However, marathon runners and middle-distance runners behaved differently during the first 20 minutes of performed exercises. OxHDLlipids levels increased more during the running test, which related to their training history.

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