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PHYSICAL ACTIVITY, CARDIORESPIRATORY FITNESS AND CARDIOVASCULAR HEALTH

The Cardiovascular Risk in Young Finns Study

Kristiina Pälve
(née Mansikkaniemi)

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To my family

ABSTRACT

Kristiina Pälve (née Mansikkaniemi). Physical activity, cardiorespiratory fitness and cardiovascular health. The Cardiovascular Risk in Young Finns Study. From the Faculty of Medicine, Cardiology and Cardiovascular Medicine, Doctoral Programme in Clinical Research, Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku and Heart Center, Turku University Hospital, Turku Finland. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Finland 2017.

Background: High physical activity and cardiorespiratory fitness may protect from the development of cardiometabolic disease outcomes but the mechanisms involved are not fully understood.

Aims: The aims of this study were to examine the associations of physical activity and cardiorespiratory fitness with traditional and novel cardiometabolic biomarkers, fatty liver and carotid artery elasticity and intima media thickness in a longitudinal population-based cohort study.

Participants and methods: This thesis is part of the Cardiovascular Risk in Young Finns Study. In 1980, 3,596 children and adolescents aged 3-18 years participated in the study. Self-reported leisure time physical activity including commuting activity was assessed by a questionnaire and blood samples were analyzed in 1986, 2001, 2007 and 2011. Carotid artery ultrasounds were examined in 2001 and 2007. A cardiopulmonary exercise test was undertaken in 2008-2009 and liver ultrasounds were performed in 2011.

Results: Physical activity and cardiorespiratory fitness were inversely associated with adiposity, heart rate, smoking, serum insulin, insulin resistance and C-reactive protein levels in adults. Leisure-time physical activity in boys and young adults was associated with better carotid artery elasticity later in life. Cardiorespiratory fitness was inversely and independently related with the risk of fatty liver regardless of adiposity.

Conclusions: Physical activity and cardiorespiratory fitness are favorably and independently associated with several cardiometabolic risk markers. These observations offer novel mechanistic insights into the beneficial effects of high physical activity and cardiorespiratory fitness on cardiometabolic disease outcomes.

Keywords: Physical activity, cardiorespiratory fitness, risk marker, carotid artery distensibility, fatty liver

TIIVISTELMÄ

Kristiina Pälve (o.s. Mansikkaniemi). Liikunta, hengitys- ja verenkiertoelimistön kunto ja sydän- ja verisuoniterveys. Lasten Sepelvaltimotaudin Riskitekijät (LASERI) -tutkimus. Turun yliopisto, Lääketieteellinen tiedekunta, Kardiologia ja kardiovaskulaarilääketiede, Turun yliopiston kliininen tohtoriohjelma, Sydäntutkimuskeskus ja Sydänkeskus, Turun yliopistollinen keskussairaala. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Finland 2017.

Tausta: Runsas fyysinen aktiivisuus ja hyvä hengitys- ja verenkiertoelimistön kunto voivat suojella sydän- ja verisuonisairauksilta, mutta mekanismeja ei vielä täysin tunneta.

Tavoitteet: Tämän tutkimuksen tarkoituksena oli tutkia fyysisen aktiivisuuden ja hengitys- ja verenkiertoelimistön kunnon yhteyttä perinteisiin ja uusiin kardiometabolisiin riskitekijöihin, rasvamaksaan ja kaulasuonen venyvyyteen sekä seinämäpaksuuteen pitkittäisessä seurantatutkimuksessa.

Menetelmät: Tämä väitöskirja on osa Lasten Sepelvaltimotaudin Riskitekijät – tutkimusta. Vuonna 1980 tutkimukseen osallistui 3596, iältään 3-18 vuotta lasta ja nuorta. Liikuntatottumukset selvitettiin kyselykaavakkeella ja verinäytteet analysoitiin vuosina 1986, 2001, 2007 ja 2011. Kaulavaltimoiden ultraäänitutkimukset tehtiin vuosina 2001 ja 2007. Kliininen rasituskoe hengityskaasumittauksin tehtiin vuosina 2008-2009. Maksan ultraäänikuvaukset tehtiin vuonna 2011.

Tulokset: Fyysinen aktiivisuus oli käänteisesti yhteydessä lihavuusmuuttujiin, sydämen leposykkeeseen, tupakointiin, seerumin insuliinitasoon, insuliiniresistenssiin ja tulehdusmuuttujaan aikuisilla. Vapaa-ajan liikunta oli yhteydessä myöhemmin elämässä mitattuun parempaan kaulasuonen venyvyyteen pojilla ja nuorilla aikuisilla. Maksimaalinen hapenottokyky oli yhteydessä pienempään rasvamaksan riskiin lihavuudesta riippumatta.

Päätelmät: Fyysinen aktiivisuus ja hengitys- ja verenkiertoelimistön kunto ovat itsenäisesti ja suotuisasti yhteydessä useisiin kardiometabolisiin riskitekijöihin. Nämä havainnot lisäävät mekanistista ymmärrystä fyysisen aktiivisuuden ja hengitys- ja verenkiertoelimistön kunnon suotuisista vaikutteista kardiometabolisiin sairauksiin.

Avainsanat: Fyysinen aktiivisuus, liikunta, hengitys- ja verenkiertoelimistön kunto, riskitekijä, kaulavaltimon venyvyys, rasvamaksa

TABLE OF CONTENTS

ABBREVIATIONS

	Correlation coefficient
ADMA	Asymmetric dimethyl arginine
AHT	Antihypertensive treatment
ALAT	Alanine aminotransferase
apoA1	Apolipoprotein A1
apoB	Apolipoprotein B
ASAT	Aspartate aminotransferase
BMI	Body mass index
BP	Blood pressure
Cdist	Carotid artery distensibility
CRP	C-reactive protein
DBP	Diastolic blood pressure
D_d	Diastolic diameter
D_s	Systolic diameter
GT	Gamma-glutamyltransferase
HDL	High density lipoprotein
HOMA-IR	Homeostasis model assessment of insulin resistance
IMT	Intima-media thickness
LDL	Low density lipoprotein
MET	Metabolic equivalent
oxLDL	oxidized-LDL
PA	Physical activity
PAI	Physical activity index
P_d	Diastolic blood pressure
P_s	Systolic blood pressure
SBP	Systolic blood pressure
SD	Standard deviation
SDMA	Symmetric dimethyl arginine
SE	Standard error
sPLA2	Secretory phospholipase A2
VLDL	Very low density lipoprotein
VO_{2peak}	Peak oxygen uptake
WHR	Waist-to-hip ratio

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. In addition, previously unpublished data are presented.

I **Mansikkaniemi** K, Juonala M, Taimela S, Hirvensalo M, Telama R, Huupponen R, Saarikoski L, Hurme M, Mallat Z, Benessiano J, Jula A, Taittonen L, Marniemi J, Kähönen M, Lehtimäki T, Rönnemaa T, Viikari J, Raitakari OT. Cross-sectional associations between physical activity and selected coronary heart disease risk factors in young adults. The Cardiovascular Risk in Young Finns Study. **Ann Med. 2012;44:733-44.**

II **Pälve** KS*, Pahkala K, Magnussen CG, Koivistoinen T, Juonala M, Kähönen M, Lehtimäki T, Rönnemaa T, Viikari JS, Raitakari OT. Association of physical activity in childhood and early adulthood with carotid artery elasticity 21 years later. The Cardiovascular Risk in Young Finns Study. **J Am Heart Assoc. 2014;3:e000594.**

III Hulkkonen J, Aatola H, **Pälve** K*, Lehtimäki T, Hutri-Kähönen N, Viikari JS, Raitakari OT, Kähönen M. Determinants of exercise peak arterial blood pressure, circulatory power, and exercise cardiac power in a population based sample of Finnish male and female aged 30 to 47 years: the Cardiovascular Risk in Young Finns Study. **BMC Cardiovasc Disord. 2014;14:35.**

IV **Pälve** KS*, Pahkala K, Suomela E, Aatola H, Hulkkonen J, Juonala M, Lehtimäki T, Rönnemaa T, Viikari JS, Kähönen M, Hutri-Kähönen N, Telama R, Tammelin T, Raitakari OT. Cardiorespiratory fitness and risk of fatty liver: The Young Finns Study. **Med Sci Sports Exerc. 2017;49:1834-41.**

* née Mansikkaniemi

1. INTRODUCTION

According to the World Health Organization's latest review, cardiovascular diseases are the number one global cause of death. These diseases are responsible for 17.5 million deaths, most of which could be prevented by addressing behavioral risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol by adopting population-wide strategies (WHO 2016).

One interesting way to examine the biological and lifestyle factors underlying cardiovascular diseases would involve their assessment in a young and healthy cohort even before cardiovascular disease end-points emerge. This would make it possible to evaluate their association with traditional cardiovascular disease risk markers and to identify novel attributes contributing to the risk. The traditional risk markers for cardiovascular disease are considered to be older age, male sex, family history of cardiovascular diseases at a young age, smoking, elevated blood pressure, high values of low density lipoprotein (LDL) and low high density (HDL) cholesterol and impaired glycemic control (D'Agostino RB *et al.* 2008). However, new risk markers have been discovered, such as phospholipase A2 enzymes (Mallat *et al.* 2010), serum amyloid A (Ridker *et al.* 2000) and asymmetric dimethylarginine (Lu *et al.* 2003). Increased artery intima-media thickness and decreased elasticity are recognized as surrogate markers of atherosclerosis (Arnett *et al.* 1994, de Groot *et al.* 2004). Fatty liver has also been established as a risk marker for cardiovascular disease (Adams *et al.* 2017).

It has been known for millennia that physical activity has a positive effect on health. Already Susruta from India prescribed moderate daily exercise for his patients 600 years Before Common Era (BCE), Hippocrates from Greece (460-370 BCE) wrote an exercise prescription for a patient suffering from consumption, and Claudius Galenus from Rome (129–210 CE) recommended exercise for patients to alleviate the symptoms of diseases (Tipton 2014). The protective mechanisms of physical activity on cardiovascular disease may include multiple influences on many risk markers. Physical activity is associated with reduced smoking rates (Kujala *et al.* 2007), favorable changes in lipid levels (Herzig *et al.* 2014), reduced insulin resistance (Schmidt *et al.* 2008) and less inflammation (Palmefors *et al.* 2014). High levels of physical activity have been associated with lower aortic intima-media thickness in adolescents (Järvisalo *et al.* 2001, Pahkala *et al.* 2011) and in cross-sectional studies, physical activity has improved arterial elasticity (Moreau *et al.* 2003, Nualnim *et al.* 2011). Previous studies have shown that cardiorespiratory fitness is inversely associated with

fatty liver in adult males although this association has been dependent on adiposity (Nguyen-Duy *et al.* 2003, Church *et al.* 2006, Minder *et al.* 2014).

The main objectives of this thesis were to examine the associations of physical activity and cardiorespiratory fitness with traditional and novel risk markers and fatty liver in a rather large cohort including both males and females, and to study the effect of physical activity in children and young adults on carotid artery elasticity measured 21 years later.

2. REVIEW OF THE LITERATURE

2.1. Physical activity

2.1.1. Definition of physical activity

Physical activity has been defined as any bodily movement produced by skeletal muscles that results in increased energy expenditure (Caspersen *et al.* 1985). Physical activity can be categorized in different ways; e.g. occupational, transportational (e.g. commuting) and leisure-time physical activity.

2.1.2. Assessment of physical activity

Physical activity can be assessed with several methods. The doubly labelled water technique is considered to be the most accurate way of assessing energy expenditure, but this procedure is expensive, difficult and the energy expenditure caused specifically by physical activity cannot be directly ascertained (Lamonte and Ainsworth 2001). Indirect calorimetry is also used to measure energy expenditure but it is difficult to accommodate into normal everyday life; it has been used to validate heart rate monitors, pedometers and accelerometers (Armstrong and Welsman 2006).

The use of wearable monitors to directly measure various components of physical activity has increased. Heart rate monitors, pedometers and accelerometers are now widely available for assessing physical activity. However, although heart rate is not a direct measure of physical activity, it does provide an indication of the stress placed upon the cardiorespiratory system by physical activity (Armstrong 1998). Nevertheless, there is a strong linear relationship between heart rate and energy expenditure in the range between the moderate and more vigorous intensity physical activity, although this relationship is not as evident in the light intensity range (Butte *et al.* 2012).

Pedometers, or stepcounters, count each step the subject takes by detecting the motion of the subject's hip or foot. The accuracy of pedometers has improved within the past 10 years; they are now excellent for measuring steps at walking speeds > 2 mph (Crouter *et al.* 2003, Melanson *et al.* 2004). It has been proposed that about 10,000 steps per day is a sufficient number for a normally healthy adult (Tudor-Locke and Bassett 2004). Accelerometers record accelerations in gravitational units on one or more planes at sampling rates >1 time/second (typically 40–100 hz). Captured accelerations are then processed at a lower resolution (i.e., some fixed epoch) and then calibrated to a known criterion measure (e.g. doubly-labeled water). Most of the existing calibration studies rely

on a unitless intensity metric or "counts" and then apply thresholds to the collected data to output the duration and frequency of physical activity into sedentary, light, moderate, and vigorous intensities. There is still no consensus about how accelerometers should be used and how the output should be interpreted (Ainsworth *et al.* 2015).

Objective methods are a more accurate way of measuring physical activity but most of these methods are also very expensive and time-consuming. Therefore, subjective methods, i.e. questionnaires, logs and diaries are often used to assess physical activity in large population-based studies. These subjective methods are relatively simple, inexpensive and non-invasive ways to assess physical activity. The limitation with the use of questionnaires, logs, and diaries is related to the accuracy of recall as well as the well documented reporting bias. In a review by Ainsworth et al., self-report questionnaires were shown to be accurate when describing high intensity physical activity but not when reporting low-to-moderate intensity physical activity (Ainsworth *et al.* 1999). Similar findings were shown in the report published by Strath and colleagues (Strath *et al.* 2004).

2.2. Cardiorespiratory fitness

Physical fitness can be expressed in five major components: (1) a morphological component (body mass for height, body composition, subcutaneous fat distribution, abdominal visceral fat, bone density and flexibility); (2) a muscular component (power or explosive strength, isometric strength, muscular endurance); (3) a motor component (agility, balance, co-ordination, speed of movement); (4) a cardiorespiratory component (endurance or submaximal exercise capacity, maximal aerobic power, heart function, lung function, blood pressure); and (5) a metabolic component (glucose tolerance, insulin sensitivity, lipid and lipoprotein metabolism, substrate oxidation characteristics) (Bouchard and Shephard 1994, Vanhees *et al.* 2005).

2.2.1. Definition of cardiorespiratory fitness

In 1967, an international committee selected maximum or peak oxygen uptake as the reference standard of cardiorespiratory fitness (Shephard *et al.* 1968). After this selection, subsequent medical publications have equated cardiorespiratory fitness to oxygen consumption under conditions of maximal aerobic work intensity. Peak oxygen uptake is by definition, a reflection of maximal aerobic capacity during working conditions involving the continuous performance of the large muscle groups (Leaf 1985).

2.2.2. Assessment of cardiorespiratory fitness

Cardiorespiratory fitness can be assessed with several methods. One of the earliest testing methods included stair climbing in which patients climbed as many stairs as possible to the end point of the exercise, or termination due to dizziness, chest pain, or, more typically, dyspnea and leg fatigue. The results were recorded variably as the number of stairs climbed, the flights of stairs ascended, or the time required to ascend the designated number of stairs (Olsen *et al.* 1991). The test was introduced in the 1960s as a preoperative assessment tool for thoracic surgery patients (Van Nostrand *et al.* 1968). Walking and running tests can be used to assess cardiorespiratory fitness. Different protocols have been developed, e.g. shuttle walk test (Singh *et al.* 1992), the 12 minute field performance test introduced by Cooper (Cooper 1968), 6 minute walk test (Butland *et al.* 1982), 2 km walking test (Laukkanen *et al.* 1992). These earlier mentioned tests are relatively simple "field tests", requiring minimal equipment and technical support, and are easily implemented. However, the cardiopulmonary exercise test, involving the noninvasive assessment of inspired oxygen, and expired carbon dioxide, is uniquely able to evaluate the contribution of respiratory, cardiovascular, and peripheral tissue function in support of maximal exercise (Pichurko 2012) making it the gold standard, as any type of exercise involves a coupling of respiratory function, cardiovascular performance and ultimately oxygen uptake and utilization by peripheral tissues.

2.2.2.1. Cardiopulmonary exercise test

The cardiopulmonary exercise test represents a non-invasive global assessment of the integrative exercise response involving the pulmonary, cardiovascular, hematopoietic, neuropsychological and skeletal muscle systems (American Thoracic Society and American College of Chest Physicians 2003). The test can be performed on a treadmill or a cycle ergometer. On a treadmill, increasing exercise stress is produced by increasing the speed and grade. Walking and running on a treadmill are more strenuous and maximal oxygen uptake is reported to be 5–10% higher on a treadmill than on a cycle ergometer (Hermansen and Saltin 1969, McKay and Banister 1976). The cycle ergometer is generally less expensive and requires less space than the treadmill. It is also less prone to introduce movement or noise artifacts into measurements (e.g., ECG and blood pressure auscultation are generally easier to record on a cycle) and the rate at which external work is performed is easily quantitated (American Thoracic Society and American College of Chest Physicians 2003). A low cardiorespiratory fitness level independently predicts cardiovascular events, morbidity and mortality later in life (Blair *et al.* 1996, Blair 1996, Barlow *et al.* 2006, Arena *et al.* 2008, Arena *et al.* 2011, Harber *et al.* 2017).

2.3. Physical activity and cardiorespiratory fitness

Physical activity and cardiorespiratory fitness are closely related but are not synonymous (Bouchard and Shephard 1994). Physical activity is a behavior and cardiorespiratory fitness is an attribute. In general, physical activity increases cardiorespiratory fitness but physical activity needs to be of sufficient intensity to improve cardiorespiratory fitness. Furthermore, both physical activity and cardiorespiratory fitness are influenced by genetics (Bray *et al.* 2009, Rankinen *et al.* 2010) and it is suggested that there is shared genetics behind physical activity and cardiorespiratory fitness (Karvinen *et al.* 2015). There is also evidence that similar regular physical activity improves cardiorespiratory fitness with considerable heterogeneity (Bouchard and Rankinen 2001, Timmons *et al.* 2010).

There is little data on which specific genetic factors influence physical activity levels in humans. The physical activity level seems to be significantly lower in mitochondrial disease patients than in healthy subjects and higher physical activity has been associated with a lower clinical disease burden (Apabhai *et al.* 2011). A large twin study has revealed that genetic factors play an important role in explaining individual differences in participation in physical activity (Stubbe *et al.* 2006). De Moor and colleagues have reported 37 single nucleotide polymorphisms in the PAPSS2 gene and in 2 intergenic regions on chromosomes 2q33.1 and 18p11.32 that were associated with exercise participation (De Moor *et al.* 2009).

Skeletal muscle consists of slow-twitch (type I) and fast-twitch (types IIa and IIb) muscle fibres. High endurance performance is associated with a high proportion of type I fibres and high speed and power capacities with type II fibres (Bergh *et al.* 1978). It has become evident that the A allele of monocarboxylate transporter 1 gene, which catalyzes the transport of lactate into myocytes for oxidation, is more prevalent among endurance athletes than among non-athletes (Fedotovskaya *et al.* 2014) and thus may thus indicate better cardiorespiratory fitness.

According to Blair and colleagues' extensive review, it is impossible to say which is more important for health, physical activity or cardiorespiratory fitness (Blair *et al.* 2001). The association between physical activity and health outcomes is weaker than that between cardiorespiratory fitness and health outcomes (Blair *et al.* 2001, DeFina *et al.* 2015). However, this could be due to problems in assessing physical activity in comparison with the more precise measurements of cardiorespiratory fitness (Blair *et al.* 2001). Nonetheless, because participation in physical activity is a behavior, this means that it can be

modified. As a consequence of modifying physical activity, cardiorespiratory fitness can be modified and the subject's health improved.

2.4. Subclinical markers of arterial health

Atherosclerosis starts to develop early in life; intimal thickening, lamellar irregularities and ruptures, foam cells, and fat have been discovered to exist already in infants' carotid arteries (Bland *et al.* 1986, Pesonen *et al.* 1996, Weninger *et al.* 1999). These subclinical markers of atherosclerosis include thickening and stiffening of large artery walls, coronary artery calcification and endothelium-dependent vasoactivity. In this thesis subclinical atherosclerosis was evaluated by carotid artery intima-media thickness with elasticity being assessed by ultrasound.

2.4.1. Arterial elasticity

Central arteries, such as the aorta and carotid, lose their elasticity with age (Vaitkevicius *et al.* 1993, Tanaka *et al.* 1998, Juonala *et al.* 2008). It has been shown that arterial elasticity decreases with age already between ages 11 and 19 years and this decrease is more pronounced in boys than girls (Mikola *et al.* 2015). Decreased arterial elasticity is a risk factor for several cardiovascular outcomes: hypertension, atherosclerosis and coronary heart disease (Arnett *et al.* 1994). It has been shown in cross-sectional studies that high levels of habitual physical activity is associated with improved carotid artery elasticity (Moreau *et al.* 2003, Nualnim *et al.* 2011) and reduced arterial stiffening in older adults (Gando *et al.* 2010, Nualnim *et al.* 2011). Van de Laar et al. have shown that lifetime vigorous, but not light-to-moderate, habitual physical activity is favorably associated with brachial and femoral artery elasticity (van de Laar *et al.* 2011). Chen et al. have demonstrated that a decrease in physical activity in males was associated with increased arterial stiffness (Chen *et al.* 2012). Physical activity reduces sympathetic activity which may lessen the peripheral vasoconstriction and therefore the elasticity of the arteries is maintained (Wilkinson and McEniery 2004). There is also evidence that physical activity may reduce oxidative stress and increase the expression and activation of nitric oxide synthase enhancing endothelium-dependent dilation of the arteries (Kingwell *et al.* 1997). Cardiorespiratory fitness has been associated with aortic elasticity in adolescents (Pahkala *et al.* 2013) and carotid artery elasticity in adults (Ferreira *et al.* 2003).

2.5. Cardiovascular disease risk markers

Atherosclerosis is a multifactorial disease. Already in 1981, 246 risk markers for cardiovascular diseases had been identified (Hopkins and Williams 1981). The traditional risk markers for cardiovascular disease are considered to be older age, male sex, family history of cardiovascular diseases at young age, smoking, elevated blood pressure, high LDL and total cholesterol and impaired glycemic control (D'Agostino RB *et al.* 2008). In addition to these classical risk markers, many etiologic markers have been identified i.e. low socioeconomic status (Beauchamp *et al.* 2010), unhealthy diet (Gidding *et al.* 2009), heart rate (Bohm *et al.* 2015), air pollution (Koulova and Frishman 2014), homocysteine (Braunwald 1997), lipoprotein(a) (Saleheen *et al.* 2017) and chronic kidney disease (Sarnak *et al.* 2003) to name but a few.

It has been previously shown in prospective studies that physical activity is inversely related to the incidence of coronary heart disease and its related mortality (Kannel *et al.* 1986, Leon and Connett 1991). Atherosclerotic cardiovascular disease has its roots in childhood, and exposure to risk markers may exert long-term adverse health effects (Taimela *et al.* 1996, Cleeman 1997, LaRosa 2001, Zieske *et al.* 2002). The level of habitual physical activity tracks significantly from adolescence to young adulthood (Raitakari *et al.* 1994, Telama *et al.* 2005, Telama *et al.* 2006), and that during the transition from childhood to early adulthood, persistent physical activity is associated with beneficial changes in other health-related behaviors and several biological risk factors (Raitakari *et al.* 1994, Raitakari *et al.* 1996). Participation in long-term vigorous physical activity has been associated with a lower risk for cardiovascular diseases such as ischaemic heart disease and stroke (Kettunen *et al.* 2015).

According to the World Health Organization (World Health Organization 2010) children and youth aged 5 to 17 years should accumulate at least 60 minutes of moderate- to vigorous-intensity physical activity every day. Adults should undertake at least 150 minutes of moderate-intensity aerobic physical activity throughout the week or do at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate- and vigorous-intensity activity. In Finnish adolescents, it was claimed that only 10 % of girls and 23 % of boys met the current recommendations for health-related physical activity (Tammelin *et al.* 2007). Physical activity was inversely associated with the time spent viewing TV, using the computer, and playing video games in both genders (Tammelin *et al.* 2007). A survey conducted in Finland in 2013 revealed that only 10 % of adults were as physically active as recommended by the World Health Organization (World Health Organization 2010). Subjects with higher education, i.e. total school years more than 16, were

physically more active than subjects with lower education, i.e. total school years 9 or less, even though higher educated individuals spent more time seated during working hours (Husu *et al.* 2014). Subjects with lower education sat more during leisure-time (Husu *et al.* 2014).

2.5.1. Smoking

There is a consensus that smoking contributes significantly to cardiovascular morbidity and mortality. Smoking impacts on all phases of atherosclerosis from endothelial dysfunction to acute clinical events, the latter being largely thrombotic, and both active and passive (environmental) cigarette smoke exposure predispose to cardiovascular events (Solberg and Strong 1983, Ambrose and Barua 2004). It has been proposed that acute and long-term exposure to cigarette smoke leads to changes in the balance of the autonomic nervous system, resulting in a sympathetic predominance, which in turn may cause atrial and ventricular arrhythmias, sudden death, and acute myocardial infarction and causes hemodynamic changes that exacerbate heart failure (Middlekauff *et al.* 2014). Smoking and passive smoking have been associated with intima-media thickness in adults (Raitakari *et al.* 2003, Chen *et al.* 2015) and the risk of type 2 diabetes (Patja *et al.* 2005). Parental smoking in childhood or adolescence have been shown to have an irreversible effect on carotid intima-media thickness and reduced flow-mediated dilatation in brachial artery in adulthood (Juonala *et al.* 2012, Gall *et al.* 2014, Chen *et al.* 2015).

Several studies have reported that high levels of physical activity are associated with reduced smoking rates (Marti *et al.* 1988, Dannenberg *et al.* 1989, Yancey *et al.* 2004, Kujala *et al.* 2007). Physical activity reduces the risk of myocardial infarction even in smoking females (Nayak *et al.* 2013) and males (Frank *et al.* 1966). Physical activity may also protect from intima-media thickening in smokers (Katano *et al.* 2013). Persistent physical activity is related to less smoking in adolescents and young adults (Raitakari *et al.* 1994). Moderate- to vigorous-intensity physical activity has been shown to significantly associate with a reduction in cigarette cravings (Glass and Maher 2014, Haasova *et al.* 2014). It has also been suggested that physical activity might increase dopamine levels in the brains thus providing an endogenous reward, making smoking less rewarding (Audrain-McGovern *et al.* 2006). There are also behavioral norms and attitudes among physically active people; smoking is inconsistent with physical performance and physically active people do not smoke.

2.5.2. Obesity

In this thesis, obesity was studied by measuring waist and hip circumference and by calculating BMI and waist-to-hip ratio.

It is well known that obesity is associated with hypertension, type 2 diabetes, dyslipidemia and major cardiovascular events i.e. non-fatal myocardial infarction, non-fatal stroke and cardiovascular death (Martin *et al.* 2008, Lavie *et al.* 2009, Cronin *et al.* 2013). Obesity tracks significantly from youth to adulthood (Raitakari *et al.* 2005) and parental BMI significantly predicts the offspring's BMI in adulthood (Serlachius *et al.* 2016). Even babies born large-for-gestational-age are more likely to be obese in adulthood and have increased carotid intima-media thickness, a marker of subclinical atherosclerosis (Skilton *et al.* 2014).

Physical activity reduces total body adiposity and abdominal and visceral fat (Ross and Janssen 2001). Obesity in adulthood is directly related to adult physical activity (Yang *et al.* 2007). Physical activity has been shown to attenuate the association of the fat mass and obesity-associated gene with the odds of obesity, highlighting the importance of physical activity, in particular in those genetically predisposed to obesity (Kilpeläinen *et al.* 2011). One of the main aims of this thesis was to examine if physical activity would be associated with cardiovascular disease risk markers independently of obesity.

2.5.3. Blood pressure

Increased blood pressure is generally associated with cardiovascular mortality and cardiovascular events (Kannel *et al.* 2003, Conen and Bamberg 2008, Ward *et al.* 2012). There are reports that elevated blood pressure in adolescence seems to track significantly to adulthood (Bao *et al.* 1995, Chen and Wang 2008, Juhola *et al.* 2011) and elevated blood pressure is associated with increased carotid artery intima-media thickness in adolescents and adults (Polak *et al.* 2010, Kollias *et al.* 2013). Childhood systolic blood pressure was associated with high adult carotid intima-media thickness (Li *et al.* 2003, Raitakari *et al.* 2005) but this risk became reduced if the elevated blood pressure during childhood resolved by adulthood (Juhola *et al.* 2013). Elevated blood pressure in youth was also reported to be predictive of elevated adult arterial stiffness and endothelial dysfunction (Vos *et al.* 2003, Li *et al.* 2004, Juonala *et al.* 2006a, Ferreira *et al.* 2012, Aatola *et al.* 2013).

Physical activity is frequently associated with lower blood pressure and a reduced risk for the development of hypertension in older people, hypertensive adults and obese individuals (Arrol and Beaglehole 1992). In hypertensive

patients, physical activity reduces the risk of cardiovascular mortality (Rossi *et al.* 2012). However, in younger subjects, no association has been generally observed between physical activity and blood pressure among normotensive individuals (Alpert and Wilmore 1994). Physical activity is also associated with lower blood pressure in children with hypertension and/or obesity (Andersen *et al.* 2011).

2.5.4. Lipids and apolipoproteins

In this thesis, lipids were studied by measuring the concentrations of total cholesterol, HDL cholesterol, triglycerides and oxidized LDL and by calculating LDL cholesterol. The apolipoprotein metabolism was studied by measuring apolipoprotein AI and B.

An increased concentration of LDL cholesterol has been associated with an increased risk of myocardial infarction and vascular death (Prospective Studies Collaboration *et al.* 2007). Findings from classic genetic studies suggest that early exposure to excessive LDL cholesterol, which is the result of mutations of the LDL receptor, results in markedly early atherosclerosis (Ridker 2014). Genetic defects that result in loss of function within the LDL receptor are a major determinant of inherited hyperlipidaemias, e.g. familial hypercholesterolemia, which lead to early atherosclerosis and a high risk of cardiovascular disease (Ridker 2014). Childhood LDL cholesterol is independently associated with adult carotid intima-media thickness (Li *et al.* 2003, Raitakari *et al.* 2005).

Oxidized LDL (oxLDL), inducer of oxidative stress in endothelial cells, smooth muscle cells and macrophages and releaser of inflammatory cytokines, is associated with cardiovascular diseases (Ishigaki *et al.* 2009, Verhoye *et al.* 2009). Vigorous physical activity seems to induce lipid oxidation (Fogarty *et al.* 2011), but on the other hand, prolonged physical activity reduces oxidized LDL (Vuorimaa *et al.* 2005).

High plasma levels of apolipoprotein B (LDL's and very low density lipoprotein (VLDL)'s apolipoprotein) is a risk factor for atherosclerosis (Lusis 2000). It has been suggested that apolipoprotein B could be a better predictor of the cardiovascular risk than the concentrations of cholesterol in the LDL fraction as apolipoprotein B is a measure of the total number of atherogenic particles (including LDL, intermediate-density lipoprotein, VLDL, chylomicrons, and chylomicron remnants) (Benn 2009).

It has been noted that many individuals with high triglyceride concentrations do not develop atherosclerosis and cardiovascular disease (Nordestgaard and Varbo 2014). This paradox may be explained by the fact that at greatly elevated

concentrations, triglyceride lipoproteins are too large to enter into the arterial intima and therefore cannot lead to the development of atherosclerosis, but at mild–to–moderately raised triglyceride concentrations, lipoproteins are small enough to enter into the arterial wall and thus have the potential to accumulate and trigger atherosclerosis (Nordestgaard and Varbo 2014). It has been proposed that the cholesterol content of triglyceride-rich lipoproteins (remnant cholesterol) is more likely to be the cause of atherosclerosis and cardiovascular disease rather than the elevated levels of triglycerides per se (Nordestgaard and Varbo 2014). However, Do et al. have shown that genetically determined elevated triglyceride levels were independently and strongly associated with cardiovascular disease (Do *et al.* 2013).

It has been established that high levels of HDL cholesterol are associated with a lower risk of cardiovascular disease (Gordon *et al.* 1977, Assmann *et al.* 1996, Barter *et al.* 2007, deGoma *et al.* 2008). However, recent studies have cast major doubts on the conventional HDL hypothesis. Large randomized clinical trials of HDL cholesterol -raising drugs have shown no benefit from the increased HDL cholesterol level on the incidence of major vascular events (Siddiqi *et al.* 2015). Human genetic studies have also failed to support the conventional HDL hypothesis (Siddiqi *et al.* 2015). Do et al. have shown that genetically determined low levels of HDL cholesterol levels were not associated with cardiovascular disease (Do *et al.* 2013). It was postulated that the functional properties of HDL would be more important in protection from atherosclerosis (Siddiqi *et al.* 2015), but presently these atheroprotective properties of HDL are not fully understood. One of the protective mechanisms could be HDL's ability to promote efflux of cholesterol from cells, including macrophages (Rader *et al.* 2009). The cholesterol efflux capacity has been shown to associate inversely with the incidence of cardiovascular disease (Rohatgi *et al.* 2014). Low levels of HDL's major apolipoprotein A1 have also been associated with a higher risk of cardiovascular disease (Contois *et al.* 1996, Andrikoula and McDowell 2008).

Physical activity is favorably associated with lipids and apolipoproteins. Physical activity lowers LDL cholesterol (Lehtonen and Viikari 1978a, Ahmed *et al.* 2012), oxidized LDL (Vasankari *et al.* 2000, Park *et al.* 2011), serum triglycerides level (Raitakari *et al.* 1997, Ahmed *et al.* 2012) and apolipoprotein B levels (Rönnemaa *et al.* 1980, Hostmark *et al.* 1992, Ahmed *et al.* 2012). The direct relationship between physical activity and HDL cholesterol levels is also well established (Lehtonen and Viikari 1978b, Raitakari *et al.* 1997, Ahmed *et al.* 2012). Increased physical activity seems to increase the level of apolipoprotein A1 (MacAuley *et al.* 1996, Luc *et al.* 2000, Ahmed *et al.* 2011). Physical activity increases skeletal muscle lipoprotein lipase activity, which in turn leads to a reduction in the concentrations of the apolipoprotein-B and triglycerides and an

increase of HDL concentrations (Senti *et al.* 2001, Ahmad *et al.* 2011). In rats (Alessio and Goldfarb 1988) and in humans (Viinikka *et al.* 1984), physical training and adaptation to muscle work of long duration may alter the response of lipid peroxidation to exercise and thus lower the oxidized LDL concentrations. High levels of cardiorespiratory fitness have been shown to be associated with a reduced polygenic risk for hypertriglyceridemia (Tanisawa *et al.* 2014) by affecting lipoprotein lipase activity.

2.5.5. Glucose homeostasis

In this thesis, glucose homeostasis was studied by measuring serum glucose and insulin and calculating the homeostasis model assessment of insulin resistance (HOMA-IR).

Hyperglycemia and insulin resistance are associated with the development of atherosclerosis and its complications. Patients with type 2 diabetes have a two-fold increase in all-cause mortality and a three-fold increase in cardiovascular mortality (Taylor *et al.* 2013). It has been shown that diabetic patients without a previous myocardial infarction carry as high a risk of myocardial infarction as nondiabetic patients with a previous myocardial infarction (Haffner *et al.* 1998). A reduced insulin sensitivity is associated with higher carotid artery intima-media thickness in males, and furthermore in females, carotid artery intima-media thickness is independently associated with fasting glucose (Kozakova *et al.* 2013b). Children with diabetes have increased aortic and carotid intima-media thickness and endothelial dysfunction (Järvisalo *et al.* 2001, Harrington *et al.* 2010, Trigona *et al.* 2010).

Higher levels physical activity are generally related with increased insulin sensitivity independent of obesity indices (Rizzo *et al.* 2008, Johannsen *et al.* 2016) and several studies have revealed that regular physical activity can substantially reduce the risk of type 2 diabetes independent of obesity (Jeon *et al.* 2007, Johannsen *et al.* 2016). It has been shown that in children with type 1 diabetes, physical activity improves endothelial function (Trigona *et al.* 2010). Physical activity may influence glucose homeostasis favorably by increasing muscle glucose transporter type 4 content thus increasing glucose absorption (Dela *et al.* 1994), increasing the activity of muscle mitochondrial enzymes (Short *et al.* 2003) and improving the actions of insulin in skeletal muscle (Cox *et al.* 1999).

2.5.6. Adipokines

Adipokines are immune-modulatory proteins secreted by adipose tissue. It has been proposed that obesity leads to increased expression of pro-inflammatory adipokines and diminished expression of anti-inflammatory adipokines, resulting in the development of a chronic, low-grade inflammatory state; this adipokine imbalance is thought to be a key event in promoting both systemic metabolic dysfunction and cardiovascular disease (Nakamura *et al.* 2014). In this thesis, adipokines were studied by measuring serum adiponectin and leptin.

Adiponectin is one of the anti-inflammatory adipokines. There is evidence suggesting that adiponectin possesses anti-atherogenic properties by improving endothelial function and displaying anti-inflammatory effects in the vascular wall. In addition, adiponectin can modify vascular intracellular redox signalling and exerts indirect antioxidant effects on human myocardium (Antoniades *et al.* 2009). Low levels of adiponectin are associated with coronary artery disease, hypertension, left ventricular hypertrophy and a greater risk of myocardial infarction (Nakamura *et al.* 2014). Low serum adiponectin levels in childhood and adolescence also predict increased intima-media thickness in adulthood (Saarikoski *et al.* 2017). The association between physical activity and adiponectin is not well established; there are several studies in which physical activity was inversely associated with adiponectin concentrations (Metcalf *et al.* 2009, Kozakova *et al.* 2013a). One possibility is that increased physical activity might increase adiponectin levels through weight loss (Kim *et al.* 2007).

Leptin is one of the pro-inflammatory adipokines. Increased circulating levels of leptin, a marker of leptin resistance, are common in obesity and independently associated with insulin resistance and cardiovascular disease (Martin *et al.* 2008). There is evidence indicating that central leptin resistance causes obesity and that obesity-induced leptin resistance damages numerous peripheral tissues, including liver, pancreas, platelets, vasculature, and myocardium (Martin *et al.* 2008). Increased physical activity is independently associated with lower circulating levels of leptin (Franks *et al.* 2003, Esteghamati *et al.* 2010, Jimenez-Pavon *et al.* 2012). The effect of physical activity on the sympathetic nervous system and circulating catecholamine levels, may exert suppressive effects on leptin level (Scriba *et al.* 2000).

2.5.7. Inflammatory markers

In this thesis, inflammation was studied by measuring the levels of C-reactive protein (CRP), secreted phospholipase A2 type IIA and its activity and serum amyloid A.

CRP, a plasma protein synthetized by the liver, is a sensitive and dynamic systemic marker of inflammation (Pepys and Hirschfield 2003) which exhibits an independent association with cardiovascular events (Ridker *et al.* 2000, Ridker 2004). The assay of CRP is very sensitive allowing detection of very low concentrations, and increases in CRP within the normal range seem to predict future vascular events in apparently healthy asymptomatic individuals (Ridker *et al.* 2002). Childhood CRP values predict weakly but significantly adult CRP; this association seems to be independent of other metabolic risk factors (Juonala *et al.* 2006b). An increased level of CRP was reported to predict independently progression of carotid artery intima-media thickness (Toprak *et al.* 2011). It has been shown that CRP decreases endothelial nitric oxide synthase expression (Venugopal *et al.* 2002) and it induces the release of biomarkers of endothelial dysfunction, suggesting that CRP has also an active role in endothelial dysfunction (Devaraj *et al.* 2011, Kusche-Vihrog *et al.* 2011). Increased physical activity and cardiorespiratory fitness (Church *et al.* 2002, Borodulin *et al.* 2006, Plaisance and Grandjean 2006) lead to lower circulating levels of C-reactive protein.

Phospholipase A2 enzymes hydrolyze phospholipids to generate lysophospholipids and fatty acids, leading to the activation of various immunoinflammatory processes involved in the pathogenesis of atherosclerosis (Dennis 1994, Hurt-Camejo *et al.* 2001, Mallat *et al.* 2010). They are classified into six main groups based on size, location, function, substrate specificity and calcium requirements. Several studies have shown increased levels of secreted phospholipase A2 type IIA or increased secreted phospholipase A2 activity in patients with cardiovascular disease (Kugiyama *et al.* 1999, Liu *et al.* 2003, Boekholdt *et al.* 2005). There is no convincing evidence that physical activity would be associated with secreted phospholipase A2; in one study, secreted phospholipase A2 type IIA was linked with physical inactivity in females (Rana *et al.* 2011).

Serum amyloid A is also a marker of inflammation; it is mainly synthetized in the liver in times of acute inflammation and in adipose tissue under noninflammatory conditions (Uhlar and Whitehead 1999). The serum level of amyloid A is significantly associated with the risk of cardiovascular events (Ridker *et al.* 2000, Kosuge *et al.* 2007) and with metabolic risk factors (Jylhävä *et al.* 2009). High levels of physical activity have been associated with lower levels of serum amyloid A (Panagiotakos *et al.* 2005, Pitsavos *et al.* 2005).

The anti-inflammatory effect of physical activity might be partly explained by changes in visceral fat mass with a subsequent decreased release of adipokines from adipose tissue (Gleeson *et al.* 2011). Vigorous and/or moderate physical

activity may induce an anti-inflammatory effect by releasing interleukin-6 into the circulation from contracting muscle fibers and subsequently increasing circulating levels of interleukin-10 and interleukin-1 receptor antagonists, by increasing the circulating number of interleukin-10-secreting regulatory T cells, by downregulating Toll-like receptor expression on monocytes and inhibiting the production of pro-inflammatory cytokine, preventing antigen presentation and lowering the expression co-stimulatory molecule, by reducing the circulating numbers of pro-inflammatory monocytes and inhibiting monocyte and/or macrophage infiltration into adipose tissue (Gleeson *et al.* 2011). Physical activity has also been reported to increase the secretion of the adrenal hormones cortisol and adrenaline which also have anti-inflammatory effects (Gleeson *et al.* 2011).

2.5.8. Arginine metabolites

In this thesis, arginine metabolites were studied by measuring asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA). ADMA and SDMA are methylated metabolites of L-arginine; a semi-essential amino acid (Martens-Lobenhoffer and Bode-Boger 2007). ADMA is an endogenous analogue of L-arginine that may interfere with nitric oxide metabolism by acting as a competitive inhibitor of nitric oxide synthase, the enzyme synthesizing nitric oxide (Lu *et al.* 2003). SDMA is an isomer of ADMA that is not directly capable of inhibiting nitric oxide synthase (Cooke 2004), but it may indirectly limit the generation of nitric oxide by reducing the intracellular availability of L-arginine (Beltowski and Kedra 2006), as SDMA may act as a competitor of L-arginine transport (Closs *et al.* 1997, Bode-Boger *et al.* 2006).

ADMA correlates with traditional and nontraditional cardiovascular risk factors and is a strong predictor of cardiovascular events and death not only in patients with chronic kidney disease but also in the general population (Schepers *et al.* 2014). The association between SDMA and cardiovascular disease has been less extensively studied but there does not seem to be any connection (Willeit *et al.* 2015). Physical activity may decrease ADMA levels (Tsarouhas *et al.* 2011), but the association needs to be confirmed. **Table 1** has gathered examples of studies showing the associations of physical activity and cardiorespiratory fitness with cardiovascular disease risk markers.

Table 1. Examples of studies expressing the associations of physical activity and cardiorespiratory fitness on cardiovascular disease risk markers.

Risk marker	Physical activity	Cardiorespiratory fitness
Smoking	Inverse association[1, 2, 3]	Inverse association[4, 5]
Obesity	Inverse association[6, 7]	Inverse association[8, 9]
Blood pressure	No association in normotensive[10] Inverse association in hypertensive[11, 12] Reduces risk of hypertension[11]	Reduces risk of hypertension[13]
LDL cholesterol	Inverse association[14, 15, 16]	Inverse association[17, 18, 19]
oxLDL	Inverse association[20, 21]	Inverse association[17]
Apolipoprotein B	Inverse association[15, 22]	Inverse association[23] No association[24]
Triglycerides	Inverse association[14, 22, 25, 26, 27]	Inverse association[19, 28]
HDL cholesterol	Direct association[14, 16, 25, 26, 29]	Direct association[17, 19, 30]
Apolipoprotein A1	Direct association[31, 32, 33]	-*
Glucose	Inverse association[34, 35]	Inverse association[36, 37]
Insulin	Inverse association[34, 35]	Inverse association[36, 37]
Adiponectin	Direct association[38, 39] Inverse association[40, 41, 42]	Inverse association[43, 44]
Leptin	Inverse association[45, 46, 47]	Inverse association[44, 48]
CRP	Inverse association[42, 49, 50]	Inverse association[49, 51]
Secreted phospholipase A2 type IIA	Inverse association[42]	-*
Serum amyloid A	Inverse association[52, 53] No association[54]	-*
ADMA	Inverse association[55] No association[56]	-*
SDMA	No association[56]	-*

[1](Tuomilehto *et al.* 1987); [2](Marti *et al.* 1987); [3](Kujala *et al.* 2007); [4](Lee and Blair 2002); [5](Benck *et al.* 2017); [6](Ross and Janssen 2001); [7](Yang *et al.* 2007); [8](Pandey *et al.* 2015); [9](Mondal and Mishra 2017); [10](Alpert and Wilmore 1994); [11](Arrol and Beaglehole 1992); [12](Andersen *et al.* 2011); [13](Rankinen *et al.* 2007); [14](Lehtonen and Viikari 1978a); [15](Rönnemaa *et al.* 1980); [16](Wanne *et al.* 1983); [17](Vasankari *et al.* 1998); [18](Jago *et al.* 2010); [19](Breneman *et al.* 2016); [20](Vasankari *et al.* 2000); [21](Park *et al.* 2011); [22](Ahmed *et al.* 2012); [23](Kawano *et al.* 2009); [24](Abdulnour *et al.* 2016); [25](Lehtonen and Viikari 1978b); [26](Välimäki *et al.* 1980); [27](Raitakari *et al.* 1997); [28](Tanisawa *et al.* 2014); [29](Lehtonen and Viikari 1980); [30](Wanne *et al.* 1984); [31](MacAuley *et al.* 1996); [32](Luc *et al.* 2000); [33](Ahmed *et al.* 2011); [34](Rizzo *et al.* 2008); [35](Johannsen *et al.* 2016); [36](Solomon *et al.* 2015); [37](Lidegaard *et al.* 2015); [38](Ischander *et al.* 2007); [39](Ring-Dimitriou *et al.* 2006); [40](Metcalf *et al.* 2009); [41](Kozakova *et al.* 2013a); [42](Rana *et al.* 2011); [43](Agostinis-Sobrinho *et al.* 2017); [44](Martinez-Gomez *et al.* 2012); [45](Franks *et al.* 2003); [46](Esteghamati *et al.* 2010); [47](Jimenez-Pavon *et al.* 2012); [48](Yang *et al.* 2017); [49](Borodulin *et al.* 2006); [50](Plaisance and Grandjean 2006); [51](Church *et al.* 2002); [52](Pitsavos *et al.* 2005); [53](Panagiotakos *et al.* 2005); [54](Verdaet *et al.* 2004); [55](Tsarouhas *et al.* 2011); [56](Niebauer *et al.* 2005).
*No studies found.

2.6. Fatty liver

Non-alcoholic fatty liver disease (later simply called fatty liver) is a major public health concern. The global prevalence of fatty liver is thought to be around 20% (Chalasani *et al.* 2012). It is the most common cause of chronic liver disease in Western countries and it has been predicted to become also the most frequent

indication for liver transplantation by 2030 (Byrne and Targher 2015). The etiology of fatty liver is multifactorial including both genetic and metabolic factors (Dongiovanni *et al.* 2015). Fatty liver is also associated with a wide range of metabolic disturbances and an increased risk of cardiovascular diseases and type 2 diabetes (Targher and Arcaro 2007, Kotronen and Yki-Järvinen 2008, Fabbrini *et al.* 2009). There is evidence that fatty liver is associated with carotid intima-media thickness, coronary calcification, endothelial dysfunction and arterial stiffness (Oni *et al.* 2013). However, it is unclear whether this association is related to excess fat liver storage per se or to the metabolic abnormalities that typically accompany fatty liver. It seems that there is shared genetics behind fatty liver and certain metabolic disturbances such as obesity, dyslipidemia and insulin resistance (Di Costanzo *et al.* 2017). It has been suggested that there might be two different forms of fatty liver disease: one associated mainly with metabolic abnormalities, leading to cardiovascular diseases, and another due primarily to genetic factors, which might carry a higher risk of progressive liver damage (Yki-Järvinen 2016, Di Costanzo *et al.* 2017).

2.6.1. Assessment of fatty liver

Fatty liver can be assessed non-invasively with ultrasound, computed tomography and magnetic resonance imaging, proton magnetic resonance spectroscopy and magnetic resonance elastography. Liver biopsy is the gold standard, but it is invasive and the procedure may result in severe complications (Joy *et al.* 2003). Computed tomography exposes the patient to radiation, lacks sensitivity in detecting small amounts of fat and is susceptible to inter-device variability and furthermore, computed tomography is not clinically reliable in the diagnosis of mild-to-moderate hepatic steatosis (Schwenzer *et al.* 2009). Magnetic resonance offers imaging and spectroscopic methods for quantification of fat with relatively high accuracy without any invasive procedures or radiation exposure (Schwenzer *et al.* 2009). However, these examinations are relatively costly and are not suitable for patients with claustrophobia, implanted electronic devices or certain metal implants (Schwenzer *et al.* 2009).

Ultrasound is accepted as an initial screening approach for fatty liver since it is non-invasive, inexpensive and widely available (Schwenzer *et al.* 2009). It has been shown that ultrasonography has a sensitivity of 60–94% and a specificity of 66–95% in detecting fatty liver (Debongnie *et al.* 1981, Saverymuttu *et al.* 1986). Ultrasound was the method used in this thesis.

2.6.2. Factors affecting fatty liver

Increased alcohol consumption has been associated with liver disease morbidity and mortality (Hart *et al.* 2010). It has been shown that the intake of saturated fat and cholesterol is higher with subjects with fatty liver than without, and conversely, the intakes of polyunsaturated fatty acids, fiber, and the antioxidant vitamins C and E and zinc are lower (Musso *et al.* 2003, Cortez-Pinto *et al.* 2006, Toshimitsu *et al.* 2007). A daily intake of sugar-sweetened soft drinks increases the prevalence of fatty liver compared with milk, diet cola and water (Maersk *et al.* 2012). Increased fructose consumption has also been linked with fatty liver and fructose intake should be limited by decreasing foods and drinks high in added (fructose-containing) sugars (Abdelmalek *et al.* 2010, Vos and Lavine 2013). However, there are also conflicting studies stating that a higher fructose intake is inversely associated with fatty liver (Kanerva *et al.* 2014). It has also been proposed that alterations in the intestinal microbiome may play a role in the development of fatty liver (Mouzaki *et al.* 2013, Raman *et al.* 2013).

Some previous studies have examined the association between physical activity and fatty liver. Vigorous, but not light or moderate physical activity, is favorably associated with fatty liver independent of obesity (Kistler *et al.* 2011, Long *et al.* 2015, Oni *et al.* 2015). Cardiorespiratory fitness has been shown to associate inversely with liver fat in males (Nguyen-Duy *et al.* 2003, McMillan *et al.* 2007, O'Donovan *et al.* 2009, Haufe *et al.* 2010) but no association has been detected in females (Kuk *et al.* 2004, Haufe *et al.* 2010). The role of visceral adipose tissue or waist circumference in mediating the association between cardiorespiratory fitness and fatty liver is controversial as some studies have detected an independent association (McMillan *et al.* 2007, Haufe *et al.* 2010) while in others, the association has disappeared when adjustment is made for these adiposity measures (Nguyen-Duy *et al.* 2003, O'Donovan *et al.* 2009).

3. AIMS OF THE STUDY

The present thesis is based on the findings from the Cardiovascular Risk in Young Finns Study. The purpose was to examine the associations between physical activity and cardiovascular risk factors and early markers of atherosclerosis, and cardiorespiratory fitness and the risk of fatty liver.

The major aims of this thesis were as follows:

1. To study the associations between physical activity and cardiorespiratory fitness and selected cardiovascular risk markers among Finnish adults.

2. To examine the effect of physical activity in children and young adults on carotid artery intima media thickness and elasticity measured 21 years later.

3. To evaluate the effects of cardiorespiratory fitness and physical activity on metabolic health by examining the association between peak oxygen uptake and the metabolic equivalent (MET) index with fatty liver.

4. PARTICIPANTS AND METHODS

4.1. Description of the Cardiovascular Risk in Young Finns Study

The Cardiovascular Risk in Young Finns Study is a prospective, multicenter follow-up study of atherosclerosis risk factors from youth. The first cross-sectional survey was conducted in 1980. The original sample size was 4,320 children and adolescents aged 3, 6, 9, 12, 15, and 18 years. Of these, 3,596 participated (83.2% of those invited) in 1980 (Raitakari *et al.* 2008). The whole cohort was followed up in 1983, 1986, 2001, 2007 and 2011. The participation rates in the follow-up studies have been relatively high. In 1986, 78% of the original cohort returned the questionnaire and 70% participated in the clinical study. In 2001, 76% from the original cohort returned the questionnaire and 66% participated in the clinical study. In 2007, 66% from the original cohort returned the questionnaire and 65% participated in the clinical study. In 2011, 61% from the original cohort returned the questionnaire and 62% participated in the clinical study. Clinical measurements and physical activity data collections were performed simultaneously in each follow-up session, during the winter months, to minimize seasonal variation. Vascular ultrasound measurements were performed in 2001 and 2007. Cardiorespiratory fitness was assessed during a separate study visit in 2008-2009. Ultrasonic assessment of liver fat accumulation was performed as a part of the follow-up study in 2011.

4.2. Study design and participants

In study I, the associations between physical activity and a range of risk markers of coronary heart disease were studied in adults aged 24 to 39 years. A total of 2,268 participants in 2001 were included in the cross-sectional analyses.

In study II, longitudinal data on the effect of physical activity in youth on adult arterial elasticity was examined. The sample for this analysis included those subjects who took part in the study in 1986 and in 2007 (N=2,416). Participants were aged 9 to 24 years at baseline and 30 to 45 years at follow-up.

In study III, we assessed peak arterial blood pressure, circulatory power and exercise cardiac power with the cycle ergometer exercise test in 538 participants aged 30 to 47 years.

In study IV, the association between cardiorespiratory fitness and fatty liver was studied in 463 adults aged 30 to 47 years. Cardiorespiratory fitness was measured

with a cycle ergometer exercise test as peak oxygen uptake and fatty liver with ultrasonographic imaging.

Summary of the study design is shown in **Figure 1**.

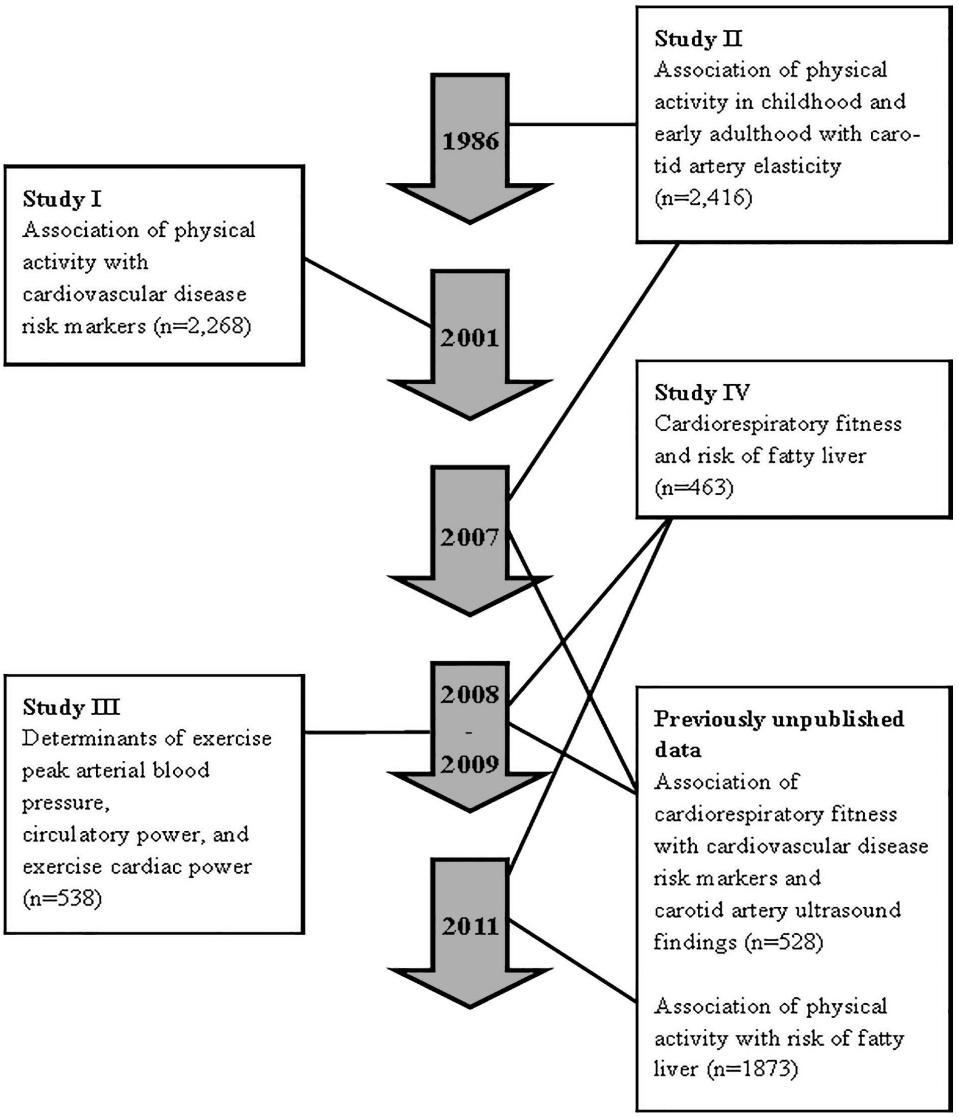

Figure 1. Description of substudies and number of participants.

4.3. Physical activity

Self-reported leisure time physical activity including commuting activity was assessed by a questionnaire (Telama *et al.* 1997). Participants were asked the frequency of their participation in physical activity, its intensity during leisure

time and its duration. In addition, commuting to work and participation in organized physical activity were assessed. Intensity was evaluated by asking how the participant usually performed the physical exercise: (1) usually not becoming out of breath or sweating, (2) becoming out of breath and sweating slightly, (3) becoming out of breath and sweating considerably. Becoming out of breath and sweating were considered to be rigorous physical activity. We found direct Spearman's rank order correlations with the intensity category and the frequencies of engaging in high intensity sports, such as jogging ($r=0.23$, $P<0.001$), ball games ($r=0.18$, $P<0.001$), racket games ($r=0.15$, $P<0.001$) and ice-hockey ($r=0.15$, $P<0.001$). Frequency was assessed by the question: How often do you engage in rigorous physical activity? (1) not at all, (2) once a month, (3) once a week, (4) 2-3 times a week, (5) 4-6 times a week, (6) daily. Duration was assessed by inquiring about the average duration of a single session of physical exercise: (1) under 20 minutes, (2) 20-40 minutes, (3) 40-60 minutes, (4) over 60 minutes. When estimating the physical activity during commuting to the workplace, the length of the journey and whether it was traveled by foot or by bicycle was considered. Subjects could also choose public transportation or their own car. This was asked separately for winter and summer times. Participation in organized physical activity was assessed by the question: Do you participate in organized physical activity? (1) not at all, (2) occasionally, (3) regularly, about once per week, (4) several hours and times per week. The duration of weekly physical activity was assessed by asking how many hours per week the participant expended on rigorous physical activity: (1) not at all, (2) 30-60 minutes, (3) 1 hour, (4) 2-3 hours, (5) 4-6 hours, (6) over 7 hours.

A metabolic equivalent (MET) index for physical activity (later "MET-index") was calculated from the product of intensity*frequency*duration and commuting physical activity (METh/wk). A MET-index for leisure-time physical activity was calculated from the product of intensity*frequency*duration (METh/wk). The coefficients for the variables were estimated from existing tables (Ainsworth *et al.* 1993). One MET is the consumption of 1 kcal of an individual per weight kilogram per hour at rest.

In addition to the MET-index, a sum index of physical activity (PAI) was calculated with five variables; intensity, frequency, duration, participation in organized physical activity and duration of weekly physical activity. The index ranged from 5 to 15. The lowest scores designated passive, and the highest scores were indicative of physical activity (Telama *et al.* 1997, Telama *et al.* 2005).

An experimental study was performed in order to validate the physical activity questionnaire, with 45 young adults (age range 23 to 55, 48% females) being included. The participants filled in the questionnaire, and their physical activity

was measured with accelerometers and pedometers for a period of one week. The MET-index and PAI and their main components, i.e. intensity, frequency, and duration correlated significantly with the volume of movement assessed with accelerometers (r-values 0.26-0.45) and the number of steps measured with pedometers (r-values 0.30-0.49). The Spearman's rank order correlation coefficients derived in this validation study were of the same magnitude as those demonstrated in other similar studies (Tudor-Locke *et al.* 2004).

In addition, step data was collected using validated pedometers in 1934 individuals of the whole study population. Participants wore an Omron Walking Style One (HJ-152R-E) step counter for a period of one week. Similarly, as is the small validation study done in an independent population, significant Spearman's rank order correlations were seen between the number of steps and the MET-index, as well as between the number of steps and individual components of the MET-index **(Table 2)**. The correlation between the number of steps measured with pedometers and the amount of movement measured with ActiGraph accelerometers (GT1M) was r=0.966 (p<0.001, N=45).

Table 2. Spearman's rank order correlations between MET-index, its individual components, and the pedometer measurements of total steps and aerobic steps (aerobic steps are those taken during activities that last for at least ten minutes without interruption).

	Total steps r value	Aerobic steps r value
Frequency of rigorous participation	0.297‡	0.429‡
Intensity level	0.054*	0.100‡
Duration of participation	0.188‡	0.217‡
Commuting during summer	0.238‡	0.297‡
Commuting during winter	0.240‡	0.274‡
MET-index (METh/wk)	0.323‡	0.423‡

*P<0.05; ‡P<0.0001; MET=Metabolic equivalent; Reproduced from Annals of Medicine (Study I) with permission of Taylor and Francis.

The intensity question and its validity were also examined. The associations between intensity and different sports were studied by calculating Spearman's correlation coefficients (**Table 3**). Participants were asked about their engagement in gym exercise, muscle strengthening exercise, ball games, racket games, ice-hockey, aerobics, dancing, high jump or long jump or pole vault, combat sports, walking, jogging, rowing, skiing, swimming or bicycling during leisure-time or commuting.

Table 3. Spearman's rank order correlations between intensity and different sports.

	Intensity r value
Gym exercise	0.10†
Muscle strengthening exercise	0.12†
Ball games	0.18‡
Racket games	0.15‡
Ice-hockey	0.15‡
Aerobics	0.06
Dancing	-0.08*
High jump or long jump or pole vault	-0.01
Combat sports	0.10†
Walking	-0.24‡
Jogging	0.23‡
Rowing	0.04
Skiing	0.10†
Swimming	-0.05
Bicycling	0.13‡

*P<0.05; †P<0.01; ‡P<0.0001

These data demonstrated that the intensity question had higher correlations with high intensity sports. This provided evidence that the intensity question had good construct validity which according to Tudor-Locke et al. (Tudor-Locke *et al.* 2004) can be evaluated by measuring how well the instrument (intensity question in this case) corresponds with other measures of theoretically-related parameters.

4.4. Cardiorespiratory fitness

Cardiorespiratory fitness was assessed during a separate study visit in 2008-2009. Participants from study centers in Turku and Tampere were invited, and 538 completed the cardiorespiratory fitness test (age range 30-47 years, 52% females). The exercise tests were performed on electronically braked cycle ergometers (Lode Corival 906900, Lode BV, Groningen, Netherlands) according to the American Thoracic Society guidelines and the American College of Chest Physicians Joint Statement on Cardiopulmonary Exercise Testing (American Thoracic Society and American College of Chest Physicians 2003). In brief, after a 10-minute rest and a warm-up period of 10–60 seconds, the subjects performed an incremental test with 1-minute intervals and increments of 15 W/minute for females and 20 W/minute for males until exhaustion limited maximal power output. Otherwise, objective test termination criteria (established in ref. (American Thoracic Society and American College of Chest Physicians 2003)) were applied by the observers. Standard 12-lead electrocardiography was recorded during the test (Corina ECG amplifier and CardioSoft acquisition software ver. 4.2, GE Medical Systems, Freiburg, Germany). Blood pressure was

measured with the cuff method by means of auscultation. Peak heart rate was defined as the maximal heart rate achieved during the exercise. The rating of perceived exertion was obtained using the Borg category scale (Borg 1982). Breath-by-breath measurements of oxygen uptake and carbon dioxide output as well as an assessment of ventilatory parameters were performed with computerized analyzers (V-max 29C, SensorMedics, Yorba Linda, CA, USA and Jaeger Oxycon Pro, VIASYS Healthcare GmbH, Hoechberg, Germany). As two different analyzers were used, the linearity of the gas measurement devices was checked prior to the analyses with a metabolic simulator (VacuMed Syringe model 17050 calibration kit, Ventura, CA, USA) at ventilation levels of 34–90 liters/min. This calibration method has been proven valid (Huszczuk *et al.* 1990). The correlation between expected and measured values in this test range revealed the good linearity of both devices (R^2 values for oxygen uptake and carbon dioxide output measurements > 0.99). The inter-instrument difference varied from 1% to 3% for tidal volume measurements, oxygen uptake and carbon dioxide output which is within the expected range (American Thoracic Society and American College of Chest Physicians 2003). After calibration measurements, oxygen and carbon dioxide test gases were used daily in order to check the reproducibility of the measurements. The final tabular and graphic averaged data were collected at 30-second intervals as recommended (American Thoracic Society and American College of Chest Physicians 2003). Peak oxygen uptake was determined as the oxygen uptake during the last 30 seconds of exercise.

While all subjects carried out the cycle ergometer test and reached the objective maximum (based on respiratory quotient > 1.0, peak heart rate at the range of expected maximum of heart rate ± 10 or Borg category scale >19), we decided to exclude gas measurement data for 8 subjects (1.5% of tested individuals). The reason for this was technical error such as mask leakage or computer failure. In one subject, the gas measurements had to be interrupted, as the subject could not tolerate to wear the mask.

4.5. Physical examination and questionnaires

Weight was measured in light clothing without shoes with a digital scale, with an accuracy of 0.1 kg and height was measured by a wall-mounted statiometer with 0.1 cm accuracy. BMI was calculated with the formula: BMI=weight (kg)/[height (m)]^2. Waist circumference was measured midway between iliac crest and the lowest rib at the midaxillary line with a non-stretchable plastic covered cloth measuring tape to an accuracy of 0.1 cm. Blood pressure was measured with a random zero sphygmomanometer. Korotkoff's fifth sound was used as the sign

of diastolic blood pressure (DBP) and the first sound as the sign of systolic blood pressure (SBP). Readings to the nearest even number of millimeters of mercury were taken at least three times on each subject. The average of these measurements was used in the analyses. Heart rate was measured simultaneously.

Total energy intake was assessed by a food frequency questionnaire. Alcohol consumption was enquired by standardized questionnaires and calculated in standard doses (12 g pure ethanol) per day by dividing the total number of doses consumed per week (0.33 l doses of beer or cider, 0.12 l doses of wine and 0.04 l doses of hard liquor) by 7. Smoking habits were assessed by a self-administered questionnaire beginning at age 12 years. Those smoking daily were considered as smokers. Pack-years of smoking were calculated. Socioeconomic status was assessed by the level of education (total school years). The use of medications was gathered from self-administered questionnaire.

4.6. Biochemical analyses

Venous blood samples were drawn after an overnight fast.

4.6.1. Lipid and apolipoprotein measurements

All lipid and apolipoprotein determinations were performed using standard methods (Porkka *et al.* 1997, Juonala *et al.* 2004). Serum cholesterol and triglyceride concentrations were determined enzymatically (Olympus System Reagent; Olympus Diagnostica GmbH, Hamburg, Germany) in a clinical chemistry analyzer (AU400; Olympus Optical Ltd, Mishima, Japan). HDL cholesterol was analyzed after precipitation of VLDL and LDL with dextrane sulphate 500 000 (Kostner 1976). The concentration of LDL cholesterol was calculated using the Friedewald formula (Friedewald *et al.* 1972). Subjects with triglycerides above 4 mmol/l were excluded from this analysis. Serum apolipoproteins A1 and B were analyzed immunoturbidometrically (Orion Diagnostica, Espoo, Finland) (Viikari *et al.* 1991, Porkka *et al.* 1997). The oxLDL concentrations were determined using a Mercodia test (Holvoet *et al.* 2008).

4.6.2. Glucose and insulin measurements

Serum glucose concentrations were analyzed enzymatically (Olympus Diagnostica GmbH, Hamburg, Germany). Serum insulin was measured by microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot) (Juonala *et al.* 2004). The HOMA-IR method was used to

estimate insulin resistance as described [(fasting insulin mU/ml x fasting glucose mmol/l)/22.5] (Matthews *et al.* 1985).

4.6.3. Inflammatory markers

Serum high sensitive CRP was analyzed by an automated analyzer (Olympus AU400, Olympus, USA) and a highly sensitive turbidimetric immunoassay kit ("CRP-UL"-assay, Wako Chemicals, Neuss, Germany) (Raitakari *et al.* 2003, Juonala *et al.* 2004). The concentration of secretory phospholipase A2 (sPLA2) was measured with a sandwich-type enzyme-linked immunosorbent assay and sPLA2 activity by a selective fluorometric assay by using fluorescent substrate 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3 phosphomethanol, sodium salt (Interchim, Montluçon, France) (Mallat *et al.* 2007). The minimum detectable activity was 0.10 nmol/min/ml and the intra- and inter-assay coefficient of variation (CV) were <10%. Serum amyloid A concentrations were measured with an ELISA kit with a detection limit of <0.004 mg/L (Human serum amyloid A, Biosource International, Camarillo, CA).

4.6.4. Arginine metabolites

Serum l-arginine, ADMA and SDMA levels were determined by a high-performance liquid chromatography method with precolumn *o*-phthaldialdehyde derivatization (Saarelainen *et al.* 2008). The precision (CV) for a plasma pool (n=77) for arginine, ADMA and SDMA within series was 7.5%, 5.7% and 6.5% and between series 12.9%, 10.6% and 12.1%, respectively.

4.6.5. Adipokines

The serum leptin and adiponectin concentrations were determined in duplicate using a commercially available double-antibody radioimmunoassay kit (Human Leptin RIA Kit and Human Adiponectin RIA Kit; Linco Research, St. Charles, MO) (Hakanen *et al.* 2004). The inter-assay coefficient of variation was 5.5-11.9% for adiponectin and 7-9% for leptin.

4.6.6. Liver enzymes

Serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and GT concentrations were measured by enzymatic methods (ALAT, ASAT and GT System Reagent, Beckman Coulter Biomedical, Ireland) on an automatic analyzer (AU400, Olympus, Japan) (Suomela *et al.* 2014).

4.7. Ultrasound studies

4.7.1. Carotid artery ultrasound

The left common carotid artery was scanned by ultrasound technicians following a similar, standardized protocol in the five study centers (Juonala *et al.* 2008). A high-resolution ultrasound imaging device (Sequoia 512, Acuson, CA) with a 13MHz linear-array transducer was used to assess arterial elasticity and intima-media thickness (IMT). The image was focused on the posterior (far) wall and gain settings were used to optimize image quality. Measurements were made offline from stored digital images. One reader blinded to the subjects' details analyzed all of the ultrasound scans. To assess carotid artery elasticity indices and intima-media thickness, the best-quality cardiac cycle was selected from a continuous 5-second image file. From this image, at least four measurements of the common carotid far wall were taken approximately 10 mm proximal to the bifurcation to derive mean carotid intima-media thickness. The common carotid diameter was measured at least twice during end diastole and end systole. The means of the measurements were used as the end-diastolic and end-systolic diameters (Raitakari *et al.* 2003, Juonala *et al.* 2005). Ultrasound and concomitant brachial blood pressure measurements were used to calculate the following indices of arterial elasticity: carotid artery distensibility (Cdist; %/10 mmHg) = $[(D_s–D_d)/D_d]/(P_s–P_d)$, Young's elastic modulus (kPa) = $[(P_s–P_d)D_d^2]/[2(D_s–D_d)IMT]$ and stiffness index (unitless) = $\ln(P_s/P_d)/[(D_s–D_d)/D_d]$ where D_d is diastolic diameter, D_s is systolic diameter, P_s is systolic blood pressure, P_d is diastolic blood pressure (Salomaa *et al.* 1995). Note that higher levels of both the Young's elastic modulus and stiffness index represent decreased arterial elasticity while a higher carotid artery distensibility value represents a better arterial elasticity.

4.7.2. Liver ultrasound

Ultrasonographic imaging of the liver was performed in 2011 by using Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA) with 4.0 MHz adult abdominal transducers. Evaluation of fatty liver was performed according to the liver-to-kidney contrast, parenchymal brightness, deep beam attenuation and bright vessel walls (Saverymuttu *et al.* 1986). The liver-to-kidney contrast was determined as a clear ultrasonographic contrast between the hepatic parenchyma and the right renal cortex. Parenchymal brightness was defined as hyperechogenic liver tissue with fine, tightly packed echoes on ultrasound examination. Deep beam attenuation was defined as an indistinguishable diaphragm line and bright vessel walls were considered to represent the presence

of the brightly visible walls of small intrahepatic vessels. According to these criteria, the presence of fatty liver was assessed visually from non-blinded images by a trained ultrasonographer. According to the ultrasonic parameters, the participants were categorized into two groups; those with (N=80) and without (N=383) fatty liver (prevalence of fatty liver 17.3 %).

4.8. Statistical analyses

The difference in risk marker levels between sexes was tested by using a t-test, non-parametric median test, generalized linear model or chi-square test, as appropriately (**Tables 5-9 and 13**). To study the effect of secular change on different variables, repeated measurements modeling was employed separately in males and females and Bonferroni corrected P values were calculated. The Bonferroni corrected statistically significant P value was 0.0005 in **Tables 5-9** and 0.0008 in **Table 13.** As the outcome variables, all the variables mentioned in **Tables 5-9**, measured at two or three time points were modeled. The covariates included age and study years. In the MET-index, daily smoking, pack-years of smoking, alcohol consumption and education repeated measurements modeling could not be used as a result of skewness, which could not be normalized. Therefore, changes in values of these variables between measurement times (eg. ΔMET=MET-index 2001 - MET-index 2007) was calculated and these differences were tested with sequentially rejective Bonferroni test.

In study I, the participants were divided into age and sex specific MET-index tertiles. The tertile cut-points by age group and sex are shown in **Table 4**. The same cut-points were used in 2007 and 2011 to demonstrate the effect of time on different risk markers (**Table 10**). The associations between tertiles of physical activity and risk markers were evaluated by calculating Spearman's correlation coefficients. Adjustment for waist circumference and smoking was done by calculating partial correlation coefficients. Regression modeling was used to assess whether a difference existed between males and females in terms of physical activity. To examine whether the associations between physical activity and risk markers were similar in males and females, statistical interactions were tested with a regression model. The regression models included each risk variable as the outcome variable and MET-index, sex and MET-index*sex interaction term as independent variables. If the MET-index*sex interaction term was significant ($p<0.05$), the association between a risk marker and physical activity tertiles was calculated separately for males and females.

Table 4. MET-index (METh/wk) tertile cut-points in each age group in males and females.

Females		
Age	33.3 percentile	66.7 percentile
24	12	28
27	6	20
30	9	21
33	5	20
36	5	20
39	5	20

Males		
Age	33.3 percentile	66.7 percentile
24	10	33
27	4	22
30	5	29
33	3	20
36	3	20
39	3	20

MET=Metabolic equivalent; Reproduced from Annals of Medicine (Study I) with permission of Taylor and Francis.

In study II, the 1986 leisure-time MET-index could not be normalized due to the discontinuity and it was therefore categorized into tertiles that reflected meaningful differences in activity levels to ease the interpretation of the regression coefficients. Similar relationships were obtained when the continued leisure-time MET-index was used in the regression models in place of the categorized index. The tertile cut-points were 3 METh/wk and 12 METh/wk. For example, a value of 3 METh/wk corresponds to moderate intensity physical activity for 60 minutes once a week; walking for 1 h at a speed of 4 km/h. Similarly, 12 METh/wk corresponds to 4 h/wk of moderate intensity physical activity or 1-2 h/wk of vigorous physical activity (running for 1.5 h at the speed of 8 km/h). Males and females aged 9 to 15 years were analyzed separately because there was an interaction between sex and leisure-time MET-index tertiles when carotid artery distensibility measured in 2007 (P=0.047) was the explanatory variable. In young adults, there was no sex-by-MET-index interaction (P=0.23) and thus males and females were combined in the analyses. The association between 1986 leisure-time MET-index tertiles and carotid artery elasticity indices and intima-media thickness were examined separately for children (aged 9 to 15 years) and young adults (aged 18 to 24 years) using multivariate regression analysis. Age in 2007, sex, BMI in 1986 and in 2007, systolic blood pressure in 1986 and in 2007, smoking in 1986 and in 2007, HDL cholesterol in 1986 and in 2007, LDL cholesterol in 1986 and in 2007, triglycerides in 1986 and in 2007, insulin in 1986 and in 2007, glucose in 1986 and in 2007, 21-year change in leisure-time MET-indexes (ΔMET) and leisure-

time MET-index in 2007 were used to adjust the analyses. Due to the skewness of triglyceride and insulin levels, Young's elastic modulus and stiffness index, a natural logarithm transformation was performed. To illustrate the associations between leisure-time MET-index tertiles and carotid artery distensibility in 2007 (**Figures 2 and 3**), the adjusted means (SE) were calculated by using ANCOVA with Tukey-Kramer adjusted multiple comparison.

Risk ratios (RR) between physical activity and fatty liver were examined with linear multivariable Poisson regression analysis **(Table 12)**. RR for 1 unit increase in MET-index (METh/wk) and fatty liver was also analyzed using age, sex, BMI, waist circumference, pack-years of smoking, alcohol consumption, HDL cholesterol, LDL cholesterol, triglycerides, insulin, glucose, hs-CRP and physical activity as covariates. Also quadratic multivariable Poisson regression analysis was used to examine whether a non-linear association existed between physical activity and fatty liver. This association was not found (data not shown). To study the prevalence of fatty liver in different physical activity groups, the subjects were first divided into sex and age specific quartiles according to the MET-index. The number of participants in the MET-index quartiles was 426, 444, 490 and 519 from the lowest to the highest MET-index quartile, respectively. Secondly, a similar physical activity classification according to quartiles was also used among obese participants (N=277, 264, 280, and 291 from the lowest to the highest MET-index quartile, respectively). Third, to study the combined effect of physical activity and obesity on fatty liver, low and high activity groups were formed using age and sex specific median for MET-index as the cut-off point. The median in low active group was 3 METh/wk corresponding to walking for 1 h at a speed of 4 km/h once a week and 31 METh/wk in the high active group corresponding to running for 1.5 h at a speed of 8 km/h 5 times a week. Obese and non-obese participants were determined according to sex specific cut-off points for waist circumference according the criteria of central obesity by the International Diabetes Federation (94 cm for males and 80 cm for females; prevalence of obesity 61.4%). The difference in fatty liver between MET-index quartiles was studied with logistic regression model (**Figure 4**). In **Figures 4 and 6**, logistic regression was used to calculate pre-specified contrasts to examine how an increase from one MET-index quartile to the next influences the prevalence of fatty liver (i.e. Q1 vs. Q2, Q2 vs. Q3 and Q3 vs. Q4). In **Figure 5**, the pre-specified comparisons were performed using the Chi-Square test between MET-index medians within the obese and non-obese groups.

In study III, the Pearson product–moment correlation coefficient was calculated. After non-parametric analyses, variables potentially explaining peak oxygen uptake were entered into a multivariable linear regression model. The original covariates in the models were sex, age, height, weight, waist-to-hip ratio,

physical activity index, smoking, fasting insulin and glucose, and the anamnestic use of antihypertensive treatment. Values for insulin and glucose were log-transformed before analyses, because of the skewed distributions.

The participants were divided into age and sex specific cardiorespiratory fitness tertiles in order to examine the association between cardiorespiratory fitness and cardiovascular risk markers. The associations between tertiles of cardiorespiratory fitness and risk markers were estimated by calculating Spearman's correlation coefficients. Adjustment for waist circumference and smoking was done by calculating partial correlation coefficients. To examine whether the associations between cardiorespiratory fitness and risk markers were similar in males and females, statistical interactions were tested by the regression model. The regression models included each risk variable as the outcome variable and cardiorespiratory fitness, sex and cardiorespiratory fitness*sex interaction term as independent variables. If the cardiorespiratory fitness*sex interaction term was significant ($p<0.05$), the association between a risk marker and cardiorespiratory fitness tertiles was calculated separately for males and females.

The association between cardiorespiratory fitness and carotid artery elasticity indices and intima-media thickness were examined using linear and quadratic multivariate regression analysis. Age, sex and BMI were used to adjust the analyses. No non-linear associations was found (data not shown).

In study IV, males and females were analyzed combined as there was no sex-by-cardiorespiratory fitness interaction when fatty liver was the outcome variable. The interaction was tested with logistic regression model. Risk ratios (RR) between cardiorespiratory fitness and fatty liver were examined with linear multivariable Poisson regression analysis (**Table 17**). RR for 1 unit increase in VO_{2peak} and fatty liver was also analyzed using age, sex, BMI, waist circumference, pack-years of smoking, alcohol consumption, HDL cholesterol, LDL cholesterol, triglycerides, insulin, glucose, hs-CRP and physical activity as covariates. Also quadratic multivariable Poisson regression analysis was used to examine whether a non-linear association existed between cardiorespiratory fitness and fatty liver. This association was not found (data not shown). The subjects were first divided into sex and age specific quartiles according to peak oxygen uptake when evaluating the prevalence of fatty liver in different cardiorespiratory fitness groups. The numbers of participants in the cardiorespiratory fitness quartiles were 127, 134, 131 and 136 from the lowest to the highest cardiorespiratory fitness quartile, respectively. Secondly, a similar cardiorespiratory fitness classification according to quartiles was also used among obese participants (N=65, 71, 67, and 74 from the lowest to the highest

cardiorespiratory fitness quartile, respectively). Third, to study the combined effect of cardiorespiratory fitness and obesity on fatty liver, low and high fit groups were formed using age and sex specific median for peak oxygen uptake as the cut-off point. The median peak oxygen uptake was 25 ml/kg/min in low fit, corresponding to running approx. 8 min/km whereas it was 36 ml/kg/min in high fit, corresponding to running approx. 6 min/km. Obese and non-obese participants were determined according to sex specific cut-off points for waist circumference according to the criteria of central obesity by the International Diabetes Federation (94 cm for males and 80 cm for females; prevalence of obesity 59.6%). The difference in fatty liver between cardiorespiratory fitness quartiles was studied with logistic regression model (**Figure 7**). In **Figures 7 and 9**, logistic regression was used to calculate pre-specified contrasts to examine how an increase from one cardiorespiratory fitness quartile to the next would influence the prevalence of fatty liver (i.e. Q1 vs. Q2, Q2 vs. Q3 and Q3 vs. Q4). In **Figure 8**, the pre-specified comparisons were performed using Chi-Square test between cardiorespiratory fitness medians within the obese and non-obese groups.

The associations between physical activity and cardiorespiratory fitness and risk markers were studied by calculating Spearman's correlation coefficients between risk markers and physical activity and cardiorespiratory fitness tertiles. Adjustment for waist circumference and smoking was made by calculating partial correlation coefficients.

Statistical analyses were done in studies I, II and IV with the Statistical Analysis System software version 9.2 and 9.4, and Statistica for Windows 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA) and PASWS Statistics 18.0 for Windows (SPSS Inc., Chicago, Illinois, USA) in study III. Statistical significance was inferred at a 2-tailed probability value <0.05.

4.9. Ethics

The Cardiovascular Risk in Young Finns Study was approved by the Joint Commission on Ethics of University of Turku and Turku University Hospital. Participants gave written informed consent, and their parents provided it for the under-aged participants.

5. RESULTS

5.1. Physical activity

Participant characteristics in 2001, 2007 and 2011 are shown in **Tables 5-8**.

Table 5. Characteristics of participants in 2001, 2007 and 2011. Values are mean and standard deviation, unless stated otherwise.

		Females		Males	
		Mean	SD	Mean	SD
MET-index (median, range)					
	2001	14.0	0-111	12.0	0-120
	2007	13.9	0-159	11.8	0-163
	2011	19.5	0-151	18.7	0-145
Leisure-time MET-index (median, range)					
	2001	8.3	0-110	8.3	0-93
	2007	8.3	0-93	8.3	0-93
	2011	11.8	0-93	11.8	0-93
Age (years)					
	2001	31.6	5.0	31.5	5.0
	2007	37.5	5.0	37.4	5.0
	2011	41.5	5.0	41.4	5.0

MET=Metabolic equivalent.

Participants had increased their physical activity during the ten years of follow-up ($P<0.05$). Females had been more active than males. This difference was due to the fact that females were more active commuters. There was no significant difference in the MET-index when that was calculated based only on leisure-time physical activities (**Table 5**). For instance in 2001, 18.2 % of females reported that they walked and 25.1 % bicycled to work during the summer time. During winter time, 25.0 % of females walked and 4.2 % bicycled to work; the corresponding values for males were 9.4 % walking and 7.4 % cycling to work during winter time.

Table 6. Characteristics of participants in 2001, 2007 and 2011 continue. Values are mean and standard deviation, unless stated otherwise.

		Females		Males	
		Mean	SD	Mean	SD
Waist circumference (cm)					
	2001	79	11	90	11
	2007	84	13	94	12
	2011	88	14	97	13
BMI (kg/m2)					
	2001	24.3	4.4	25.8	3.9
	2007	25.4	5.1	26.7	4.2
	2011	26.1	5.5	27.0	4.4
Systolic blood pressure (mmHg)					
	2001	113	12	122	12
	2007	117	14	126	13
	2011	116	14	123	13
Diastolic blood pressure (mmHg)					
	2001	69	10	73	11
	2007	73	11	79	11
	2011	72	10	78	11
Heart rate/min					
	2001	68	9	65	9
	2007	69	9	67	10
	2011	68	10	66	11
Daily smokers (%)					
	2001	18		28	
	2007	15		23	
	2011	14		18	
Pack-years of smoking (years)					
	2001	2.1	4.5	4.6	7.5
	2007	2.8	5.7	5.7	9.2
	2011	3.8	7.1	7.3	11.0
Alcohol consumption (dose per day)					
	2001	0.5	0.8	1.2	1.5
	2007	0.5	0.7	1.4	1.8
	2011	0.5	0.7	1.2	1.5
Education (school years)					
	2001	14.9	3.0	14.2	3.1
	2007	15.8	3.4	14.8	3.6
	2011	15.7	3.4	14.9	3.8

BMI=Body mass index. All differences between sexes $P<0.0001$, except in daily smoking in 2011.

During the follow-up, both sexes had increased significantly their waist circumference ($P<0.05$) and education level ($P<0.0001$). Daily smoking ($P<0.0001$) had decreased.

Table 7. Characteristics of participants in 2001, 2007 and 2011 continue. Values are mean and standard deviation.

	Females		Males	
	Mean	SD	Mean	SD
Total cholesterol (mmol/l)				
2001	5.1	0.9	5.3	1.0
2007	4.9	0.9	5.2	0.9
2011	5.1	0.9	5.3	1.0
LDL cholesterol (mmol/l)				
2001	3.2	0.8	3.4	0.9
2007	2.9	0.7	3.3	0.8
2011	3.1	0.8	3.4	0.9
HDL cholesterol (mmol/l)				
2001	1.4	0.3	1.2	0.3
2007	1.4	0.3	1.2	0.3
2011	1.4	0.3	1.2	0.3
LDL/HDL-ratio (unitless)				
2001	2.4	0.8	3.1	1.2
2007	2.2	0.8	2.9	1.0
2011	2.3	0.8	3.0	1.0
oxidized LDL (U/l)				
2001	79.4	23.4	88.6	25.9
Triglycerides (mmol/l)				
2001	1.2	0.7	1.5	1.0
2007	1.2	0.7	1.7	1.1
2011	1.1	1.2	1.6	1.2
ApoA1 (g/l)				
2001	1.6	0.3	1.4	0.2
2007	1.7	0.3	1.5	0.2
2011	1.7	0.2	1.5	0.2
ApoB (g/l)				
2001	1.0	0.2	1.1	0.3
2007	0.9	0.2	1.1	0.3
2011	1.0	0.3	1.2	0.3
ApoB/ApoA1-ratio (unitless)				
2001	0.7	0.2	0.8	0.2
2007	0.6	0.2	0.8	0.2
2011	0.6	0.2	0.8	0.2

LDL= low density lipoprotein, HDL=high density lipoprotein, ApoA1=apolipoprotein A1, ApoB=apolipoprotein B. All differences between sexes P<0.0001.

Total cholesterol (P<0.0001), LDL cholesterol (P<0.0001), LDL/HDL-ratio (P<0.0001), apolipoprotein B (P<0.0001) and ApoB/ApoA1-ratio (P<0.0001) had decreased and apolipoprotein A1 (P<0.001) had increased in both sexes during the follow-up. HDL cholesterol levels increased only in females (P<0.05) and triglycerides decreased in males (P<0.05).

Table 8. Characteristics of participants in 2001, 2007 and 2011 continue. Values are mean and standard deviation.

		Females		Males	
		Mean	SD	Mean	SD
Glucose (mmol/l)					
	2001	4.9	0.7	5.2	1.0
	2007	5.2	0.8	5.5	1.0
	2011	5.3	1.0	5.5	0.8
Insulin (mU/l)					
	2001	7.9	6.0	7.7	5.8
	2007	8.7	8.3	10.2	22.6
	2011	9.5	15.6	10.6	11.7
HOMA-IR					
	2001	1.8	1.9	1.9	2.1
	2007	2.1	3.1	2.6	4.6
	2011	2.6	8.3	2.8	4.4
hs-CRP (mg/l)					
	2001	2.3	4.4	1.5	3.3
	2007	2.1	3.6	1.6	4.1
	2011	1.8	2.7	1.6	3.8
Serum Amyloid A-1 (µg/l)					
	2001	25.6	88.8	20.8	77.7
Homocysteine (µmol/l)					
	2001	9.0	3.3	10.8	4.0
sPLA2 type IIA (ng/ml)					
	2001	4.7	3.2	3.0	2.3
sPLA2 activity (nmol/ml/min)					
	2001	1.8	0.6	1.4	0.5
Leptin (ng/ml)					
	2001	15.9	10.2	5.5	4.3
Adiponectin (µg/ml)					
	2001	11.0	4.5	7.4	3.3
	2007	12.2	5.4	7.7	3.7
ADMA (µmol/l)					
	2001	0.6	0.2	0.6	0.1
SDMA (µmol/l)					
	2001	0.4	0.1	0.4	0.1
L-Arginine (µmol/l)					
	2001	116.5	38.4	111.1	37.8

HOMA-IR=homeostasis model assessment of insulin resistance; hs-CRP=high sensitive C-reactive protein; sPLA2=phospholipase A; ADMA=asymmetric dimethyl arginine; SDMA=symmetric dimethyl arginine. All differences between sexes $P<0.0001$, except for insulin, HOMA-IR in 2001 and 2007, hs-CRP in 2011, ADMA, and L-Arginine.

Glucose levels decreased significantly in females during the follow-up ($P<0.05$). Insulin levels and HOMA-IR increased significantly in males ($P<0.05$). Adiponectin had increased in both sexes during follow-up ($P<0.05$).

Table 9. Characteristics of carotid artery ultrasound findings in participants in 2001 and 2007. Values are mean and standard deviation.

		Females		Males	
		Mean	SD	Mean	SD
Intima-media thickness (mm)					
	2001	0.57	0.08	0.59	0.10
	2007	0.61	0.09	0.64	0.11
Carotid artery distensibility (%/10mmHg)					
	2001	2.3	0.8	2.0	0.7
	2007	2.0	0.7	1.7	0.6
Young's elastic modulus (kPa)					
	2001	327	174	391	168
	2007	380	1025	430	267
Stiffness index (unitless)					
	2001	5.3	2.3	5.6	2.0
	2007	6.6	17.7	6.5	3.4

All differences between sexes $P<0.0001$.

In six years, the intima-media thickness had significantly increased in both sexes ($P<0.05$).

5.1.1. The associations between physical activity and cardiovascular disease risk markers

Physical activity was inversely associated with waist circumference, BMI, resting heart rate, daily smoking, pack-years of smoking, serum triglycerides, apolipoprotein B, apolipoprotein B/apolipoprotein A1 ratio, glucose, insulin, HOMA-IR, and hs-CRP in all three follow-up studies. When controlling for waist circumference and daily smoking, the association remained significant with resting heart rate, insulin, HOMA-IR and hs-CRP. The detailed results are shown in **Table 10**.

Interaction analysis suggested significant sex differences in the relations of physical activity with pack-years of smoking (stronger in males) and adiponectin (only in females) in each follow-up year. The detailed results are shown in **Table 11**.

Table 10. Associations between physical activity level (Low-Moderate-High) and selected cardiovascular disease risk markers in 2001, 2007 and 2011.

		MET-index tertiles									
		Low*		Moderate†		High‡		P value	P value adjusted for waist	P value adjusted for waist and smoking	P value sex-interaction term
		mean	(95% CI)	mean	(95% CI)	mean	(95% CI)				
Age (years)											
	2001	31.8	(31.4-32.1)	31.3	(30.9-31.7)	31.6	(31.2-31.9)				
	2007	37.1	(36.7-37.4)	38.1	(37.7-38.5)	37.9	(37.5-38.3)				
	2011	41.3	(40.9-41.7)	42.0	(41.6-42.4)	41.8	(41.5-42.2)				
Waist circumference (cm)											
	2001	86	(85-87)	85	(84-86)	82	(82-83)	<0.0001			0.51
	2007	90	(89-91)	88	(87-89)	87	(86-88)	<0.001			0.88
	2011	94	(92-95)	92	(90-93)	90	(89-91)	<0.0001			0.66
BMI (kg/m2)											
	2001	25.3	(25.0-25.6)	25.0	(24.7-25.3)	24.6	(24.3-24.8)	<0.01			0.10
	2007	26.4	(26.0-26.8)	25.8	(25.5-26.2)	25.6	(25.3-25.9)	<0.05			0.27
	2011	27.1	(26.6-27.5)	26.5	(26.0-26.9)	25.9	(25.6-26.2)	<0.001			0.13
Systolic blood pressure (mmHg)											
	2001	116	(115-117)	117	(116-118)	117	(116-118)	0.19			0.70
	2007	120	(119-121)	121	(120-122)	121	(120-122)	0.75			0.57
	2011	119	(118-120)	119	(118-121)	118	(117-119)	0.13			0.69
Diastolic blood pressure (mmHg)											
	2001	70	(70-71)	72	(71-72)	71	(70-72)	0.64			0.33
	2007	75	(75-76)	76	(75-77)	76	(75-77)	0.41			0.07
	2011	75	(74-76)	75	(74-76)	74	(73-75)	<0.05	0.93		0.79
Resting heart rate/min											
	2001	68	(68-69)	67	(66-68)	65	(64-65)	<0.0001	<0.0001	<0.0001	<0.05
	2007	70	(69-71)	69	(68-69)	66	(65-67)	<0.0001	<0.0001	<0.0001	0.10
	2011	68	(68-69)	67	(67-68)	64	(64-65)	<0.0001	<0.0001	<0.0001	<0.05
Daily smokers (%)											
	2001	30	(27-33)	23	(20-26)	14	(12-17)	<0.0001	<0.0001		0.17
	2007	24	(21-27)	16	(13-19)	12	(10-15)	<0.0001	<0.0001		<0.01
	2011	22	(18-25)	14	(11-17)	9	(7-12)	<0.0001	<0.0001		<0.05
Pack-years of smoking (years)											
	2001	4.2	(3.7-4.7)	2.9	(2.5-3.4)	1.7	(1.4-2.0)	<0.0001	<0.0001		<0.0001
	2007	4.8	(4.2-5.5)	3.5	(2.9-4.1)	3.1	(2.7-3.6)	<0.001	<0.01		<0.0001
	2011	6.7	(5.7-7.8)	5.0	(4.2-5.8)	3.5	(2.9-4.1)	<0.001	<0.01		<0.05

		MET-index tertiles									
		Low*		Moderate†		High‡		P value	P value adjusted for waist	P value adjusted for waist and smoking	P value sex-interaction term
		mean	(95% CI)	mean	(95% CI)	mean	(95% CI)				
Alcohol consumption (dose per day)											
	2001	1.8	(1.8-1.9)	1.9	(1.8-1.9)	1.9	(1.8-1.9)	0.31			0.15
	2007	1.0	(0.9-1.1)	0.9	(0.8-1.0)	1.0	(0.9-1.1)	0.52			0.51
	2011	0.8	(0.7-0.9)	0.8	(0.7-0.9)	0.8	(0.7-0.8)	0.16			0.09
Education (school years)											
	2001	14.1	(13.9–14.3)	14.7	(14.5–14.9)	15.0	(14.8–15.2)	0.0001	0.0001	0.0001	0.17
	2007	15.0	(14.7-15.2)	15.6	(15.3-15.8)	15.9	(15.7-16.2)	<0.0001	<0.0001	<0.0001	<0.01
	2011	15.2	(14.9-15.4)	15.3	(15.0-15.6)	15.8	(15.5-16.1)	<0.01	<0.01	0.14	0.25
Total cholesterol (mmol/l)											
	2001	5.24	(5.17-5.32)	5.16	(5.09-5.24)	5.10	(5.02-5.17)	<0.01	0.22		0.09
	2007	5.06	(5.00-5.13)	5.03	(4.95-5.10)	5.02	(4.95-5.09)	0.26			<0.05
	2011	5.27	(5.19-5.35)	5.12	(5.04-5.19)	5.15	(5.08-5.22)	0.06			0.91
LDL cholesterol (mmol/l)											
	2001	3.36	(3.29-3.42)	3.27	(3.20-3.33)	3.22	(3.16-3.29)	<0.01	0.05		0.18
	2007	3.10	(3.04-3.16)	3.08	(3.02-3.14)	3.07	(3.02-3.13)	0.56			0.08
	2011	3.35	(3.28-3.42)	3.23	(3.16-3.30)	3.24	(3.18-3.30)	0.06			0.53
HDL cholesterol (mmol/l)											
	2001	1.27	(1.25-1.29)	1.27	(1.25-1.30)	1.33	(1.31-1.35)	0.0001	<0.01	<0.05	0.59
	2007	1.32	(1.29-1.34)	1.33	(1.30-1.35)	1.36	(1.34-1.39)	<0.05	0.53		0.87
	2011	1.30	(1.27-1.33)	1.30	(1.28-1.33)	1.37	(1.34-1.39)	<0.001	0.08		0.54
LDL/HDL-ratio (unitless)											
	2001	2.8	(2.7-2.9)	2.7	(2.6-2.8)	2.6	(2.5-2.7)	<0.0001	<0.001	<0.01	0.27
	2007	2.5	(2.4-2.6)	2.5	(2.4-2.5)	2.4	(2.3-2.5)	0.07			0.08
	2011	2.7	(2.6-2.8)	2.6	(2.5-2.7)	2.5	(2.4-2.6)	<0.001	0.07		0.84
oxidized LDL (U/l)											
	2001	86.1	(84.3-88.0)	83.7	(81.7-85.7)	80.9	(79.0-82.8)	<0.0001	<0.05	<0.05	0.47
Triglycerides (mmol/l)											
	2001	1.4	(1.3-1.5)	1.4	(1.3-1.4)	1.2	(1.2-1.3)	<0.01	0.22		0.16
	2007	1.5	(1.4-1.6)	1.4	(1.3-1.5)	1.3	(1.2-1.4)	<0.001	<0.05	<0.05	<0.05
	2011	1.4	(1.34-1.55)	1.3	(1.23-1.38)	1.2	(1.13-1.36)	<0.0001	<0.01	<0.05	0.20

		MET-index tertiles									
		Low*		Moderate†		High‡		P value	P value adjusted for waist	P value adjusted for waist and smoking	P value sex-interaction term
		mean	(95% CI)	mean	(95% CI)	mean	(95% CI)				
ApoA1 (g/l)											
	2001	1.48	(1.46-1.50)	1.49	(1.47-1.51)	1.52	(1.50-1.54)	<0.01	<0.05	0.07	0.20
	2007	1.59	(1.57-1.61)	1.59	(1.57-1.61)	1.61	(1.59-1.63)	0.15			0.75
	2011	1.58	(1.56-1.60)	1.57	(1.56-1.59)	1.61	(1.59-1.63)	<0.05	0.24		0.56
ApoB (g/l)											
	2001	1.09	(1.07-1.11)	1.07	(1.05-1.09)	1.03	(1.01-1.05)	<0.0001	<0.05	<0.05	0.12
	2007	1.04	(1.02-1.06)	1.02	(1.00-1.04)	1.00	(0.99-1.02)	<0.05	0.33		<0.05
	2011	1.09	(1.07-1.12)	1.05	(1.02-1.07)	1.03	(1.01-1.05)	0.0001	0.15		0.69
ApoB/ApoA1-ratio											
	2001	0.76	(0.74-0.77)	0.74	(0.72-0.75)	0.70	(0.68-0.72)	<0.0001	<0.001	<0.01	0.32
	2007	0.67	(0.66-0.69)	0.66	(0.64-0.67)	0.64	(0.62-0.65)	<0.05	0.50		<0.05
	2011	0.71	(0.69-0.73)	0.68	(0.66-0.70)	0.66	(0.64-0.67)	<0.0001	<0.05	0.15	0.46
Glucose (mmol/l)											
	2001	5.1	(5.0-5.2)	5.1	(5.0-5.1)	5.0	(4.9-5.0)	<0.01	<0.05	0.08	0.33
	2007	5.4	(5.3-5.5)	5.3	(5.3-5.4)	5.3	(5.2-5.3)	<0.001	<0.05	<0.05	0.45
	2011	5.4	(5.3-5.5)	5.4	(5.3-5.5)	5.3	(5.2-5.3)	<0.01	0.39		0.74
Insulin (mU/l)											
	2001	8.6	(8.0-9.1)	8.0	(7.6-8.4)	6.8	(6.5-7.1)	<0.0001	<0.0001	<0.0001	0.09
	2007	11.0	(9.2-12.7)	8.8	(8.2-9.3)	8.0	(7.3-8.8)	<0.0001	<0.0001	<0.0001	0.48
	2011	11.4	(10.3-12.5)	9.4	(8.5-10.2)	8.0	(7.5-8.5)	<0.0001	<0.0001	<0.0001	0.16
HOMA-IR (unitless)											
	2001	2.1	(1.9-2.3)	1.9	(1.7-2.0)	1.5	(1.4-1.6)	<0.0001	<0.0001	<0.0001	0.08
	2007	2.7	(2.4-3.0)	2.2	(2.0-2.3)	2.1	(1.8-2.4)	<0.0001	<0.0001	<0.0001	0.43
	2011	3.1	(2.6-3.7)	2.4	(2.1-2.7)	1.9	(1.8-2.1)	<0.0001	<0.0001	<0.0001	0.18
hs-CRP (mg/l)											
	2001	2.1	(1.8-2.4)	2.0	(1.6-2.3)	1.6	(1.4-1.9)	<0.0001	<0.01	<0.05	0.21
	2007	1.9	(1.7-2.1)	2.1	(1.6-2.5)	1.6	(1.4-1.8)	<0.001	<0.01	<0.05	0.21
	2011	1.9	(1.7-2.1)	1.6	(1.4-1.8)	1.5	(1.3-1.7)	<0.0001	<0.01	<0.01	<0.05
Serum Amyloid A-1 (µg/l)											
	2001	25.4	(19.0-31.9)	25.9	(17.2-34.7)	18.6	(16.0-21.1)	<0.05	0.17		1.00
Homocysteine (µmol/l)											
	2001	9.9	(9.6-10.2)	9.9	(9.5-10.2)	9.7	(9.5-10.0)	0.45			0.61

		MET-index tertiles									
		Low*		Moderate†		High‡		P value	P value adjusted for waist	P value adjusted for waist and smoking	P value sex-interaction term
		mean	(95% CI)	mean	(95% CI)	mean	(95% CI)				
sPLA2 type IIA (ng/ml)											
	2001	3.99	(3.77-4.22)	3.88	(3.64-4.12)	3.97	(3.75-4.18)	0.88			0.94
sPLA2 activity (nmol/ml/min)											
	2001	1.65	(1.61-1.70)	1.61	(1.56-1.66)	1.60	(1.55-1.64)	<0.05	<0.05	0.07	0.98
Leptin (ng/ml)											
	2001	12.3	(11.6-13.1)	11.0	(10.2-11.7)	9.9	(9.2-10.6)	<0.0001	<0.0001	<0.0001	0.35
Adiponectin (µg/ml)											
	2001	9.1	(8.8-9.4)	9.1	(8.8-9.4)	9.8	(9.4-10.1)	<0.01	0.37		<0.01
	2007	10.1	(9.7-10.5)	10.2	(9.8-10.6)	10.3	(9.9-10.7)	0.72			<0.05
ADMA (µmol/l)											
	2001	0.60	(0.59-0.61)	0.59	(0.57-0.60)	0.59	(0.57-0.60)	0.16			0.10
SDMA (µmol/l)											
	2001	0.39	(0.38-0.40)	0.38	(0.38-0.39)	0.40	(0.39-0.41)	0.47			<0.01
L-Arginine (µmol/l)											
	2001	116.6	(113.8-119.4)	113.2	(110.2-116.2)	111.9	(108.9-114.9)	<0.05	<0.05	<0.05	0.66

*Number of participants varied between 427 and 808; †Number of participants varied between 405 and 732; ‡Number of participants varied between 457 and 728; MET=Metabolic equivalent; 95%CI=95% confidence interval for the mean; BMI=Body mass index; LDL=low density lipoprotein; HDL=high density lipoprotein; oxLDL=oxidized low density lipoprotein. ApoA1=apolipoprotein A1; ApoB=apolipoprotein B; HOMA-IR=homeostasis model assessment of insulin resistance; hs-CRP=high sensitive C-reactive protein; sPLA2=serum phospholipase A2; ADMA=asymmetric dimethyl arginine; SDMA=symmetric dimethyl arginine.

Table 11. Sex-stratified analysis for physical activity and risk markers in which the MET-index × sex interaction term was significant (p<0.05).

		MET-index tertiles									
		Low		Moderate		High		P value	P value adjusted for age	P value adjusted for age and waist	P value adjusted for age, waist and smoking
Females		mean	(95% CI)	mean	(95% CI)	mean	(95% CI)				
Heart rate/min											
	2001	69	(68-70)	68	(67-69)	67	(66-68)	<0.01	<0.001	<0.01	<0.001
	2011	69	(68-70)	68	(67-69)	66	(65-67)	<0.0001	<0.0001	<0.0001	<0.0001
Daily smokers (%)											
	2007	19	(15-23)	11	(8-15)	12	(9-16)	<0.001	<0.001	<0.01	
	2011	16	(12-20)	13	(10-18)	10	(7-13)	<0.001	<0.001	<0.001	
Pack-years of smoking (years)											
	2001	2.3	(1.8-2.7)	2.0	(1.6-2.5)	1.4	(1.0-1.7)	<0.001	<0.001	<0.05	
	2007	2.7	(2.2-3.2)	2.0	(1.5-2.6)	2.9	(2.3-3.6)	0.12			
	2011	4.5	(3.4-5.6)	3.4	(2.6-4.3)	2.9	(2.2-3.6)	<0.05	<0.05	0.06	
Education (school years)											
	2007	15.6	(15.3-15.9)	16.0	(15.6-16.4)	16.1	(15.8-16.4)	<0.01	<0.01	<0.01	<0.05
Total cholesterol (mmol/l)											
	2007	4.93	(4.85-5.01)	4.85	(4.76-4.94)	4.97	(4.88-5.06)	0.83			
Triglycerides (mmol/l)											
	2007	1.3	(1.2-1.3)	1.1	(1.1-1.2)	1.2	(1.1-1.2)	<0.01	<0.01	0.09	
ApoB (g/l)											
	2007	0.96	(0.94-0.99)	0.92	(0.90-0.95)	0.95	(0.92-0.97)	<0.05	<0.05	0.55	
ApoB/ApoA1-ratio											
	2007	0.59	(0.58-0.61)	0.57	(0.55-0.59)	0.57	(0.56-0.59)	<0.05	<0.05	0.72	
hs-CRP (mg/l)											
	2011	2.2	(1.9-2.5)	1.8	(1.5-2.1)	1.5	(1.3-1.8)	<0.0001	<0.0001	0.0001	0.0001
Adiponectin (µg/ml)											
	2001	10.5	(10.1-11.0)	10.7	(10.2-11.1)	11.8	(11.3-12.3)	<0.0001	<0.001	<0.05	<0.05
	2007	11.9	(11.4-12.4)	12.4	(11.8-13.0)	12.5	(12.0-13.1)	<0.05	<0.05	0.56	
SDMA (µmol/l)											
	2001	0.38	(0.37-0.39)	0.38	(0.37-0.39)	0.38	(0.37-0.39)	0.47			

Males		MET-index tertiles: Low mean	Low (95% CI)	Moderate mean	Moderate (95% CI)	High mean	High (95% CI)	P value	P value adjusted for age	P value adjusted for age and waist	P value adjusted for age, waist and smoking
Heart rate/min											
	2001	67	(66-68)	66	(65-67)	61	(60-62)	<0.0001	<0.0001	<0.0001	<0.0001
	2011	67	(66-69)	67	(66-68)	62	(61-63)	<0.0001	<0.0001	<0.0001	<0.0001
Daily smokers (%)											
	2007	32	(27-37)	21	(17-26)	13	(9-17)	<0.0001	<0.0001	<0.0001	
	2011	30	(24-36)	15	(11-19)	9	(6-13)	<0.0001	<0.0001	<0.0001	
Pack-years of smoking (years)											
	2001	6.7	(5.7-7.6)	3.9	(3.2-4.6)	2.2	(1.7-2.7)	<0.0001	<0.0001	<0.0001	
	2007	7.7	(6.4-8.9)	4.9	(4.0-5.9)	3.4	(2.7-4.1)	<0.0001	<0.0001	<0.0001	
	2011	9.4	(7.7-11.2)	6.9	(5.4-8.3)	4.2	(3.1-5.3)	<0.0001	<0.0001	<0.0001	
Education (school years)											
	2007	14.1	(13.7-14.5)	15.1	(14.7-15.5)	15.7	(15.3-16.1)	<0.0001	<0.0001	<0.0001	<0.0001
Total cholesterol (mmol/l)											
	2007	5.24	(5.14-5.35)	5.21	(5.10-5.32)	5.08	(4.97-5.18)	<0.001	<0.001	<0.01	<0.05
Triglycerides (mmol/l)											
	2007	1.8	(1.6-1.9)	1.7	(1.5-1.8)	1.5	(1.4-1.6)	<0.0001	<0.0001	<0.01	<0.01
ApoB (g/l)											
	2007	1.14	(1.11-1.17)	1.12	(1.08-1.15)	1.08	(1.05-1.11)	<0.0001	<0.0001	<0.001	<0.01
ApoB/ApoA1-ratio											
	2007	0.79	(0.76-0.81)	0.75	(0.72-0.77)	0.72	(0.70-0.74)	<0.0001	<0.0001	<0.001	<0.01
hs-CRP (mg/l)											
	2011	1.5	(1.2-1.8)	1.4	(1.2-1.7)	1.5	(1.1-1.8)	<0.01	<0.05	0.61	
Adiponectin (µg/ml)											
	2001	7.2	(6.9-7.6)	7.5	(7.1-7.9)	7.3	(7.0-7.7)	0.45			
	2007	7.6	(7.2-8.0)	7.9	(7.5-8.3)	7.5	(7.0-7.9)	0.50			
SDMA (µmol/l)											
	2001	0.40	(0.39-0.41)	0.39	(0.38-0.40)	0.42	(0.41-0.44)	<0.01	<0.01	<0.05	<0.05

MET=Metabolic equivalent; 95%CI=95% confidence interval for the mean; ApoB=apolipoprotein B; ApoA1=apolipoprotein A1; hs-CRP=high sensitive C-reactive protein; SDMA=symmetric dimethyl arginine.

5.1.2. Physical activity in childhood and carotid artery elasticity in adults

Physical activity in childhood was measured in 1986 by a questionnaire as mentioned in the Methods section. There was no association between leisure-time physical activity and carotid artery elasticity indices measured in 2001 in either sex. Among 9 to 15 year-old males, leisure-time physical activity was associated with better carotid artery elasticity indicated by carotid artery distensibility, Young's elastic modulus and stiffness index as measured in 2007. In multivariate analyses, leisure-time physical activity remained a significant determinant of adult carotid artery distensibility, Young's elastic modulus and stiffness index after adjustments for covariates (Study II, Table 3) **(Figure 2.**).

Among 9 to 15-year old females, there was no association between leisure-time physical activity in childhood and adult carotid artery distensibility, Young's elastic modulus or stiffness index.

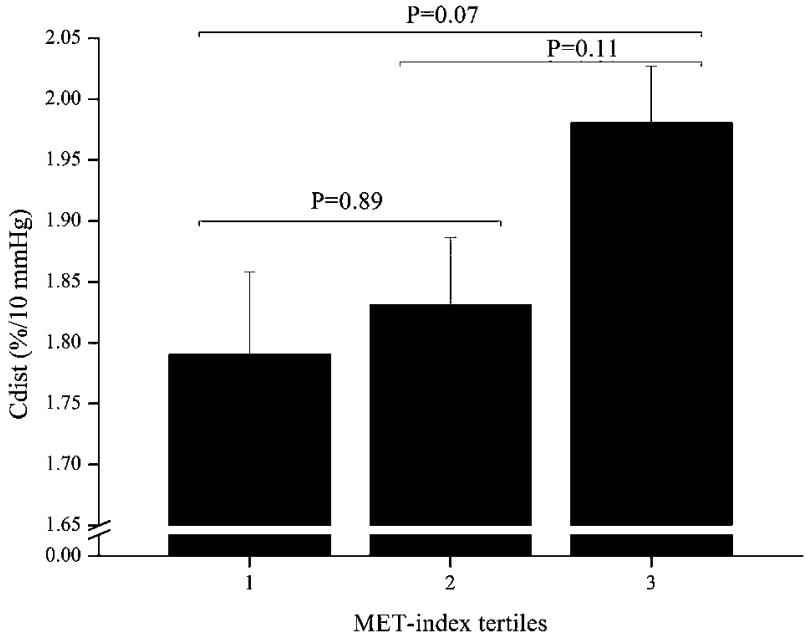

Figure 2. Association of carotid artery distensibility (Cdist) with leisure-time metabolic equivalent (MET) index tertiles in males aged 9 to 15 years in 1986. P values shown are from ANCOVA with Tukey–Kramer multiple comparison. Descriptive data are given as estimated means (SE) adjusted for age in 2007, BMI in 1986 and in 2007, SBP in 1986 and in 2007, smoking in 1986 and in 2007, HDL cholesterol measured in 1986 and in 2007, LDL cholesterol measured in 1986 and in 2007, triglycerides measured in 1986 and in 2007, insulin measured in 1986 and in 2007, glucose measured in 1986 and in 2007, and ΔMET. BMI indicates body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure. Reproduced from Journal of American Heart Association (Study II) with permission of Wiley-Blackwell.

5.1.3. Physical activity in young adulthood and carotid artery elasticity in adults

Physical activity in young adulthood was measured in 1986 by a questionnaire. Leisure-time physical activity was not associated with carotid artery indices as measured in 2001. However, leisure-time physical activity of young adults aged 18 to 24 years was directly associated with future carotid artery distensibility and inversely with Young's elastic modulus and stiffness index measured in 2007, indicating better carotid artery elasticity. In the multivariable analyses, leisure-time physical activity remained a significant determinant of carotid artery distensibility, Young's elastic modulus and stiffness index after adjustments for covariates (Study II, Table 4) **(Figure 3)**.

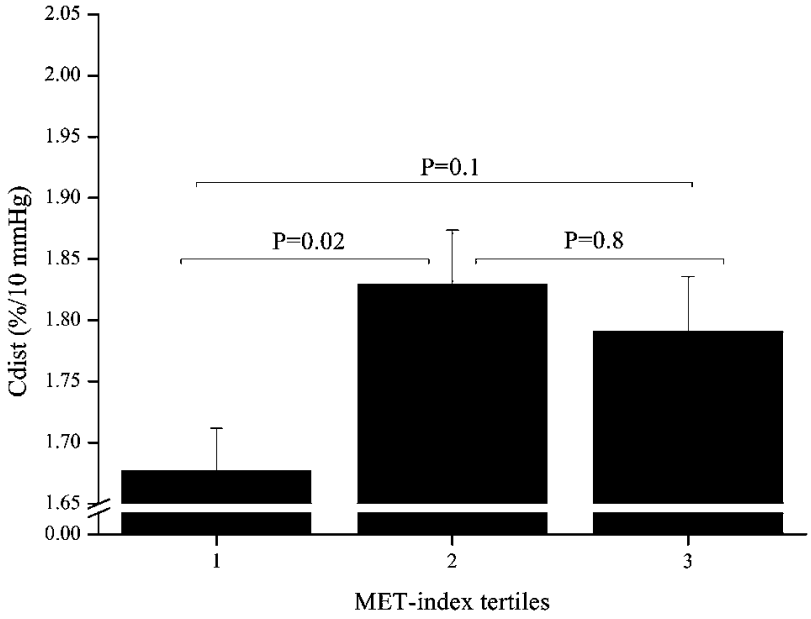

Figure 3. Association of carotid artery distensibility (Cdist) with leisure-time metabolic equivalent (MET) index tertiles in young adults aged 18 to 24 years in 1986. P values shown are from ANCOVA with Tukey–Kramer multiple comparison. Descriptive data are given as estimated means (SE) adjusted for age in 2007, sex, BMI in 1986 and in 2007, SBP in 1986 and in 2007, smoking in 1986 and in 2007, HDL cholesterol measured in 1986 and in 2007, LDL cholesterol measured in 1986 and in 2007, triglycerides measured in 1986 and in 2007, insulin measured in 1986 and in 2007, glucose measured in 1986 and in 2007, and ΔMET. BMI indicates body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure. Reproduced from Journal of American Heart Association (Study II) with permission of Wiley-Blackwell.

5.1.4. Physical activity in 1986 and carotid artery intima-media thickness in adults

Among 9 to 15 year old males, leisure-time physical activity was not associated with adult intima-media thickness measured in 2001 or 2007. The same was true among 9 to 15 year old females and 18 to 24 year old young adults.

5.1.5. Physical activity and risk of fatty liver in 2011

Higher physical activity was associated with a lower risk of fatty liver. When physical activity was adjusted for BMI or waist circumference, pack-years of smoking, alcohol consumption, HDL cholesterol, LDL cholesterol, triglycerides, insulin, glucose and hs-CRP, the risk reduction in fatty liver remained statistically significant. When cardiorespiratory fitness was added to the model, the effect became attenuated (**Table 12**).

Table 12. Risk ratios between physical activity and fatty liver.

Model	RR	CI	P
A: Age and sex-adjusted MET-index	0.98	0.97-0,99	<0.0001
B1: A + BMI	0.99	0.98-0.99	<0.0001
B2: A + waist circumference	0.99	0.98-0.99	<0.001
C: B1 + pack-years of smoking	0.99	0.98-1.00	<0.01
D: C + alcohol consumption	0.99	0.98-1.00	<0.01
E: D + HDL cholesterol	0.99	0.98-1.00	<0.01
F: E + LDL cholesterol	0.99	0.98-1.00	<0.05
G: F + triglycerides	0.99	0.98-1.00	<0.05
H: G + insulin	0.99	0.98-1.00	<0.05
I: H + glucose	0.99	0.98-1.00	<0.05
J: I + hs-CRP	0.99	0.98-1.00	<0.05
K: J + cardiorespiratory fitness	0.99	0.98-1.01	0.53

RR=risk ratio; CI=confidence interval; MET=metabolic equivalent; BMI=body mass index; HDL=high density lipoprotein; LDL=low density lipoprotein; hs-CRP=high sensitive C-reactive protein.

When physical activity was categorized into quartiles, an increase from the second to the third quartile was associated with decreased prevalence of fatty liver (**Figure 4**). With a further increase in the cardiorespiratory fitness quartiles no significant decrease in the prevalence of fatty liver was found.

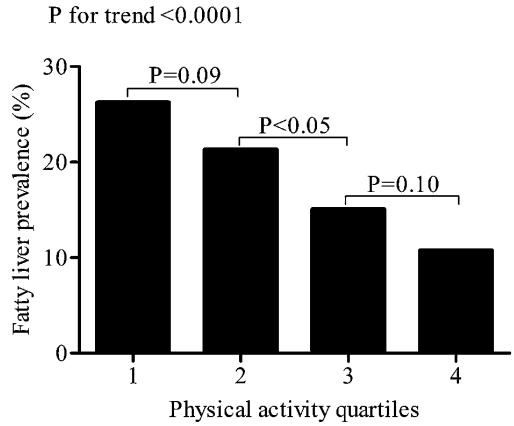

Figure 4. Prevalence of fatty liver according to physical activity quartiles.

When participants were categorized according to physical activity and obesity, the prevalence of fatty liver was the highest in the obese and poorly physically active participants (**Figure 5**). The non-obese and highly physically active participants had the lowest prevalence of fatty liver; almost half of the corresponding value in individuals who were non-obese and poorly physically active.

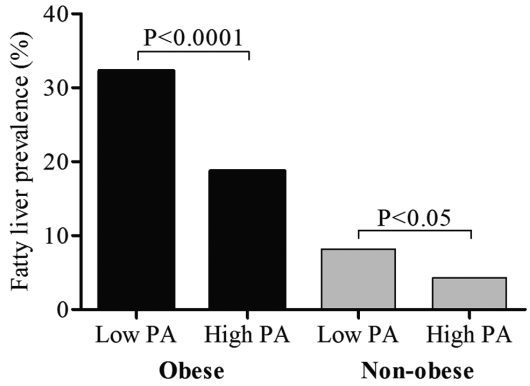

Figure 5. Prevalence of fatty liver in obese and non-obese participants with high or low physical activity. PA=Physical activity.

The association of physical activity and prevalence of fatty liver among obese participants was studied more specifically by forming physical activity quartiles (**Figure 6**). An increase in physical activity was associated with lower prevalence of fatty liver (P for linear trend <0.0001).

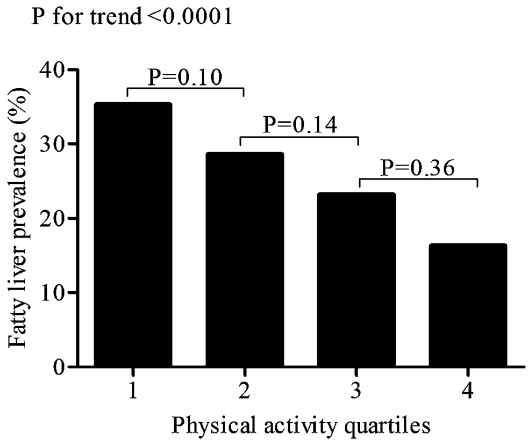

Figure 6. Prevalence of fatty liver according to physical activity quartiles among obese participants.

5.2. Cardiorespiratory fitness

The characteristics of subjects who took part in the cardiopulmonary exercise test in 2008-2009 (N=538) and follow-ups in 2007 and 2011 are shown in **Tables 13 and 14**. Ultrasound revealed that females had less fatty liver findings than males.

Table 13. Characteristics of cardiopulmonary exercise test participants in 2007, 2008-2009 and 2011. Values are mean and standard deviation, unless stated otherwise.

		Females		Males	
		Mean	SD	Mean	SD
Age (years)					
	2007	38	5	37	5
	2008-2009	39	5	38	5
	2011	42	5	41	5
Height (cm)					
	2007	166	5	180	6
	2008-2009	166	5	180	6
	2011	166	6	180	6
Weight (kg)					
	2007	69	13	86	14
	2008-2009	71	14	87	15
	2011	71	14	87	14
BMI (kg/m2)					
	2007	25.1	4.7	26.5	4.1
	2008-2009	25.7	5.0	26.9	4.2
	2011	25.9	5.1	26.8	4.1
Waist circumference (cm)					
	2007	86	12	95	11
	2011	87	13	97	11
Waist-to-hip ratio (unitless)					
	2007	0.8	0.1	0.9	0.1
	2011	0.8	0.1	1.0	0.1
Systolic blood pressure (mmHg)					
	2007	112	13	122	13
	2011	117	14	124	14
Diastolic blood pressure (mmHg)					
	2007	68	11	74	11
	2011	74	10	79	11
Daily smokers (%)					
	2007	11		16	
	2011	11		11	
Pack-years of smoking (years)					
	2007	2.7	6.1	3.9	7.3
	2011	3.5	6.5	4.6	8.5
Alcohol consumption (dose per day)					
	2007	0.6	0.8	1.4	2.2
	2011	0.5	0.7	1.1	1.3
Physical activity index (unitless)*					
	2007	8.8	1.7	8.7	1.9
	2011	9.2	1.8	8.8	1.9
MET-index (median, range)					
	2007	12	0-153	12	0-138
	2011	20	0-117	12	0-119
Leisure-time MET-index (median, range)					
	2007	5	0-52	8	0-93
	2011	12	0-93	12	0-93

		Females		Males	
		Mean	SD	Mean	SD
Total cholesterol (mmol/l)					
	2007	4.9	0.9	5.1	1.0
	2011	5.0	0.9	5.2	1.0
LDL cholesterol (mmol/l)					
	2007	2.9	0.7	3.2	0.9
	2011	3.1	0.8	3.3	0.9
HDL cholesterol (mmol/l)					
	2007	1.4	0.3	1.2	0.3
	2011	1.4	0.3	1.2	0.3
LDL/HDL-ratio (unitless)					
	2007	2.1	0.8	2.8	1.0
	2011	2.3	0.9	2.9	1.0
Triglycerides (mmol/l)					
	2007	1.2	0.6	1.6	1.1
	2011	1.1	0.6	1.5	1.0
ApoA1 (g/l)					
	2007	1.7	0.3	1.5	0.2
	2011	1.6	0.2	1.5	0.2
ApoB (g/l)					
	2007	0.9	0.2	1.1	0.3
	2011	1.0	0.3	1.1	0.3
ApoB/ApoA1-ratio (unitless)					
	2007	0.6	0.2	0.7	0.2
	2011	0.6	0.2	0.8	0.2
Glucose (mmol/l)					
	2007	5.2	0.7	5.5	0.7
	2011	5.3	0.9	5.5	0.6
Insulin (mU/l)					
	2007	8.6	7.0	9.3	10.9
	2011	9.8	17.8	9.7	10.9
HOMA-IR (unitless)					
	2007	2.1	2.1	2.5	5.2
	2011	2.9	13.5	2.6	4.3
hs-CRP (mg/l)					
	2007	1.9	2.6	1.7	3.9
	2011	1.7	2.7	1.5	2.9
GT (U/l)					
	2007	19	14	37	26.0
	2011	24	32	41	34.1
ALAT (U/l)					
	2007	13	6	24	16.3
	2011	13	9	23	19.9
ASAT (U/l)					
	2011	20	11	27	14
Fatty liver in ultrasound (%)					
	2011	10		25	

BMI=Body mass index MET=Metabolic equivalent; LDL=low density lipoprotein; HDL=high density lipoprotein; ApoA1=apolipoprotein A1, ApoB=apolipoprotein B; HOMA-IR=homeostasis model assessment of insulin resistance; hs-CRP=high sensitive C-reactive protein; GT= gamma-glutamyltransferase; ALAT=alanine aminotransferase; ASAT=aspartate aminotransferase. Differences between sexes $P<0.0008$ except for age, daily smoking, pack-years of smoking, physical activity index in 2007, MET-index, leisure-time MET-index, insulin levels, HOMA-IR and hs-CRP in 2011. *Range 5 to 15.

Females had lower peak oxygen uptake, work rate, blood pressure and heart rate parameters than males in the cardiopulmonary exercise test.

Table 14. Cardiopulmonary exercise test parameters in males and females in 2008-2009. Values are mean and standard deviation.

	Females		Males	
	Mean	SD	Mean	SD
Peak oxygen uptake (ml/kg/min)	26,6	6,9	35,0	7,8
Systolic blood pressure at rest (mmHg)	123	14	133	14
Peak systolic blood pressure (mmHg)	189	23	215	24
Diastolic blood pressure at rest (mmHg)	79	9	84	11
Peak diastolic blood pressure (mmHg)	91	13	94	13
Mean arterial blood pressure at rest (mmHg)	94	10	100	10
Peak mean arterial blood pressure (mmHg)	124	14	134	14
Resting heart rate (beats/min)	84	13	82	13
Peak heart rate (beats/min)	175	12	177	14
Peak work rate (W)	162	28	260	48

Differences between sexes P<0.05. Modified from Study III.

5.2.1. Determinants of peak oxygen uptake

Peak oxygen uptake was associated with sex, height, weight, physical activity index, smoking and serum insulin levels. The detailed results are shown in **Table 15**.

Table 15. Determinants of peak oxygen uptake.

Parameter	VO_{2peak}	
	β	SE
Sex	8.8	1.0‡
Age	-0.08	0.06
Height	0.26	0.05‡
Weight	-0.22	0.02‡
Waist-to-hip ratio	-2.96	5.98
Physical activity index	0.94	0.15‡
Smoking	-2.98	0.82†
Insulin	-0.10	0.04*
Glucose	0.77	0.51
Antihypertensive treatment	-0.51	1.26
Whole model R^2	51 %	

VO_{2peak}=peak oxygen uptake (ml/kg/min); β=regression coefficient for a 1-unit change in parameter; SE: standard error.
*p<0.05; †p<0.01; ‡p<0.0001

5.2.2. The associations between cardiorespiratory fitness and cardiovascular disease risk markers

Cardiorespiratory fitness was inversely associated with waist circumference, BMI, systolic and diastolic blood pressure, resting heart rate, daily smoking, pack-years of smoking, total cholesterol, LDL cholesterol, LDL/HDL-ratio, serum triglycerides, apolipoprotein B, apolipoprotein B/apolipoprotein A1 ratio, glucose, insulin, HOMA-IR, and hs-CRP. When controlling for waist circumference and daily smoking, the association remained significant with diastolic blood pressure, resting heart rate, serum triglycerides, insulin, HOMA-IR, hs-CRP. The detailed results are shown in **Table 16**.

Interaction analysis suggested significant sex differences in the relationships between cardiorespiratory fitness with daily smoking (only in males).

Table 16. Associations between cardiorespiratory fitness level (Low-Moderate-High) and cardiovascular disease risk markers in 2007.

	Age and sex specific cardiorespiratory fitness tertiles									
	Low*		Moderate†		High‡		P value	P value adjusted for waist	P value adjusted for waist and smoking	P value sex-interaction term
	mean	(95% CI)	mean	(95% CI)	mean	(95% CI)				
Age (years)	38	(37-38)	38	(37-38)	38	(37-39)				
Waist circumference (cm)	96	(94-98)	91	(89-92)	85	(83-86)	<0.0001			0.90
BMI (kg/m2)	28	(27-28)	26	(25-27)	24	(23-24)	<0.0001			0.41
Systolic blood pressure (mmHg)	119	(117-121)	118	(116-120)	113	(111-115)	<0.0001	0.39		0.24
Diastolic blood pressure (mmHg)	75	(73-76)	72	(70-73)	67	(66-69)	<0.0001	<0.001	<0.001	0.15
Resting heart rate (beats/min)	69	(68-71)	66	(65-68)	65	(64-67)	<0.0001	0.0001	<0.0001	0.77
Daily smokers (%)	22	(16-29)	9	(6-15)	9	(5-14)	<0.001	<0.001		<0.05
Pack-years of smoking (years)	4.6	(3.4-5.9)	3.5	(2.4-4.6)	1.9	(1.2-2.7)	<0.01	<0.05		0.08
Alcohol consumption (dose per day)	1.0	(0.8-1.1)	1.0	(0.8-1.2)	1.0	(0.6-1.3)	0.12			0.70
Education (school years)	15.2	(14.6-15.7)	15.2	(14.8-15.7)	16.1	(15.5-16.7)	<0.05	0.49		0.73
Total cholesterol (mmol/l)	5.2	(5.1-5.4)	5.0	(4.8-5.1)	4.8	(4.7-4.9)	<0.0001	0.16		0.46
LDL cholesterol (mmol/l)	3.2	(3.1-3.4)	3.0	(2.9-3.2)	2.9	(2.8-3.0)	<0.01	0.81		0.31
HDL cholesterol (mmol/l)	1.3	(1.2-1.3)	1.3	(1.3-1.4)	1.4	(1.3-1.4)	<0.001	0.81		0.12
LDL/HDL-ratio (unitless)	2.7	(2.5-2.9)	2.5	(2.3-2.6)	2.2	(2.1-2.4)	<0.001	0.90		0.12
Triglycerides (mmol/l)	1.7	(1.5-1.8)	1.4	(1.3-1.6)	1.1	(1.0-1.2)	<0.0001	<0.001	<0.01	0.42
ApoA1 (g/l)	1.6	(1.5-1.6)	1.6	(1.5-1.6)	1.6	(1.6-1.6)	0.18			0.08
ApoB (g/l)	1.1	(1.1-1.1)	1.0	(1.0-1.1)	0.9	(0.9-0.9)	<0.0001	0.10		0.26
ApoB/ApoA1-ratio (unitless)	0.7	(0.7-0.8)	0.7	(0.6-0.7)	0.6	(0.6-0.6)	<0.0001	0.37		0.15
Glucose (mmol/l)	5.5	(5.3-5.6)	5.3	(5.3-5.4)	5.2	(5.2-5.3)	<0.01	0.60		0.76
Insulin (mU/l)	11.6	(9.7-13.5)	8.7	(7.6-9.8)	6.4	(5.7-7.2)	<0.0001	<0.01	<0.01	0.18
HOMA-IR (unitless)	3.2	(2.2-4.1)	2.1	(1.8-2.5)	1.5	(1.3-1.8)	<0.0001	<0.01	<0.01	0.19
hs-CRP (mg/l)	2.4	(1.8-3.0)	1.8	(1.3-2.2)	1.2	(0.8-1.6)	<0.0001	0.0001	0.0001	0.68
Adiponectin (µg/ml)	9.3	(8.6-10.1)	9.8	(9.0-10.5)	11.1	(10.2-11.9)	<0.001	0.53		0.91

*Number of participants varied between 155 and 180; †Number of participants varied between 149 and 175; ‡Number of participants varied between 162 and 173. 95%CI=95% confidence interval for the mean; BMI=Body mass index; LDL=low density lipoprotein; HDL=high density lipoprotein; oxLDL=oxidized low density lipoprotein. ApoA1=apolipoprotein A1; ApoB=apolipoprotein B; HOMA-IR=homeostasis model assessment of insulin resistance; hs-CRP=high sensitive C-reactive protein

5.2.3. Cardiorespiratory fitness and carotid artery ultrasound findings

Cardiorespiratory fitness was directly associated with carotid artery distensibility (β=0.014, SE=0.004, P<0.001) and inversely with Young's elastic modulus (β=-0.010, SE=0.003, P<0.001) and stiffness index (β=-0.008, SE=0.003, P<0.01) as measured in 2007, indicating better carotid artery elasticity, but when controlling for BMI all these associations were attenuated (P=0.11, P=0.08 and P=0.17, respectively). Cardiorespiratory fitness was not associated with intima-media thickness (β=-0.002, SE=0.005, P=0.64).

5.2.4. Cardiorespiratory fitness and risk of fatty liver

Higher cardiorespiratory fitness was associated with lower risk of fatty liver. When cardiorespiratory fitness was adjusted for BMI or waist circumference, pack-years of smoking, alcohol consumption, HDL cholesterol, LDL cholesterol, triglycerides, insulin, glucose, hs-CRP and physical activity, the risk reduction in fatty liver remained significant (**Table 17**).

Table 17. Risk ratios between cardiorespiratory fitness and fatty liver.

Model	RR	CI	P
A: Age and sex-adjusted VO_{2peak} 1 ml/kg/min increase	0.90	0.88-0.93	<0.0001
B1: A + BMI	0.94	0.90-0.97	<0.001
B2: A + waist circumference	0.94	0.91-0.97	<0.001
C: B1 + pack-years of smoking	0.93	0.89-0.97	<0.01
D: C + alcohol consumption	0.94	0.90-0.98	<0.01
E: D + HDL cholesterol	0.93	0.89-0.97	<0.01
F: E + LDL cholesterol	0.93	0.89-0.98	<0.01
G: F + triglycerides	0.93	0.88-0.98	<0.01
H: G + insulin	0.92	0.87-0.97	<0.01
I: H + glucose	0.92	0.87-0.97	<0.01
J: I + hs-CRP	0.92	0.87-0.98	0.01
K: J + physical activity	0.92	0.86-0.98	0.01

VO_{2peak}=peak oxygen uptake; RR=risk ratio; CI=95% confidence interval; BMI=body mass index; HDL =high density lipoprotein; LDL=low density lipoprotein; hs-CRP=high sensitive C-reactive protein. Reproduced from Medicine & Science in Sports & Exercise (Study IV) with permission of Wolters Kluwer Health.

When cardiorespiratory fitness was categorized into quartiles, an increase from the lowest to the second quartile was associated with decreased prevalence of fatty liver (**Figure 7**). With a further increase in the cardiorespiratory fitness quartiles, no significant decrease was found in the prevalence of fatty liver.

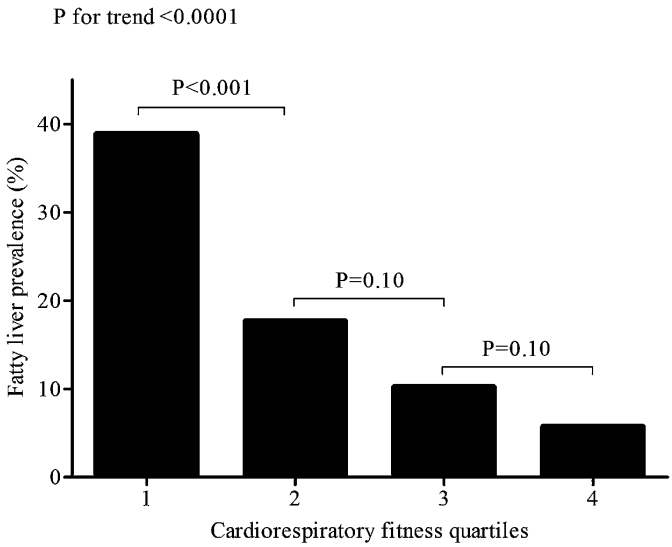

Figure 7. Prevalence of fatty liver according to cardiorespiratory fitness quartiles. Reproduced from Medicine & Science in Sports & Exercise (Study IV) with permission of Wolters Kluwer Health.

When participants were categorized according to cardiorespiratory fitness and obesity, the prevalence of fatty liver was the highest in the obese and low fit participants (**Figure 8**). Participants who were obese but high fit had a three times lower prevalence of fatty liver than those who were obese and low fit. The non-obese and high fit participants had the lowest prevalence of fatty liver. Among those non-obese individuals there was no difference in the prevalence of fatty liver depending on cardiorespiratory fitness.

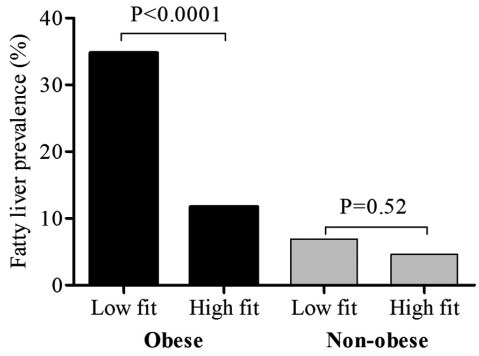

Figure 8. Prevalence of fatty liver in obese and non-obese participants with high or low cardiorespiratory fitness. Reproduced from Medicine & Science in Sports & Exercise (Study IV) with permission of Wolters Kluwer Health.

Among the obese participants, cardiorespiratory fitness quartiles for peak oxygen uptake were formed to examine more specifically the association of

cardiorespiratory fitness and prevalence of fatty liver in this group (**Figure 9**). The increase in cardiorespiratory fitness from the lowest quartile to the second lowest was associated with a pronounced decrease in the prevalence of fatty liver. A further increase in the cardiorespiratory fitness level was not associated with a decreased prevalence of fatty liver.

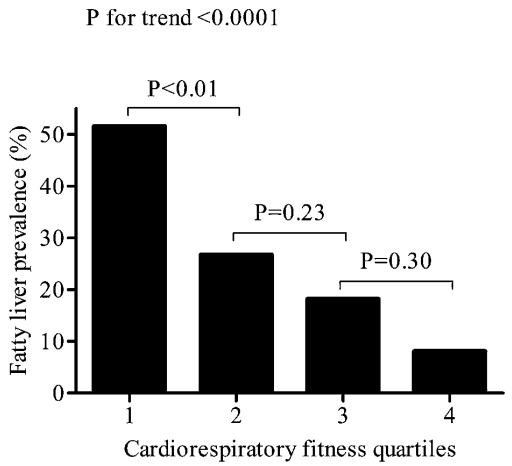

Figure 9. Prevalence of fatty liver according to cardiorespiratory fitness quartiles among obese participants. Reproduced from Medicine & Science in Sports & Exercise (Study IV) with permission of Wolters Kluwer Health.

5.3. Physical activity, cardiorespiratory fitness and cardiovascular disease risk markers

504 study participants had data on both physical activity and cardiorespiratory fitness. Physical activity correlated strongly with cardiorespiratory fitness (ρ=0.22, P<0.0001, **Figure 10**). The association between physical activity tertiles, cardiorespiratory fitness tertiles and cardiovascular disease markers in those individuals are displayed in **Table 18**.

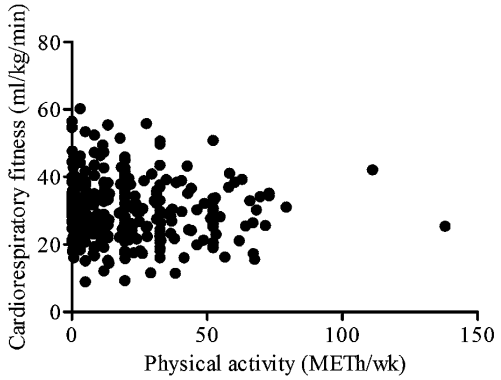

Figure 10. Correlation of physical activity with cardiorespiratory fitness.

Table 18. Physical activity and cardiorespiratory fitness and their association with cardiovascular risk markers measured in 2007.

	Physical activity tertiles		Cardiorespiratory fitness tertiles	
	ρ	P value	ρ	P value
Physical activity tertiles			0.24	<0.0001
Cardiorespiratory fitness tertiles	0.24	<0.0001		
Waist circumference (cm)	-0.14	<0.01	-0.38	<0.0001
BMI (kg/m2)	-0.10	<0.05	-0.40	<0.0001
Systolic blood pressure (mmHg)	0.005	0.92	-0.18	<0.0001
			-0.02*	0.60*
Diastolic blood pressure (mmHg)	-0.02	0.59	-0.27	<0.0001
			-0.15*	<0.001*
			-0.16†	<0.001†
Resting heart rate/min	-0.21	<0.0001	-0.20	<0.0001
	-0.21	<0.0001*	-0.19	<0.0001*
	-0.21	<0.0001†	-0.19†	<0.0001†
Daily smoking	-0.04	0.34	-0.14	<0.01
			-0.14*	<0.01*
Pack-years of smoking (years)	-0.03	0.50	-0.15	<0.01
			-0.10*	<0.05*
Total cholesterol (mmol/l)	-0.11	<0.05	-0.17	<0.001
	-0.07*	0.14*	-0.04*	0.34*
LDL cholesterol (mmol/l)	-0.07	0.15	-0.11	<0.05
			0.02*	0.68*
HDL cholesterol (mmol/l)	-0.001	0.97	0.16	<0.001
			-0.01*	0.88*
LDL/HDL-ratio (unitless)	-0.05	0.30	-0.16	<0.001
			0.02*	0.62*
Triglycerides (mmol/l)	-0.10	<0.05	-0.34	<0.0001
	-0.03*	0.4*	-0.17*	<0.001*
			-0.16†	<0.001†
ApoA1 (g/l)	-0.04	0.37	0.06	0.18
ApoB (g/l)	-0.10	<0.05	-0.25	<0.0001
	-0.03*	0.54*	-0.06*	0.20*
ApoB/ApoA1-ratio (unitless)	-0.07	0.12	-0.24	<0.0001
			-0.03*	0.52*
Glucose (mmol/l)	-0.08	0.09	-0.11	<0.05
			0.04*	0.39*
Insulin (mU/l)	-0.18	<0.0001	-0.31	<0.0001
	-0.12*	<0.01*	-0.14*	<0.01*
	-0.12†	<0.01†	-0.15†	<0.001†
HOMA-IR (unitless)	-0.18	<0.0001	-0.31	<0.0001
	-0.13*	<0.01*	-0.13*	<0.01*
	-0.12†	<0.01†	-0.14†	<0.01†
CRP (mg/l)	-0.03	0.57	-0.31	<0.0001
			-0.18*	<0.0001*
			-0.18†	<0.0001†
Adiponectin (µg/ml)	0.08	0.08	0.15	<0.001
			-0.04*	0.40*

ρ=Spearman correlation coefficient; *=adjusted for waist circumference; †=adjusted for waist circumference and smoking.

6. DISCUSSION

6.1. Participants and methods

6.1.1. Participants

This thesis is based on the Cardiovascular Risk in Young Finns Study, which is a multicenter, on-going follow-up study of atherosclerosis risk factors in children, adolescents and adults. Loss to follow-up is evident in all longitudinal studies, but the participant rates have been rather good as mentioned in the Methods section. The representativeness of the remaining cohort has been examined on several occasions. In the 1980s, the loss-to-follow-ups were more often men, more often smokers and from the older age groups (Porkka *et al.* 1997). In 2001, loss-to-follow-ups were more often men and more often from the younger age groups but there were no differences in either males or females in terms of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, blood pressure, BMI or physical activity measured in 1980 between participants and loss-to-follow-ups (Juonala *et al.* 2004).

The participants have been closely followed with well-established methods since a young age to adulthood and extensive measurements of risk markers, questionnaires on lifestyle factors and ultrasound studies have been performed.

6.1.2. Physical activity questionnaire

In this thesis, self-reported leisure time physical activity including commuting activity was assessed with a questionnaire that included questions on the frequency, intensity and duration of leisure-time physical activity and commuting to work. The reliability of physical activity questionnaire is evaluated in earlier phases of Cardiovascular Risk in Young Finns study project in many ways. A coefficient of internal consistency (Cronbach's alpha) has been calculated as an indicator of the reliability of leisure-time physical activity for the sum index including questions on the frequency, intensity and duration. The Spearman's rank order coefficients have varied from 0.44 to 0.76 in studies in youth (Telama *et al.* 1994, Telama *et al.* 1997, Telama and Yang 2000), and from 0.59 to 0.85 in adults from 1992 to 2001 (Yang *et al.* 2008). The reliability of the test–retest reliabilities of the coefficients within a 3-year interval of the PAI was evaluated also by reliability coefficients from a simplex model. The simplex model, that fitted data very well, provided the possibility to make a distinction between measurement error and real change. The test–retest reliabilities using the PAI values were all over 0.70 (Telama *et al.* 1996). Cronbach's Alpha (r=0.65) was

calculated to address the reliability of the MET-index. Values greater than 0.6 are commonly considered to indicate good internal consistency (Vogt 1993). There might be potential inaccuracies in the individual components of the PAI and MET-index. For example, the intensity of physical activity was estimated with a question that had only three categories. However, the intensity question correlated directly with high intensity sport activities, providing evidence of its construct validity. In the frequency question, the two categories at the high end had wide ranges: 2-3 or 4-6 times a week. Therefore, when constructing the MET-index we allocated the same coefficient for these choices so that this inaccuracy would not cause artificial differences in the index. However, similar results were seen even when different coefficients were allocated for these two choices suggesting by combining these two categories, not much information was lost. The highest category for the maximum duration of the exercise was over 60 minutes, thus we were unable to rank subjects above this cut-point. Despite these potential limitations, we observed reasonable correlations between the MET-index and objective measurements of physical activity assessed by pedometers and accelerometers. Nevertheless, the results should be interpreted with caution as the potential inaccuracies in the physical activity data may introduce underestimations of the relations between MET-index and risk markers.

6.1.3. Assessing carotid artery elasticity

In this thesis, ultrasound was used to measure changes in the diameters of the carotid artery during cardiac cycle. Carotid artery ultrasound shows similar relationships with cardiovascular risk markers as the pulse wave velocity (Boutouyrie *et al.* 1999, Oliver and Webb 2003). Three indices that measure different aspects of arterial elasticity were calculated in the assessment of arterial elasticity. Distensibility measures the ability of the arteries to expand as a response to pulse pressure caused by cardiac contraction and relaxation. Young's elastic modulus gives an estimate of arterial stiffness that is independent of intima-media thickness. The stiffness index has been developed to reduce the impact of the curvilinear pressure-stiffness relationship on arterial stiffness and is considered to be relatively independent of blood pressure. It is relatively easy to perform carotid artery ultrasound in large population studies especially as it is non-invasive. When calculating distensibility, peripheral blood pressure, instead of central, is used. It would be more optimal to study the pulse pressure from the carotid artery, because the use of brachial pressure overestimates pulse pressure in the central artery (Karamanoglu *et al.* 1993). However, the difference between central and peripheral pulse pressure is likely to be similar between study subjects within a narrow age range, as in our study and a strong correlation

between systolic (r=0.98) and diastolic (r=0.97) pressures measured in the central and peripheral arteries has been shown previously (Borow and Newburger 1982).

6.1.4. Assessing cardiorespiratory fitness

In this thesis, cardiorespiratory fitness was assessed with cycle ergometer and oxygen uptake was measured while the participants were cycling. Cardiopulmonary exercise test represents a non-invasive, easily accessible and information-intensive way to assess the cardiovascular status. Peak oxygen uptake and especially peak oxygen uptake as related to body weight, have been widely used as a tool in clinical decision-making (American Thoracic Society and American College of Chest Physicians 2003). However, the interpretation of the test may be complex as oxygen uptake is closely dependent on age, sex, body anthropometrics and lifestyle and variation in these factors may interfere with diagnostic findings (American Thoracic Society and American College of Chest Physicians 2003). In this thesis, peak oxygen uptake was dependent on sex, height, weight, physical activity, smoking and insulin levels. However, age was not significantly associated with peak oxygen uptake, but a steeper decline in peak oxygen uptake can be expected after the age of 50 years in males and 60 years in females (Fleg *et al.* 2005, Koch *et al.* 2009); in our study subjects were 30 to 47 years at time of the cardiopulmonary exercise test.

6.1.5. Assessing fatty liver

In this thesis, fatty liver was assessed visually with ultrasound from non-blinded images by a trained ultrasonographer. Evaluation of fatty liver was performed according to liver-to-kidney contrast, parenchymal brightness, deep beam attenuation and bright vessel walls. Liver ultrasound is relatively easy to perform in large population studies, is inexpensive and noninvasive.

In assessing fatty liver, liver biopsy is the gold standard because it is able to differentiate between simple steatosis and steatohepatitis based on histological evaluation of the tissue (Schwenzer *et al.* 2009). The sensitivity and specificity of ultrasound in detecting fatty liver are a matter of some debate. Saadeh et al. have estimated that ultrasound may detect fatty liver when more than 30% of the liver is affected (Saadeh *et al.* 2002). On the other hand, it has been demonstrated that ultrasonography has a sensitivity of 60–94% and a specificity of 66–95% in detecting fatty liver (Debongnie *et al.* 1981, Saverymuttu *et al.* 1986, GRAIF 2000). The specificity could be increased up to 100%, if the study subjects did not have known liver disease, drug use or alcohol consumption (Hamaguchi *et al.* 2007).

6.2. Results

6.2.1. Cardiovascular disease risk markers

6.2.1.1. Smoking

Physical activity is generally inversely associated with smoking (Marti *et al.* 1987, Tuomilehto *et al.* 1987). In this thesis, physical activity was inversely associated with smoking and this was independent of obesity variables. There are several modes of behavior that might explain this inverse association. Physical activity has been shown to be inversely associated with depression (Kaseva *et al.* 2016), whereas depression has been shown to be directly associated with smoking (Mathew *et al.* 2017). Physical activity may protect against smoking because of its beneficial effects on mood (Audrain-McGovern *et al.* 2003). It has been shown that temporary smoking abstinence leads to an increase in emotional stress, which returns to a normal level after smoking a cigarette (Steptoe and Ussher 2006). Acute aerobic exercise increases self-reported positive-activated affect (Reed and Ones 2006) and this positive effect from physical activity may decrease the urge to smoke. Both physical activity and smoking can be considered as a reward and physical activity might be so rewarding that people no longer need the reward associated with smoking (Audrain-McGovern *et al.* 2006). Raitakari et al. have shown that young subjects leading a sedentary life more often tend to start smoking compared to physically more active youths and in contrast, those remaining physically active hardly ever start to smoking during their transition from adolescence to young adulthood (Raitakari *et al.* 1994).

Furthermore, cardiorespiratory fitness was also inversely associated with smoking and again this was independent of obesity variables. In line with this finding, Benck et al. found that fit participants smoked less in young adulthood and after 20 years of follow-up (Benck *et al.* 2017). The same result emerged from Lee and Blair's large follow-up study (Lee and Blair 2002).

6.2.1.2. Hemodynamics

Physically active subjects generally have lower heart rates (Carter *et al.* 2003) as a result of increased parasympathetic tone (Yataco *et al.* 1997). One mechanism might be an improvement in the sensitivity and regulation of the cardiovagal baroreflex on the autonomic outflow induced by physical activity (Rennie *et al.* 2003). More fit individuals also tend to have lower heart rates (Cooper *et al.* 1976). In this thesis both physical activity and cardiorespiratory fitness were inversely associated with lower heart rates.

Physical activity is associated with lower blood pressure and a reduced risk for the development of hypertension in older persons, hypertensive adults and obese individuals (Arrol and Beaglehole 1992). In normotensive and younger individuals, this association is not generally observed (Alpert and Wilmore 1994). Accordingly, physical activity was not associated with blood pressure with this relatively young and normotensive population of this thesis. However interestingly, cardiorespiratory fitness was inversely associated with systolic and diastolic blood pressure. The association remained significant with diastolic blood pressure even after adjusting for waist circumference and smoking although it disappeared with systolic blood pressure. This might be partly explained by obesity indices. As seen in **Tables 10 and 17**, when moving from the lowest cardiorespiratory fitness tertile to the highest, waist circumference decreased 11 centimeters and BMI was reduced 4 units. On the other hand, when moving from the lowest physical activity tertile to the highest, waist circumference decreased only 3 centimeters and BMI declined by less than 1 unit. A larger relative reduction of body mass in cardiorespiratory fitness tertiles compared to physical activity tertiles might reflect more clearly on the association with blood pressure.

6.2.1.3. Lipids and apolipoproteins

Physical activity generally improves the lipid profile (Thompson *et al.* 2003, Ki *et al.* 2011, Herzig *et al.* 2014). In line with previous studies (Hostmark *et al.* 1992, MacAuley *et al.* 1996, Raitakari *et al.* 1997, Ahmed *et al.* 2011, Ahmed *et al.* 2012), in this thesis, physical activity was inversely associated with triglycerides, apolipoprotein B and apolipoprotein B/apolipoprotein A1 ratio in males and females in ten years' follow-up time. Physical activity was also inversely associated with oxidized LDL measured in 2001. Physical activity was associated directly with HDL cholesterol and apolipoprotein A1 measured in 2001.

In previous studies, physical activity has reduced LDL oxidation (Vasankari *et al.* 1998, Vuorimaa *et al.* 2005). The influence of physical activity on lipoprotein metabolism may be mediated through several mechanisms, including increased skeletal muscle lipoprotein lipase activity (Hamilton *et al.* 2004), decreased cholesterol ester transferase protein concentration or activity, increased lecithin cholesterol acyltransferase activity (Olchawa *et al.* 2004) and decreased hepatic triglyceride lipase activity (Pronk 1993).

Cardiorespiratory fitness was inversely associated with total cholesterol, LDL cholesterol, LDL/HDL-ratio, serum triglycerides, apolipoprotein B and apolipoprotein B/apolipoprotein A1 ratio, but when adjusting for waist

circumference and daily smoking, the association remained significant only with serum triglycerides. One mechanism could be that cardiorespiratory fitness increases lipoprotein lipase activity thus reducing the polygenic risk for hypertriglyceridemia (Tanisawa *et al.* 2014).

6.2.1.4. Glucose homeostasis

Physical activity has been reported to lessen insulin resistance (Kavouras *et al.* 2007, Schmidt *et al.* 2008) and increase insulin sensitivity (Ball *et al.* 2004, Rizzo *et al.* 2008). In this thesis, physical activity was inversely associated with insulin, glucose and HOMA-IR. When adjusted for waist circumference and smoking, the association between physical activity and insulin and HOMA-IR remained significant in every follow-up. Possible mechanisms may include increased muscle glucose transporter type 4 content, which increases glucose absorption (Dela *et al.* 1994), increased activity of muscle mitochondrial enzymes (Short *et al.* 2003) and improved insulin action in skeletal muscle (Cox *et al.* 1999).

Cardiorespiratory fitness inversely associated with glucose, serum insulin levels and HOMA-IR. When adjusting for waist circumference and daily smoking, the association remained significant with serum insulin and HOMA-IR. There are studies indicating that in genetically bred low fit rats, hepatic mitochondrial content and the capacity of mitochondria to oxidize fatty acids is reduced leading to impaired glucose metabolism (Wisloff *et al.* 2005, Morris *et al.* 2016). It may be that cardiorespiratory fitness is an attribute that may markedly influence glucose metabolism also in humans.

6.2.1.5. Inflammatory markers

There is convincing evidence that physical activity decreases CRP levels as this connection has been extensively investigated (Raitakari *et al.* 2005, Chen *et al.* 2014, Palmefors *et al.* 2014). Part of the effect of physical activity may be mediated by changes in fat mass and decreased adipocyte production of interleukin-6 (Ford 2002). In this thesis, physical activity and cardiorespiratory fitness were inversely associated with CRP and this association remained significant even after controlling for waist circumference and smoking, thus suggesting an independent effect of physical activity and cardiorespiratory fitness on the hs-CRP level. It seems that the anti-inflammatory effect of cardiorespiratory fitness is also mediated through decreased interleukin-6 levels (Park *et al.* 2017).

There is a lack of information regarding the effects of physical activity on phospholipase enzymes. In an intervention study with patients with type 2

diabetes, physical activity was not associated with secretory phospholipase A2 activity. In this thesis, physical activity was however, inversely associated with secretory phospholipase A2 activity measured in 2001 even after adjusting for waist circumference. This points to a direct effect of physical activity on secretory phospholipase activity.

There is some controversy concerning data on physical activity and serum amyloid A. In a study examining participants over 40 years of age, physical activity was inversely associated with serum amyloid A levels (Pitsavos *et al.* 2005). In a younger cohort, however, physical activity was not associated with serum levels of amyloid A (Verdaet *et al.* 2004). In this thesis, physical activity was not associated with the serum amyloid A concentration.

6.2.2. Physical activity, cardiorespiratory fitness and cardiovascular disease risk markers

The association between physical activity tertiles, cardiorespiratory fitness tertiles and cardiovascular disease markers in 504 study participants were gathered in **Table 18**. Although it would be tempting to compare which is better, physical activity or cardiorespiratory fitness, this comparison cannot be done on the basis of this table. The methodological differences are far too great as physical activity was acquired subjectively by a questionnaire whereas cardiorespiratory fitness was determined objectively as breath-by-breath measurements of oxygen uptake with cardiopulmonary exercise test.

6.2.3. Carotid artery ultrasound findings

Intima-media thickness, Young's elastic modulus and stiffness index increased and carotid artery distensibility decreased significantly between examination years 2001 and 2007. The stiffening of arteries is considered to be physiological change which comes with age and is caused by the fatigue and fracturing or matrix metallo-proteinases activity on elastin lamellae of central arteries (Greenwald 2007, O'Rourke and Hashimoto 2007). The elastin is transformed to collagen, which is 100-1000 times stiffer than elastin (Greenwald 2007). In this thesis, leisure-time physical activity in boys and young adults was associated favorably with carotid artery elasticity 21 years later.

There is very limited longitudinal data on the association of physical activity and carotid artery elasticity. It has been shown that lifetime vigorous habitual physical activity is favorably associated with brachial and femoral artery elasticity in young adults (van de Laar *et al.* 2010). A decrease in physical activity in teenaged males has been associated with increased arterial stiffness

after 3 years of follow-up (Chen *et al.* 2012). Regular physical activity was associated with increased small artery distensibility in young to middle aged males and females after 6 years of follow-up (Saladini *et al.* 2014). In cross-sectional studies, high physical activity has been associated with improved arterial elasticity in children (Nettlefold *et al.* 2012), adolescents (Ried-Larsen *et al.* 2015), young and middle aged adults (McGavock *et al.* 2006, Huynh *et al.* 2015) and in older adults (Laursen *et al.* 2015).

Although physical activity was related to arterial elasticity, there was no link between youth physical activity and adult carotid intima-media thickness. In the Special Turku Coronary Risk Factor Intervention Project –study, physical activity was inversely associated with aortic intima-media thickness in adolescents. However, aortic intima-media thickness might be a more sensitive marker of early atherosclerosis than carotid intima-media thickness (Järvisalo *et al.* 2001, Pahkala *et al.* 2011). The majority of cross-sectional studies in adults have reported inverse associations between physical activity and carotid intima-media thickness, but many studies have also failed to demonstrate this relationship. For example, physical activity was not associated with carotid intima-media thickness in over 1,500 adults participating in the NHLBI Family Heart Study (Kronenberg *et al.* 2000). There was also no difference in carotid intima-media thickness between sedentary and endurance-trained males (Tanaka *et al.* 2002). In their review, Kadoglou et al. suggested that a possible explanation for the differing results would derive from the wide variability of the different study populations and that the influence of physical activity on carotid intima-media thickness seems especially inconsistent among healthy subjects (Kadoglou *et al.* 2008). On the other hand, there are studies in which physical activity was associated with increased intima-media thickness (Cuspidi *et al.* 1996, Abergel *et al.* 1998, Casiglia *et al.* 2000). This effect could be a consequence of the increased wall stress during exercise and might prevent the excessive stretching of large arteries at high blood pressure levels (Casiglia *et al.* 2000).

There was no association between physical activity in childhood and carotid artery elasticity in the younger group of females. It is possible that there is a sex difference in the responsiveness to the exposure due to differences in physiology (e.g. hormonal differences) and/or to differences in the quality or quantity of the exposure dose, such as the amount of vigorous physical activity. It has been postulated that estrogen may protect arteries against atherogenesis (Baker *et al.* 2003). In the follow-up year, the females in the younger age group were 30 to 36 years old and the protective effect of estrogen may have masked a weaker effect associated with early physical activity exposure. There also might be a difference in the quality of physical activity in 9 to 15 year old males and females.

Cardiorespiratory fitness was not significantly associated with elasticity indices or carotid artery intima-media thickness after adjusting for BMI. In the Special Turku Coronary Risk Factor Intervention Project –study, cardiorespiratory fitness was inversely associated with aortic intima-media thickness in adolescents (Pahkala *et al.* 2013). This might again be a result of the development of atherosclerosis occurring initially in the intima of abdominal aorta (Järvisalo *et al.* 2001, Pahkala *et al.* 2011). It has been shown in subjects over the age of 40 years that cardiorespiratory fitness is inversely associated with carotid artery intima-media thickness (Lakka *et al.* 2001, Gando *et al.* 2011) but not in younger age groups, as also found in this thesis.

There are several possible mechanisms that might explain the association between physical activity with enhanced carotid artery elasticity. It has been shown that physical activity increases the elastin content of the aortic wall and probably enhances arterial dilatation and distensibility in rodents (Matsuda *et al.* 1993). In middle-aged and older adults, endurance training improves carotid artery elasticity and the effect is mediated through a reduction in alpha-adrenergic receptor-mediated vascular tone i.e. sympathetic nervous system activity is reduced (Sugawara *et al.* 2009) and conversely, parasympathetic tone (Yataco *et al.* 1997) is elevated with endurance training. High resting heart rate has been shown to associate with arterial stiffness (Quan *et al.* 2014) and as physical activity lowers heart rate (Carter *et al.* 2003) this could explain the protective mechanism of physical activity. Aerobic physical activity training may enhance the release of nitric oxide via increased shear stress during or immediately after physical activity bouts (Kingwell *et al.* 1997) and delay the age-associated reduction or fraction of elastic lamellae in the arterial wall (Schlatmann and Becker 1977). Aerobic exercise training has been shown to enhance common carotid artery elasticity and reduce the concentration of plasma endothelin-1, which is the most potent vasoconstrictor peptide secreted by vascular endothelial cells (Yanagisawa *et al.* 1988).

6.2.4. Fatty liver

In this thesis, higher cardiorespiratory fitness, independently linked with several cardiometabolic risk markers, was associated with lower risk of fatty liver. Importantly, this was evident also among those obese individuals suggesting that regardless of body weight status, attainment of even moderate fitness level may confer protection against the development of fatty liver.

In a recent review article, fatty liver has been strongly associated with cardiovascular disease, chronic kidney disease and type 2 diabetes mellitus (Adams *et al.* 2017). It has been suggested that fatty liver is associated with

colonic neoplasia and reduced bone mineral density (Adams *et al.* 2017). The benefits of physical activity on fatty liver have been extensively studied. Both aerobic and resistance training have been shown to improve fatty liver in adults (Hashida *et al.* 2017, Katsagoni *et al.* 2017). Moderate-to-vigorous exercise has been shown to decrease the risk of development of new fatty liver or improving resolution of existing fatty liver (Smart *et al.* 2016, Sung *et al.* 2016). In obese mice, moderate physical activity seems to enhance basal autophagy in the liver and this may confer protection from hepatic fat accumulation (Rosa-Caldwell *et al.* 2017). The effect of cardiorespiratory fitness on fatty liver has been less examined. Kantartzis et al. have previously shown in overweight adults that cardiorespiratory fitness is inversely associated with fatty liver independently of total adiposity, body fat distribution and exercise intensity (Kantartzis *et al.* 2009). Haufe et al. have reported similar results in an all-male cohort (Haufe *et al.* 2010). Morris et al. have shown that high fit rats are less susceptible to the ability of a high fat diet to induce fatty liver than low fit rats (Morris *et al.* 2014, Morris *et al.* 2016). In summary, it seems that both physical activity and cardiorespiratory fitness are important on cardiometabolic health.

6.3. Strengths and limitations

The major strength of this study is the large number of participants followed for 31 years. This cohort of racially homogenous males and females, was randomly selected, covering rural and urban areas in Finland; have been well phenotyped since childhood to adulthood. This study design has made it possible to examine not only cross-sectional associations but also longitudinal associations of physical activity with cardiovascular disease risk markers, subclinical markers of arterial health and fatty liver.

As the study cohort consists of white European subjects, the results may not be generalized to other ethnic groups. The cohort is still too young to have been subject to cardiovascular disease end-points, such as myocardial infarction or stroke and therefore it is not possible to examine the associations between physical activity or cardiorespiratory fitness with cardiovascular morbidity or mortality.

6.4. Clinical implications

Physical activity and cardiorespiratory fitness are inversely associated with a number of risk markers of cardiovascular diseases. This thesis supports the previous studies findings that physical activity and cardiorespiratory fitness are favorably associated with decreased smoking, glucose homeostasis, lipid and

apolipoprotein levels and hs-CRP in males and females. Physical activity already in childhood seems to exert a positive effect on arterial health and cardiorespiratory fitness is associated inversely with the risk of fatty liver, even among those individuals who are obese. Thus, it is important to encourage people to be more physically active, the long-term advantages of cardiorespiratory fitness need to be emphasized. The American Heart Association has recently issued a scientific statement of the importance of assessing cardiorespiratory fitness in clinical practice (Ross *et al.* 2016). In this statement, they suggest that low cardiorespiratory fitness should be recognized as important risk factor in a similar manner as smoking, hypertension, high cholesterol and type 2 diabetes; for this reason cardiorespiratory fitness should be assessed more frequently in clinical practice (Ross *et al.* 2016).

6.4.1. Future research needs

Among 9 to 15-year old females there was no association between leisure-time physical activity in childhood and adult carotid artery elasticity indices. The study setting should be repeated in an older cohort to examine if this association can be detected in these individuals.

Leisure-time physical activity measured in childhood or adulthood was not associated with adult carotid artery intima-media thickness. This should be examined also in an older cohort of subjects.

A prospective analysis of the predictive power of physical activity and cardiorespiratory fitness for hypertension, myocardial infarction, stroke and death should be conducted later as a part of the ongoing follow-up on this cohort.

7. SUMMARY OF RESULTS

1. Physical activity was favorably and independently associated with resting heart rate, levels of insulin, HOMA-IR and hs-CRP in all of the three follow-up studies.

2. Cardiorespiratory fitness was favorably and independently associated with diastolic blood pressure, resting heart rate, serum levels of triglycerides, insulin, HOMA-IR, hs-CRP.

3. Among 9 to 15 year-old males, leisure-time physical activity was associated with better carotid artery elasticity as indicated by carotid artery distensibility, Young's elastic modulus and stiffness index when these were measured 21 years later.

4. In young adults aged 18 to 24 years, leisure-time physical activity was associated with better carotid artery elasticity as indicated by carotid artery distensibility, Young's elastic modulus and stiffness index as measured 21 years later.

5. Physical activity was inversely associated with a lower risk of fatty liver independent of several confounding factors. However, when adjusting for cardiorespiratory fitness, the effect was attenuated.

6. Cardiorespiratory fitness was strongly, inversely and independently related with the risk of fatty liver, even among those obese individuals.

8. CONCLUSIONS

The following conclusions can be drawn from the results emerging from this thesis:

1. Physical activity and cardiorespiratory fitness are associated with a wide range of metabolic variables, including markers of glucose/insulin metabolism and inflammation and this association seems to persist after 10 years of follow-up with physical activity.

2. Leisure-time physical activity has a positive effect on carotid artery elasticity after 21 years of follow-up in 9 to 15 years old boys and 18 to 24 years old adults.

3. Cardiorespiratory fitness and physical activity are associated with a reduced risk of fatty liver even among obese subjects.

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Sincerely,

Kristiina Pälve
Turussa 21.11.2017

10. REFERENCES

Aatola, H, Magnussen, CG, Koivistoinen, T, Hutri-Kähönen, N, Juonala, M, Viikari, JS*, et al*. Simplified definitions of elevated pediatric blood pressure and high adult arterial stiffness. *Pediatrics.* 2013;132:e70-6.

Abdelmalek, MF, Suzuki, A, Guy, C, Unalp-Arida, A, Colvin, R, Johnson, RJ*, et al*. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. *Hepatology.* 2010;51:1961-71.

Abdulnour, J, Razmjou, S, Doucet, E, Boulay, P, Brochu, M, Rabasa-Lhoret, R*, et al*. Influence of cardiorespiratory fitness and physical activity levels on cardiometabolic risk factors during menopause transition: A MONET study. *Prev Med Rep.* 2016;4:277-82.

Abergel, E, Linhart, A, Chatellier, G, Gariepy, J, Ducardonnet, A, Diebold, B*, et al*. Vascular and cardiac remodeling in world class professional cyclists. *Am Heart J.* 1998;136:818-23.

Adams, LA, Anstee, QM, Tilg, H, Targher, G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut.* 2017;66:1138-53.

Agostinis-Sobrinho, C, Moreira, C, Abreu, S, Lopes, L, Oliveira-Santos, J, Steene-Johannessen, J*, et al*. Serum Adiponectin Levels and Cardiorespiratory Fitness in Nonoverweight and Overweight Portuguese Adolescents: The LabMed Physical Activity Study. *Pediatr Exerc Sci.* 2017;29:237-44.

Ahmad, T, Chasman, DI, Buring, JE, Lee, IM, Ridker, PM, Everett, BM. Physical activity modifies the effect of LPL, LIPC, and CETP polymorphisms on HDL-C levels and the risk of myocardial infarction in women of European ancestry. *Circ Cardiovasc Genet.* 2011;4:74-80.

Ahmed, HM, Blaha, MJ, Nasir, K, Rivera, JJ, Blumenthal, RS. Effects of physical activity on cardiovascular disease. *Am J Cardiol.* 2012;109:288-95.

Ahmed, K, Rask, P, Hurtig-Wennlof, A. Serum apolipoproteins, apoB/apoA-I ratio and objectively measured physical activity in elderly. *Scand Cardiovasc J.* 2011;45:105-11.

Ainsworth, B, Cahalin, L, Buman, M, Ross, R. The current state of physical activity assessment tools. *Prog Cardiovasc Dis.* 2015;57:387-95.

Ainsworth, BE, Haskell, WL, Leon, AS, Jacobs, DR,Jr, Montoye, HJ, Sallis, JF*, et al*. Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc.* 1993;25:71-80.

Ainsworth, BE, Richardson, MT, Jacobs, DR,Jr, Leon, AS, Sternfeld, B. Accuracy of recall of occupational physical activity by questionnaire. *J Clin Epidemiol.* 1999;52:219-27.

Alessio, HM and Goldfarb, AH. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. *J Appl Physiol (1985).* 1988;64:1333-6.

Alpert, BS and Wilmore, JH. Physical Activity and Blood Pressure in Adolescents. *Pediatr Exerc Sci.* 1994;4:361-80.

Ambrose, JA and Barua, RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol.* 2004;43:1731-7.

American Thoracic Society and American College of Chest Physicians. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med.* 2003;167:211-77.

Andersen, LB, Riddoch, C, Kriemler, S, Hills, AP. Physical activity and cardiovascular risk factors in children. *Br J Sports Med.* 2011;45:871-6.

Andrikoula, M and McDowell, IF. The contribution of ApoB and ApoA1 measurements to cardiovascular risk assessment. *Diabetes Obes Metab.* 2008;10:271-8.

Antoniades, C, Antonopoulos, AS, Tousoulis, D, Stefanadis, C. Adiponectin: from obesity to cardiovascular disease. *Obes Rev.* 2009;10:269-79.

Apabhai, S, Gorman, GS, Sutton, L, Elson, JL, Plotz, T, Turnbull, DM*, et al*. Habitual physical activity in mitochondrial disease. *PLoS One.* 2011;6:e22294.

Arena, R, Myers, J, Abella, J, Pinkstaff, S, Brubaker, P, Kitzman, DW*, et al*. Cardiopulmonary exercise testing is equally prognostic in young, middle-aged and older individuals diagnosed with heart failure. *Int J Cardiol.* 2011;151:278-83.

Arena, R, Myers, J, Guazzi, M. The clinical and research applications of aerobic capacity and ventilatory efficiency in heart failure: an evidence-based review. *Heart Fail Rev.* 2008;13:245-69.

Armstrong, N. Young people's physical activity patterns as assessed by heart rate monitoring. *J Sports Sci.* 1998;16 Supplement:S9-16.

Armstrong, N and Welsman, JR. The physical activity patterns of European youth with reference to methods of assessment. *Sports Med.* 2006;36:1067-86.

Arnett, DK, Evans, GW, Riley, WA. Arterial stiffness: a new cardiovascular risk factor? *Am J Epidemiol.* 1994;140:669-82.

Arrol, B and Beaglehole, R. Does physical activity lower blood pressure: A critical review of the clinical trials. *J Clin Epidemiol.* 1992;45:439-47.

Assmann, G, Schulte, H, von Eckardstein, A, Huang, Y. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis.* 1996;124, Supplement:S11-20.

Audrain-McGovern, J, Rodriguez, D, Moss, HB. Smoking progression and physical activity. *Cancer Epidemiol Biomarkers Prev.* 2003;12:1121-9.

Audrain-McGovern, J, Rodriguez, D, Wileyto, EP, Schmitz, KH, Shields, PG. Effect of team sport participation on genetic predisposition to adolescent smoking progression. *Arch Gen Psychiatry.* 2006;63:433-41.

Baker, L, Meldrum, KK, Wang, M, Sankula, R, Vanam, R, Raiesdana, A*, et al*. The role of estrogen in cardiovascular disease. *J Surg Res.* 2003;115:325-44.

Ball, GD, Shaibi, GQ, Cruz, ML, Watkins, MP, Weigensberg, MJ, Goran, MI. Insulin sensitivity, cardiorespiratory fitness, and physical activity in overweight Hispanic youth. *Obes Res.* 2004;12:77-85.

Bao, W, Threefoot, SA, Srinivasan, SR, Berenson, GS. Essential hypertension predicted by tracking of elevated blood pressure from childhood to adulthood: the Bogalusa Heart Study. *Am J Hypertens.* 1995;8:657-65.

Barlow, CE, LaMonte, MJ, Fitzgerald, SJ, Kampert, JB, Perrin, JL, Blair, SN. Cardiorespiratory fitness is an independent predictor of hypertension incidence among initially normotensive healthy women. *Am J Epidemiol.* 2006;163:142-50.

Barter, P, Gotto, AM, LaRosa, JC, Maroni, J, Szarek, M, Grundy, SM*, et al*. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med.* 2007;357:1301-10.

Beauchamp, A, Peeters, A, Wolfe, R, Turrell, G, Harriss, LR, Giles, GG*, et al*. Inequalities in cardiovascular disease mortality: the role of behavioural, physiological and social risk factors. *J Epidemiol Community Health.* 2010;64:542-8.

Beltowski, J and Kedra, A. Asymmetric dimethylarginine (ADMA) as a target for pharmacotherapy. *Pharmacol Rep.* 2006;58:159-78.

Benck, LR, Cuttica, MJ, Colangelo, LA, Sidney, S, Dransfield, MT, Mannino, DM*, et al*. Association between Cardiorespiratory Fitness and Lung Health from Young Adulthood to Middle Age. *Am J Respir Crit Care Med.* 2017;195:1236-43.

Benn, M. Apolipoprotein B levels, APOB alleles, and risk of ischemic cardiovascular disease in the general population, a review. *Atherosclerosis.* 2009;206:17-30.

Berenson, GS. Childhood risk factors predict adult risk associated with subclinical cardiovascular disease. The Bogalusa Heart Study. *Am J Cardiol.* 2002;90:3L-7L.

Bergh, U, Thorstensson, A, Sjodin, B, Hulten, B, Piehl, K, Karlsson, J. Maximal oxygen uptake and muscle fiber types in trained and untrained humans. *Med Sci Sports.* 1978;10:151-4.

Blair, SN. Physical inactivity and cardiovascular disease risk in women. *Med Sci Sports Exerc.* 1996;28:9-10.

Blair, SN, Cheng, Y, Holder, JS. Is physical activity or physical fitness more important in defining health benefits? *Med Sci Sports Exerc.* 2001;33:379-99.

Blair, SN, Kampert, JB, Kohl, HW,3rd, Barlow, CE, Macera, CA, Paffenbarger, RS,Jr*, et al*. Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *J. Am. Med. Assoc.* 1996;276:205-10.

Bland, J, Skordalaki, A, Emery, JL. Early intimal lesions in the common carotid artery. *Cardiovasc Res.* 1986;20:863-8.

Bode-Boger, SM, Scalera, F, Kielstein, JT, Martens-Lobenhoffer, J, Breithardt, G, Fobker, M*, et al*. Symmetrical dimethylarginine: a new combined parameter for renal function and extent of coronary artery disease. *J Am Soc Nephrol.* 2006;17:1128-34.

Boekholdt, SM, Keller, TT, Wareham, NJ, Luben, R, Bingham, SA, Day, NE*, et al*. Serum levels of type II secretory phospholipase A2 and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk Prospective Population Study. *Arterioscler Thromb Vasc Biol.* 2005;25:839-46.

Bohm, M, Reil, JC, Deedwania, P, Kim, JB, Borer, JS. Resting heart rate: risk indicator and emerging risk factor in cardiovascular disease. *Am J Med.* 2015;128:219-28.

Borg, GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc.* 1982;14:377-81.

Borodulin, K, Laatikainen, T, Salomaa, V, Jousilahti, P. Associations of leisure time physical activity, self-rated physical fitness, and estimated aerobic fitness with serum C-reactive protein among 3,803 adults. *Atherosclerosis.* 2006;185:381-7.

Borow, KM and Newburger, JW. Noninvasive estimation of central aortic pressure using the oscillometric method for analyzing systemic artery pulsatile blood flow: comparative study of indirect systolic, diastolic, and mean brachial artery pressure with simultaneous direct ascending aortic pressure measurements. *Am Heart J.* 1982;103:879-86.

Bots, ML, Dijk, JM, Oren, A, Grobbee, DE. Carotid intima-media thickness, arterial stiffness and risk of cardiovascular disease: current evidence. *J Hypertens.* 2002;20:2317-25.

Bouchard, C and Rankinen, T. Individual differences in response to regular physical activity. *Med Sci Sports Exerc.* 2001;33:S446-51.

Bouchard, C and Shephard, R. Physical activity, fitness and health: the model and key concepts. In: Bouchard C, Shephard R, Stephens T, eds. Physical activity, fitness and health. International proceedings and consensus statement. *Human Kinetics, Champaign, IL.* 1994;77-88.

Boutouyrie, P, Bussy, C, Lacolley, P, Girerd, X, Laloux, B, Laurent, S. Association between local pulse pressure, mean blood pressure, and large-artery remodeling. *Circulation.* 1999;100:1387-93.

Braunwald, E. Shattuck lecture--cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N Engl J Med.* 1997;337:1360-9.

Bray, MS, Hagberg, JM, Perusse, L, Rankinen, T, Roth, SM, Wolfarth, B*, et al*. The human gene map for performance and health-related fitness phenotypes: the 2006-2007 update. *Med Sci Sports Exerc.* 2009;41:35-73.

Breneman, CB, Polinski, K, Sarzynski, MA, Lavie, CJ, Kokkinos, PF, Ahmed, A*, et al*. The Impact of Cardiorespiratory Fitness Levels on the Risk of Developing Atherogenic Dyslipidemia. *Am J Med.* 2016;129:1060-6.

Butland, RJ, Pang, J, Gross, ER, Woodcock, AA, Geddes, DM. Two-, six-, and 12-minute walking tests in respiratory disease. *BMJ (Clin Res Ed).* 1982;284:1607-8.

Butte, NF, Ekelund, U, Westerterp, KR. Assessing physical activity using wearable monitors: measures of physical activity. *Med Sci Sports Exerc.* 2012;44:S5-12.

Byrne, CD and Targher, G. NAFLD: A multisystem disease. *J Hepatol.* 2015;62:S47-64.

Carter, JB, Banister, EW, Blaber, AP. Effect of endurance exercise on autonomic control of heart rate. *Sports Med.* 2003;33:33-46.

Casiglia, E, Palatini, P, Da Ros, S, Pagliara, V, Puato, M, Dorigatti, F*, et al*. Effect of blood pressure and physical activity on carotid artery intima-media thickness in stage 1 hypertensives and controls. *Am J Hypertens.* 2000;13:1256-62.

Caspersen, CJ, Powell, KE, Christenson, GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep.* 1985;100:126-31.

Chalasani, N, Younossi, Z, Lavine, JE, Diehl, AM, Brunt, EM, Cusi, K*, et al*. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology.* 2012;55:2005-23.

Chen, W, Yun, M, Fernandez, C, Li, S, Sun, D, Lai, CC*, et al*. Secondhand smoke exposure is associated with increased carotid artery intima-media thickness: The Bogalusa Heart Study. *Atherosclerosis.* 2015;240:374-9.

Chen, X and Wang, Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. *Circulation.* 2008;117:3171-80.

Chen, Y, Dangardt, F, Osika, W, Berggren, K, Gronowitz, E, Friberg, P. Age- and sex-related differences in vascular function and vascular response to mental stress. Longitudinal and cross-sectional studies in a cohort of healthy children and adolescents. *Atherosclerosis.* 2012;220:269-74.

Chen, Y, Apostolakis, S, Lip, GYH. Exercise-induced changes in inflammatory processes: Implications for thrombogenesis in cardiovascular disease. *Ann Med.* 2014;46:439-55.

Church, TS, Barlow, CE, Earnest, CP, Kampert, JB, Priest, EL, Blair, SN. Associations between cardiorespiratory fitness and C-reactive protein in men. *Arterioscler Thromb Vasc Biol.* 2002;22:1869-76.

Church, TS, Kuk, JL, Ross, R, Priest, EL, Biltoft, E, Blair, SN. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. *Gastroenterology.* 2006;130:2023-30.

Cleeman, JI. Adults aged 20 and older should have their cholesterol measured. *Am J Med.* 1997;102:31-6.

Closs, EI, Basha, FZ, Habermeier, A, Forstermann, U. Interference of L-arginine analogues with L-arginine transport mediated by the y+ carrier hCAT-2B. *Nitric Oxide.* 1997;1:65-73.

Conen, D and Bamberg, F. Noninvasive 24-h ambulatory blood pressure and cardiovascular disease: a systematic review and meta-analysis. *J Hypertens.* 2008;26:1290-9.

Contois, J, McNamara, JR, Lammi-Keefe, C, Wilson, PW, Massov, T, Schaefer, EJ. Reference intervals for plasma apolipoprotein A-1 determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. *Clin Chem.* 1996;42:507-14.

Cooke, JP. Asymmetrical dimethylarginine: the Uber marker? *Circulation.* 2004;109:1813-8.

Cooper, KH. A means of assessing maximal oxygen intake. Correlation between field and treadmill testing. *J. Am. Med. Assoc.* 1968;203:201-4.

Cooper, KH, Pollock, ML, Martin, RP, White, SR, Linnerud, AC, Jackson, A. Physical fitness levels vs selected coronary risk factors. A cross-sectional study. *J. Am. Med. Assoc.* 1976;236:166-9.

Cortez-Pinto, H, Jesus, L, Barros, H, Lopes, C, Moura, MC, Camilo, ME. How different is the dietary pattern in non-alcoholic steatohepatitis patients? *Clin Nutr.* 2006;25:816-23.

Cox, JH, Cortright, RN, Dohm, GL, Houmard, JA. Effect of aging on response to exercise training in humans: skeletal muscle GLUT-4 and insulin sensitivity. *J Appl Physiol (1985).* 1999;86:2019-25.

Cronin, O, Morris, DR, Walker, PJ, Golledge, J. The association of obesity with cardiovascular events in patients with peripheral artery disease. *Atherosclerosis.* 2013;228:316-23.

Crouter, SE, Schneider, PL, Karabulut, M, Bassett, DR,Jr. Validity of 10 electronic pedometers for measuring steps, distance, and energy cost. *Med Sci Sports Exerc.* 2003;35:1455-60.

Cuspidi, C, Lonati, L, Sampieri, L, Leonetti, G, Zanchetti, A. Similarities and differences in structural and functional changes of left ventricle and carotid arteries in young borderline hypertensives and in athletes. *J Hypertens.* 1996;14:759-64.

D'Agostino RB, S, Vasan, RS, Pencina, MJ, Wolf, PA, Cobain, M, Massaro, JM*, et al*. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation.* 2008;117:743-53.

Dannenberg, AL, Keller, JB, Wilson, PWF, Castelli, WP. Leisure time physical activity in the Framingham Offspring Study: description, seasonal variation, and risk factor correlates. *Am J Epidemiol.* 1989;129:76-88.

de Groot, E, Hovingh, GK, Wiegman, A, Duriez, P, Smit, AJ, Fruchart, JC*, et al*. Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. *Circulation.* 2004;109:III33-8.

De Moor, MH, Liu, YJ, Boomsma, DI, Li, J, Hamilton, JJ, Hottenga, JJ*, et al*. Genome-wide association study of exercise behavior in Dutch and American adults. *Med Sci Sports Exerc.* 2009;41:1887-95.

Debongnie, JC, Pauls, C, Fievez, M, Wibin, E. Prospective evaluation of the diagnostic accuracy of liver ultrasonography. *Gut.* 1981;22:130-5.

DeFina, LF, Haskell, WL, Willis, BL, Barlow, CE, Finley, CE, Levine, BD*, et al*. Physical Activity Versus Cardiorespiratory Fitness: Two (Partly) Distinct Components of Cardiovascular Health? *Prog Cardiovasc Dis.* 2015;57:324-9.

deGoma, EM, Leeper, NJ, Heidenreich, PA. Clinical Significance of High-Density Lipoprotein Cholesterol in Patients With Low Low-Density Lipoprotein Cholesterol. *J Am Coll Cardiol.* 2008;51:49-55.

Dela, F, Ploug, T, Handberg, A, Petersen, LN, Larsen, JJ, Mikines, KJ*, et al*. Physical training increases muscle GLUT4 protein and mRNA in patients with NIDDM. *Diabetes.* 1994;43:862-5.

Dennis, EA. Diversity of group types, regulation, and function of phospholipase A2. *J Biol Chem.* 1994;269:13057-60.

Devaraj, S, Kumaresan, PR, Jialal, I. C-reactive protein induces release of both endothelial microparticles and circulating endothelial cells in vitro and in vivo: further evidence of endothelial dysfunction. *Clin Chem.* 2011;57:1757-61.

Di Costanzo, A, D'Erasmo, L, Polimeni, L, Baratta, F, Coletta, P, Di Martino, M*, et al*. Non-alcoholic fatty liver disease and subclinical atherosclerosis: A comparison of metabolically- versus genetically-driven excess fat hepatic storage. *Atherosclerosis.* 2017;257:232-9.

Do, R, Willer, CJ, Schmidt, EM, Sengupta, S, Gao, C, Peloso, GM*, et al*. Common variants associated with plasma triglycerides and risk for coronary artery disease. *Nat Genet.* 2013;45:1345-52.

Dongiovanni, P, Romeo, S, Valenti, L. Genetic Factors in the Pathogenesis of Nonalcoholic Fatty Liver and Steatohepatitis. *Biomed Res Int.* 2015;2015:460190.

Eikendal, AL, Groenewegen, KA, Anderson, TJ, Britton, AR, Engstrom, G, Evans, GW, *et al*. Common carotid intima-media thickness relates to cardiovascular events in adults aged <45 years. *Hypertension.* 2015;65:707-13.

Esteghamati, A, Khalilzadeh, O, Ashraf, H, Zandieh, A, Morteza, A, Rashidi, A, *et al*. Physical activity is correlated with serum leptin independent of obesity: results of the national surveillance of risk factors of noncommunicable diseases in Iran (SuRFNCD-2007). *Metabolism.* 2010;59:1730-5.

Fabbrini, E, Magkos, F, Mohammed, BS, Pietka, T, Abumrad, NA, Patterson, BW, *et al*. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci U S A.* 2009;106:15430-5.

Fedotovskaya, ON, Mustafina, LJ, Popov, DV, Vinogradova, OL, Ahmetov, II. A common polymorphism of the MCT1 gene and athletic performance. *Int J Sports Physiol Perform.* 2014;9:173-80.

Ferreira, I, Twisk, JW, Stehouwer, CD, van Mechelen, W, Kemper, HC. Longitudinal changes in .VO2max: associations with carotid IMT and arterial stiffness. *Med Sci Sports Exerc.* 2003;35:1670-8.

Ferreira, I, van de Laar, RJ, Prins, MH, Twisk, JW, Stehouwer, CD. Carotid stiffness in young adults: a life-course analysis of its early determinants: the Amsterdam Growth and Health Longitudinal Study. *Hypertension.* 2012;59:54-61.

Fleg, JL, Morrell, CH, Bos, AG, Brant, LJ, Talbot, LA, Wright, JG, *et al*. Accelerated longitudinal decline of aerobic capacity in healthy older adults. *Circulation.* 2005;112:674-82.

Fogarty, MC, Hughes, CM, Burke, G, Brown, JC, Trinick, TR, Duly, E, *et al*. Exercise-induced lipid peroxidation: Implications for deoxyribonucleic acid damage and systemic free radical generation. *Environ Mol Mutagen.* 2011;52:35-42.

Folsom, AR, Eckfeldt, JH, Weitzman, S, Ma, J, Chambless, LE, Barnes, RW, *et al*. Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Stroke.* 1994;25:66-73.

Ford, ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. *Epidemiology.* 2002;13:561-8.

Frank, CW, Weinblatt, E, Shapiro, S, Sager, RV. Myocardial infarction in men. Role of physical activity and smoking in incidence and mortality. *J. Am. Med. Assoc.* 1966;198:1241-5.

Franks, PW, Farooqi, IS, Luan, J, Wong, MY, Halsall, I, O'Rahilly, S, *et al*. Does physical activity energy expenditure explain the between-individual variation in plasma leptin concentrations after adjusting for differences in body composition? *J Clin Endocrinol Metab.* 2003;88:3258-63.

Friedewald, WT, Levy, RI, Fredrickson, DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18:499-502.

Gall, S, Huynh, QL, Magnussen, CG, Juonala, M, Viikari, JS, Kähönen, M, *et al*. Exposure to parental smoking in childhood or adolescence is associated with increased carotid intima-media thickness in young adults: evidence from the Cardiovascular Risk in Young Finns study and the Childhood Determinants of Adult Health Study. *Eur Heart J.* 2014;35:2484-91.

Gando, Y, Yamamoto, K, Kawano, H, Murakami, H, Ohmori, Y, Kawakami, R, *et al*. Attenuated age-related carotid arterial remodeling in adults with a high level of cardiorespiratory fitness. *J Atheroscler Thromb.* 2011;18:248-54.

Gando, Y, Yamamoto, K, Murakami, H, Ohmori, Y, Kawakami, R, Sanada, K, *et al*. Longer time spent in light physical activity is associated with reduced arterial stiffness in older adults. *Hypertension.* 2010;56:540-6.

Gidding, SS, Lichtenstein, AH, Faith, MS, Karpyn, A, Mennella, JA, Popkin, B, *et al*. Implementing American Heart Association pediatric and adult nutrition guidelines: a scientific statement from the American Heart Association Nutrition Committee of the Council on Nutrition, Physical Activity and Metabolism, Council on Cardiovascular Disease in the Young, Council on Arteriosclerosis, Thrombosis and Vascular Biology, Council on Cardiovascular Nursing, Council on Epidemiology and Prevention, and Council for High Blood Pressure Research. *Circulation.* 2009;119:1161-75.

Glass, TW and Maher, CG. Physical activity reduces cigarette cravings. *Br J Sports Med.* 2014;48:1263-4.

Gleeson, M, Bishop, NC, Stensel, DJ, Lindley, MR, Mastana, SS, Nimmo, MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol.* 2011;11:607-15.

Gordon, T, Castelli, WP, Hjortland, MC, Kannel, WB, Dawber, TR. High density lipoprotein as a protective factor against coronary heart disease: The Framingham study. *Am J Med.* 1977;62:707-14.

GRAIF. Quantitative Estimation of Attenuation in Ultrasound Video Images: Correlation with Histology in Diffuse Liver Disease. *Invest Radiol.* 2000;35:319-24.

Greenwald, SE. Ageing of the conduit arteries. *J Pathol.* 2007;211:157-72.

Haasova, M, Warren, FC, Ussher, M, Janse Van Rensburg, K, Faulkner, G, Cropley, M*, et al*. The acute effects of physical activity on cigarette cravings: exploration of potential moderators, mediators and physical activity attributes using individual participant data (IPD) meta-analyses. *Psychopharmacology (Berl).* 2014;231:1267-75.

Haffner, SM, Lehto, S, Rönnemaa, T, Pyörälä, K, Laakso, M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med.* 1998;339:229-34.

Hakanen, M, Rönnemaa, T, Talvia, S, Rask-Nissilä, L, Koulu, M, Viikari, J*, et al*. Serum leptin concentration poorly reflects growth and energy and nutrient intake in young children. *Pediatrics.* 2004;113:1273-8.

Hamaguchi, M, Kojima, T, Itoh, Y, Harano, Y, Fujii, K, Nakajima, T*, et al*. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol.* 2007;102:2708-15.

Hamilton, MT, Hamilton, DG, Zderic, TW. Exercise physiology versus inactivity physiology: an essential concept for understanding lipoprotein lipase regulation. *Exerc Sport Sci Rev.* 2004;32:161-6.

Harber, MP, Kaminsky, LA, Arena, R, Blair, SN, Franklin, BA, Myers, J*, et al*. Impact of Cardiorespiratory Fitness on All-Cause and Disease-Specific Mortality: Advances Since 2009. *Prog Cardiovasc Dis.* 2017;

Harrington, J, Pena, AS, Gent, R, Hirte, C, Couper, J. Aortic intima media thickness is an early marker of atherosclerosis in children with type 1 diabetes mellitus. *J Pediatr.* 2010;156:237-41.

Hart, CL, Morrison, DS, Batty, GD, Mitchell, RJ, Davey Smith, G. Effect of body mass index and alcohol consumption on liver disease: analysis of data from two prospective cohort studies. *BMJ.* 2010;340:c1240.

Hashida, R, Kawaguchi, T, Bekki, M, Omoto, M, Matsuse, H, Nago, T*, et al*. Aerobic vs. resistance exercise in non-alcoholic fatty liver disease: A systematic review. *J Hepatol.* 2017;66:142-52.

Haufe, S, Engeli, S, Budziarek, P, Utz, W, Schulz-Menger, J, Hermsdorf, M*, et al*. Cardiorespiratory fitness and insulin sensitivity in overweight or obese subjects may be linked through intrahepatic lipid content. *Diabetes.* 2010;59:1640-7.

Hermansen, L and Saltin, B. Oxygen uptake during maximal treadmill and bicycle exercise. *J Appl Physiol.* 1969;26:31-7.

Herzig, KH, Ahola, R, Leppäluoto, J, Jokelainen, J, Jämsä, T, Keinänen-Kiukaanniemi, S. Light physical activity determined by a motion sensor decreases insulin resistance, improves lipid homeostasis and reduces visceral fat in high-risk subjects: PreDiabEx study RCT. *Int J Obes (Lond).* 2014;38:1089-96.

Holvoet, P, Lee, DH, Steffes, M, Gross, M, Jacobs, DR,Jr. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. *J. Am. Med. Assoc..* 2008;299:2287-93.

Hopkins, PN and Williams, RR. A survey of 246 suggested coronary risk factors. *Atherosclerosis.* 1981;40:1-52.

Hostmark, AT, Berg, J, Brudal, S, Berge, SR, Kierulf, P, Bjerkedal, T. Coronary risk factors in middle-aged men as related to smoking, coffee intake and physical activity. *Scand J Soc Med.* 1992;20:196-203.

Hurt-Camejo, E, Camejo, G, Peilot, H, Oorni, K, Kovanen, P. Phospholipase A(2) in vascular disease. *Circ Res.* 2001;89:298-304.

Husu, P, Tokola, K, Suni, J, Luoto, R, Sievänen, H, Mäki-Opas, T*, et al*. Istuminen ja terveysliikuntasuositusten toteutuminen suomalaisilla aikuisilla vuonna 2013 - ATH-tutkimuksen tuloksia. *Tutkimuksesta tiiviisti: 2014_005.* 2014.

Huszczuk, A, Whipp, BJ, Wasserman, K. A respiratory gas exchange simulator for routine calibration in metabolic studies. *Eur Respir J.* 1990;3:465-8.

Huynh, QL, Blizzard, CL, Raitakari, O, Sharman, JE, Magnussen, CG, Dwyer, T*, et al*. Vigorous physical activity and carotid distensibility in young and mid-aged adults. *Hypertens Res.* 2015;38:355-60.

Ischander, M, Zaldivar, F,Jr, Eliakim, A, Nussbaum, E, Dunton, G, Leu, SY*, et al*. Physical activity, growth, and inflammatory mediators in BMI-matched female adolescents. *Med Sci Sports Exerc.* 2007;39:1131-8.

Ishigaki, Y, Oka, Y, Katagiri, H. Circulating oxidized LDL: a biomarker and a pathogenic factor. *Curr Opin Lipidol.* 2009;20:363-9.

Jae, SY, Heffernan, K, Fernhall, B, Choi, YH. Cardiorespiratory fitness and carotid artery intima media thickness in men with type 2 diabetes. *J Phys Act Health.* 2012;9:549-53.

Jago, R, Drews, KL, McMurray, RG, Thompson, D, Volpe, SL, Moe, EL*, et al*. Fatness, fitness, and cardiometabolic risk factors among sixth-grade youth. *Med Sci Sports Exerc.* 2010;42:1502-10.

Jeon, CY, Lokken, RP, Hu, FB, van Dam, RM. Physical activity of moderate intensity and risk of type 2 diabetes: a systematic review. *Diabetes Care.* 2007;30:744-52.

Jimenez-Pavon, D, Ortega, FB, Artero, EG, Labayen, I, Vicente-Rodriguez, G, Huybrechts, I*, et al*. Physical activity, fitness, and serum leptin concentrations in adolescents. *J Pediatr.* 2012;160:598-603.

Johannsen, NM, Swift, DL, Lavie, CJ, Earnest, CP, Blair, SN, Church, TS. Combined Aerobic and Resistance Training Effects on Glucose Homeostasis, Fitness, and Other Major Health Indices: A Review of Current Guidelines. *Sports Med.* 2016;46:1809-18.

Joy, D, Thava, VR, Scott, BB. Diagnosis of fatty liver disease: is biopsy necessary? *Eur J Gastroenterol Hepatol.* 2003;15:539-43.

Juhola, J, Magnussen, CG, Berenson, GS, Venn, A, Burns, TL, Sabin, MA*, et al*. Combined effects of child and adult elevated blood pressure on subclinical atherosclerosis: the International Childhood Cardiovascular Cohort Consortium. *Circulation.* 2013;128:217-24.

Juhola, J, Magnussen, CG, Viikari, JS, Kähönen, M, Hutri-Kähönen, N, Jula, A*, et al*. Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: the Cardiovascular Risk in Young Finns Study. *J Pediatr.* 2011;159:584-90.

Juonala, M, Järvisalo, MJ, Mäki-Torkko, N, Kähönen, M, Viikari, JS, Raitakari, OT. Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. *Circulation.* 2005;112:1486-93.

Juonala, M, Kähönen, M, Laitinen, T, Hutri-Kähönen, N, Jokinen, E, Taittonen, L*, et al*. Effect of age and sex on carotid intima-media thickness, elasticity and brachial endothelial function in healthy adults: The Cardiovascular Risk in Young Finns Study. *Eur Heart J.* 2008;29:1198-206.

Juonala, M, Magnussen, CG, Venn, A, Gall, S, Kähönen, M, Laitinen, T*, et al*. Parental smoking in childhood and brachial artery flow-mediated dilatation in young adults: the Cardiovascular Risk in Young Finns study and the Childhood Determinants of Adult Health study. *Arterioscler Thromb Vasc Biol.* 2012;32:1024-31.

Juonala, M, Viikari, JS, Hutri-Kähönen, N, Pietikäinen, M, Jokinen, E, Taittonen, L*, et al*. The 21-year follow-up of the Cardiovascular Risk in Young Finns Study: risk factor levels, secular trends and east-west difference. *J Intern Med.* 2004;255:457-68.

Juonala, M, Viikari, JS, Rönnemaa, T, Helenius, H, Taittonen, L, Raitakari, OT. Elevated blood pressure in adolescent boys predicts endothelial dysfunction: the cardiovascular risk in young Finns study. *Hypertension.* 2006a;48:424-30.

Juonala, M, Viikari, JS, Rönnemaa, T, Taittonen, L, Marniemi, J, Raitakari, OT. Childhood C-reactive protein in predicting CRP and carotid intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *Arterioscler Thromb Vasc Biol.* 2006b;26:1883-8.

Jylhävä, J, Haarala, A, Eklund, C, Pertovaara, M, Kähönen, M, Hutri-Kähönen, N*, et al*. Serum amyloid A is independently associated with metabolic risk factors but not with early atherosclerosis: the Cardiovascular Risk in Young Finns Study. *J Intern Med.* 2009;266:286-95.

Järvisalo, MJ, Jartti, L, Näntö-Salonen, K, Irjala, K, Rönnemaa, T, Hartiala, JJ*, et al*. Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. *Circulation.* 2001;104:2943-7.

Kadoglou, NP, Iliadis, F, Liapis, CD. Exercise and carotid atherosclerosis. *Eur J Vasc Endovasc Surg.* 2008;35:264-72.

Kanerva, N, Sandboge, S, Kaartinen, NE, Männistö, S, Eriksson, JG. Higher fructose intake is inversely associated with risk of nonalcoholic fatty liver disease in older Finnish adults. *Am J Clin Nutr.* 2014;100:1133-8.

Kannel, WB, Belanger, A, D'Agostino, R, Israel, I. Physical activity and physical demand on the job and risk of cardiovascular disease and death: The Framingham Study. *Am Heart J.* 1986;112:820-5.

Kannel, WB, Vasan, RS, Levy, D. Is the relation of systolic blood pressure to risk of cardiovascular disease continuous and graded, or are there critical values? *Hypertension.* 2003;42:453-6.

Kantartzis, K, Thamer, C, Peter, A, Machann, J, Schick, F, Schraml, C*, et al*. High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. *Gut.* 2009;58:1281-8.

Karamanoglu, M, O'Rourke, MF, Avolio, AP, Kelly, RP. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J.* 1993;14:160-7.

Karvinen, S, Waller, K, Silvennoinen, M, Koch, LG, Britton, SL, Kaprio, J*, et al*. Physical activity in adulthood: genes and mortality. *Sci Rep.* 2015;5:18259.

Kaseva, K, Rosenström, T, Hintsa, T, Pulkki-Råback, L, Tammelin, T, Lipsanen, J*, et al*. Trajectories of Physical Activity Predict the Onset of Depressive Symptoms but Not Their Progression: A Prospective Cohort Study. *J Sports Med (Hindawi Publ Corp).* 2016;2016:8947375.

Katano, H, Ohno, M, Yamada, K. Protection by physical activity against deleterious effect of smoking on carotid intima-media thickness in young Japanese. *J Stroke Cerebrovasc Dis.* 2013;22:176-83.

Katsagoni, CN, Georgoulis, M, Papatheodoridis, GV, Panagiotakos, DB, Kontogianni, MD. Effects of lifestyle interventions on clinical characteristics of patients with non-alcoholic fatty liver disease: A meta-analysis. *Metabolism.* 2017;68:119-32.

Kawano, M, Shono, N, Yoshimura, T, Yamaguchi, M, Hirano, T, Hisatomi, A. Improved cardio-respiratory fitness correlates with changes in the number and size of small dense LDL: randomized controlled trial with exercise training and dietary instruction. *Intern Med.* 2009;48:25-32.

Kavouras, SA, Panagiotakos, DB, Pitsavos, C, Chrysohoou, C, Anastasiou, CA, Lentzas, Y*, et al*. Physical activity, obesity status, and glycemic control: The ATTICA study. *Med Sci Sports Exerc.* 2007;39:606-11.

Kettunen, JA, Kujala, UM, Kaprio, J, Backmand, H, Peltonen, M, Eriksson, JG*, et al*. All-cause and disease-specific mortality among male, former elite athletes: an average 50-year follow-up. *Br J Sports Med.* 2015;49:893-7.

Ki, M, Pouliou, T, Li, L, Power, C. Physical (in)activity over 20 y in adulthood: Associations with adult lipid levels in the 1958 British birth cohort. *Atherosclerosis.* 2011;219:361-7.

Kilpeläinen, TO, Qi, L, Brage, S, Sharp, SJ, Sonestedt, E, Demerath, E*, et al*. Physical activity attenuates the influence of FTO variants on obesity risk: a meta-analysis of 218,166 adults and 19,268 children. *PLoS Med.* 2011;8:e1001116.

Kim, ES, Im, JA, Kim, KC, Park, JH, Suh, SH, Kang, ES*, et al*. Improved insulin sensitivity and adiponectin level after exercise training in obese Korean youth. *Obesity (Silver Spring).* 2007;15:3023-30.

Kingwell, BA, Sherrard, B, Jennings, GL, Dart, AM. Four weeks of cycle training increases basal production of nitric oxide from the forearm. *Am J Physiol.* 1997;272:H1070-7.

Kistler, KD, Brunt, EM, Clark, JM, Diehl, AM, Sallis, JF, Schwimmer, JB*, et al*. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol.* 2011;106:460-8.

Koch, B, Schaper, C, Ittermann, T, Spielhagen, T, Dorr, M, Volzke, H*, et al*. Reference values for cardiopulmonary exercise testing in healthy volunteers: the SHIP study. *Eur Respir J.* 2009;33:389-97.

Kollias, A, Psilopatis, I, Karagiaouri, E, Glaraki, M, Grammatikos, E, Grammatikos, EE*, et al*. Adiposity, blood pressure, and carotid intima-media thickness in greek adolescents. *Obesity (Silver Spring).* 2013;21:1013-7.

Kostner, GM. Letter: Enzymatic determination of cholesterol in high-density lipoprotein fractions prepared by polyanion precipitation. *Clin Chem.* 1976;22:695.

Kosuge, M, Ebina, T, Ishikawa, T, Hibi, K, Tsukahara, K, Okuda, J*, et al*. Serum amyloid A is a better predictor of clinical outcomes than C-reactive protein in non-ST-segment elevation acute coronary syndromes. *Circ J.* 2007;71:186-90.

Kotronen, A and Yki-Järvinen, H. Fatty liver: a novel component of the metabolic syndrome. *Arterioscler Thromb Vasc Biol.* 2008;28:27-38.

Koulova, A and Frishman, WH. Air pollution exposure as a risk factor for cardiovascular disease morbidity and mortality. *Cardiol Rev.* 2014;22:30-6.

Kozakova, M, Balkau, B, Morizzo, C, Bini, G, Flyvbjerg, A, Palombo, C. Physical activity, adiponectin, and cardiovascular structure and function. *Heart Vessels.* 2013a;28:91-100.

Kozakova, M, Natali, A, Dekker, J, Beck-Nielsen, H, Laakso, M, Nilsson, P*, et al*. Insulin sensitivity and carotid intima-media thickness: relationship between insulin sensitivity and cardiovascular risk study. *Arterioscler Thromb Vasc Biol.* 2013b;33:1409-17.

Kronenberg, F, Pereira, MA, Schmitz, MK, Arnett, DK, Evenson, KR, Crapo, RO*, et al*. Influence of leisure time physical activity and television watching on atherosclerosis risk factors in the NHLBI Family Heart Study. *Atherosclerosis.* 2000;153:433-43.

Kugiyama, K, Ota, Y, Takazoe, K, Moriyama, Y, Kawano, H, Miyao, Y*, et al*. Circulating levels of secretory type II phospholipase A(2) predict coronary events in patients with coronary artery disease. *Circulation.* 1999;100:1280-4.

Kujala, UM, Kaprio, J, Rose, RJ. Physical activity in adolescence and smoking in young adulthood: a prospective twin cohort study. *Addiction.* 2007;102:1151-7.

Kuk, JL, Nichaman, MZ, Church, TS, Blair, SN, Ross, R. Liver fat is not a marker of metabolic risk in lean premenopausal women. *Metabolism.* 2004;53:1066-71.

Kusche-Vihrog, K, Urbanova, K, Blanque, A, Wilhelmi, M, Schillers, H, Kliche, K*, et al*. C-reactive protein makes human endothelium stiff and tight. *Hypertension.* 2011;57:231-7.

Laitinen, TT, Pahkala, K, Magnussen, CG, Oikonen, M, Viikari, JS, Sabin, MA*, et al*. Lifetime measures of ideal cardiovascular health and their association with subclinical atherosclerosis: The Cardiovascular Risk in Young Finns Study. *Int J Cardiol.* 2015;185:186-91.

Lakka, TA, Laukkanen, JA, Rauramaa, R, Salonen, R, Lakka, HM, Kaplan, GA*, et al*. Cardiorespiratory fitness and the progression of carotid atherosclerosis in middle-aged men. *Ann Intern Med.* 2001;134:12-20.

Lamonte, MJ and Ainsworth, BE. Quantifying energy expenditure and physical activity in the context of dose response. *Med Sci Sports Exerc.* 2001;33:S370,8; discussion S419-20.

LaRosa, JC. Prevention and treatment of coronary heart disease: who benefits? *Circulation.* 2001;104:1688-92.

Laukkanen, RM, Oja, P, Ojala, KH, Pasanen, ME, Vuori, IM. Feasibility of a 2-km walking test for fitness assessment in a population study. *Scand J Soc Med.* 1992;20:119-26.

Laursen, AS, Hansen, AL, Wiinberg, N, Brage, S, Sandbaek, A, Lauritzen, T*, et al*. Higher physical activity is associated with lower aortic stiffness but not with central blood pressure: the ADDITION-Pro Study. *Medicine (Baltimore).* 2015;94:e485.

Lavie, CJ, Milani, RV, Ventura, HO. Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. *J Am Coll Cardiol.* 2009;53:1925-32.

Leaf, DA. Fitness: a new look at an old term (measurements of human aerobic performance). *Med Hypotheses.* 1985;18:33-46.

Lee, CD and Blair, SN. Cardiorespiratory fitness and smoking-related and total cancer mortality in men. *Med Sci Sports Exerc.* 2002;34:735-9.

Lehtonen, A and Viikari, J. Serum lipids in soccer and ice-hockey players. *Metabolism.* 1980;29:36-9.

Lehtonen, A and Viikari, J. The effect of vigorous physical activity at work on serum lipids with a special reference to serum high-density lipoprotein cholesterol. *Acta Physiol Scand.* 1978a;104:117-21.

Lehtonen, A and Viikari, J. Serum triglycerides and cholesterol and serum high-density lipoprotein cholesterol in highly physically active men. *Acta Med Scand.* 1978b;204:111-4.

Leon, AS and Connett, J. Physical activity and 10.5 year mortality in the multiple risk factor intervention trial (MRFIT). *Int J Epidemiol.* 1991;20:690-7.

Li, S, Chen, W, Srinivasan, SR, Berenson, GS. Childhood blood pressure as a predictor of arterial stiffness in young adults: the bogalusa heart study. *Hypertension.* 2004;43:541-6.

Li, S, Chen, W, Srinivasan, SR, Bond, MG, Tang, R, Urbina, EM*, et al*. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. *J. Am. Med. Assoc.* 2003;290:2271-6.

Lidegaard, LP, Hansen, AL, Johansen, NB, Witte, DR, Brage, S, Lauritzen, T*, et al*. Physical activity energy expenditure vs cardiorespiratory fitness level in impaired glucose metabolism. *Diabetologia.* 2015;58:2709-17.

Liu, PY, Li, YH, Tsai, WC, Chao, TH, Tsai, LM, Wu, HL*, et al*. Prognostic value and the changes of plasma levels of secretory type II phospholipase A2 in patients with coronary artery disease undergoing percutaneous coronary intervention. *Eur Heart J.* 2003;24:1824-32.

Long, MT, Pedley, A, Massaro, JM, Hoffmann, U, Esliger, DW, Vasan, RS*, et al*. Hepatic steatosis is associated with lower levels of physical activity measured via accelerometry. *Obesity (Silver Spring).* 2015;23:1259-66.

Lorenz, MW, Markus, HS, Bots, ML, Rosvall, M, Sitzer, M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation.* 2007;115:459-67.

Lu, TM, Ding, YA, Charng, MJ, Lin, SJ. Asymmetrical dimethylarginine: a novel risk factor for coronary artery disease. *Clin Cardiol.* 2003;26:458-64.

Luc, G, Bard, JM, Evans, A, Arveiler, D, Ruidavets, JB, Amouyel, P*, et al*. The relationship between apolipoprotein AI-containing lipoprotein fractions and environmental factors: the prospective epidemiological study of myocardial infarction (PRIME study). *Atherosclerosis.* 2000;152:399-405.

Lusis, AJ. Atherosclerosis. *Nature.* 2000;407:233-41.

MacAuley, D, McCrum, EE, Stott, G, Evans, AE, Duly, E, Trinick, T*, et al*. Physical activity, lipids, apolipoproteins, and Lp(a) in the Northern Ireland Health and Activity Survey. *Med Sci Sports Exerc.* 1996;28:720-36.

Maersk, M, Belza, A, Stodkilde-Jorgensen, H, Ringgaard, S, Chabanova, E, Thomsen, H*, et al*. Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study. *Am J Clin Nutr.* 2012;95:283-9.

Mallat, Z, Benessiano, J, Simon, T, Ederhy, S, Sebella-Arguelles, C, Cohen, A*, et al*. Circulating secretory phospholipase A2 activity and risk of incident coronary events in healthy men and women: the EPIC-Norfolk study. *Arterioscler Thromb Vasc Biol.* 2007;27:1177-83.

Mallat, Z, Lambeau, G, Tedgui, A. Lipoprotein-associated and secreted phospholipases A(2) in cardiovascular disease: roles as biological effectors and biomarkers. *Circulation.* 2010;122:2183-200.

Martens-Lobenhoffer, J and Bode-Boger, SM. Chromatographic-mass spectrometric methods for the quantification of L-arginine and its methylated metabolites in biological fluids. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007;851:30-41.

Marti, B, Salonen, JT, Tuomilehto, J, Puska, P. 10-year trends in physical activity in the eastern Finnish adult population: relationship to socioeconomic and lifestyle characteristics. *Acta Med Scand.* 1988;224:195-203.

Marti, B, Tuomilehto, J, Salonen, JT, Puska, P, Nissinen, A. Relationship between leisure-time physical activity and risk factors for coronary heart disease in middle-aged Finnish women. *Acta Med Scand.* 1987;222:223-30.

Martin, SS, Qasim, A, Reilly, MP. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. *J Am Coll Cardiol.* 2008;52:1201-10.

Martinez-Gomez, D, Eisenmann, JC, Gomez-Martinez, S, Veses, A, Romeo, J, Veiga, OL*, et al*. Associations of physical activity and fitness with adipocytokines in adolescents: the AFINOS Study. *Nutr Metab Cardiovasc Dis.* 2012;22:252-9.

Mathew, AR, Hogarth, L, Leventhal, AM, Cook, JW, Hitsman, B. Cigarette smoking and depression comorbidity: systematic review and proposed theoretical model. *Addiction.* 2017;112:401-12.

Matsuda, M, Nosaka, T, Sato, M, Ohshima, N. Effects of physical exercise on the elasticity and elastic components of the rat aorta. *Eur J Appl Physiol Occup Physiol.* 1993;66:122-6.

Matthews, DR, Hosker, JP, Rudenski, AS, Naylor, BA, Treacher, DF, Turner, RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28:412-9.

McGavock, JM, Anderson, TJ, Lewanczuk, RZ. Sedentary lifestyle and antecedents of cardiovascular disease in young adults. *Am J Hypertens.* 2006;19:701-7.

McGill, HC,Jr, McMahan, CA, Herderick, EE, Tracy, RE, Malcom, GT, Zieske, AW*, et al*. Effects of coronary heart disease risk factors on atherosclerosis of selected regions of the aorta and right coronary artery. PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. *Arterioscler Thromb Vasc Biol.* 2000;20:836-45.

McKay, GA and Banister, EW. A comparison of maximum oxygen uptake determination by bicycle ergometry at various pedaling frequencies and by treadmill running at various speeds. *Eur J Appl Physiol Occup Physiol.* 1976;35:191-200.

McMillan, KP, Kuk, JL, Church, TS, Blair, SN, Ross, R. Independent associations between liver fat, visceral adipose tissue, and metabolic risk factors in men. *Appl Physiol Nutr Metab.* 2007;32:265-72.

Melanson, EL, Knoll, JR, Bell, ML, Donahoo, WT, Hill, JO, Nysse, LJ*, et al*. Commercially available pedometers: considerations for accurate step counting. *Prev Med.* 2004;39:361-8.

Metcalf, BS, Jeffery, AN, Hosking, J, Voss, LD, Sattar, N, Wilkin, TJ. Objectively measured physical activity and its association with adiponectin and other novel metabolic markers: a longitudinal study in children (EarlyBird 38). *Diabetes Care.* 2009;32:468-73.

Middlekauff, HR, Park, J, Moheimani, RS. Adverse effects of cigarette and noncigarette smoke exposure on the autonomic nervous system: mechanisms and implications for cardiovascular risk. *J Am Coll Cardiol.* 2014;64:1740-50.

Mikola, H, Pahkala, K, Rönnemaa, T, Viikari, JS, Niinikoski, H, Jokinen, E*, et al*. Distensibility of the aorta and carotid artery and left ventricular mass from childhood to early adulthood. *Hypertension.* 2015;65:146-52.

Minder, CM, Shaya, GE, Michos, ED, Keenan, TE, Blumenthal, RS, Nasir, K*, et al*. Relation between self-reported physical activity level, fitness, and cardiometabolic risk. *Am J Cardiol.* 2014;113:637-43.

Mondal, H and Mishra, SP. Effect of BMI, Body Fat Percentage and Fat Free Mass on Maximal Oxygen Consumption in Healthy Young Adults. *J Clin Diagn Res.* 2017;11:CC17-20.

Moreau, KL, Donato, AJ, Seals, DR, DeSouza, CA, Tanaka, H. Regular exercise, hormone replacement therapy and the age-related decline in carotid arterial compliance in healthy women. *Cardiovasc Res.* 2003;57:861-8.

Morris, EM, Jackman, MR, Johnson, GC, Liu, TW, Lopez, JL, Kearney, ML*, et al*. Intrinsic aerobic capacity impacts susceptibility to acute high-fat diet-induced hepatic steatosis. *Am J Physiol Endocrinol Metab.* 2014;307:E355-64.

Morris, EM, Meers, GM, Koch, LG, Britton, SL, Fletcher, JA, Fu, X*, et al*. Aerobic capacity and hepatic mitochondrial lipid oxidation alters susceptibility for chronic high-fat diet-induced hepatic steatosis. *Am J Physiol Endocrinol Metab.* 2016;311:E749-60.

Mouzaki, M, Comelli, EM, Arendt, BM, Bonengel, J, Fung, SK, Fischer, SE*, et al*. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology.* 2013;58:120-7.

Musso, G, Gambino, R, De Michieli, F, Cassader, M, Rizzetto, M, Durazzo, M*, et al*. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology.* 2003;37:909-16.

Nakamura, K, Fuster, JJ, Walsh, K. Adipokines: a link between obesity and cardiovascular disease. *J Cardiol.* 2014;63:250-9.

Nayak, RK, Zdravkovic, S, Janzon, E. Incidence of myocardial infarction among Swedish and immigrant smoking women: can physical activity modify the risk? An epidemiological study on the Malmo Diet and Cancer Study. *Scand J Public Health.* 2013;41:672-9.

Nettlefold, L, McKay, HA, Naylor, PJ, Bredin, SS, Warburton, DE. The relationship between objectively measured physical activity, sedentary time, and vascular health in children. *Am J Hypertens.* 2012;25:914-9.

Nguyen-Duy, TB, Nichaman, MZ, Church, TS, Blair, SN, Ross, R. Visceral fat and liver fat are independent predictors of metabolic risk factors in men. *Am J Physiol Endocrinol Metab.* 2003;284:E1065-71.

Niebauer, J, Clark, AL, Webb-Peploe, KM, Boger, R, Coats, AJ. Home-based exercise training modulates pro-oxidant substrates in patients with chronic heart failure. *Eur J Heart Fail.* 2005;7:183-8.

Nordestgaard, BG and Varbo, A. Triglycerides and cardiovascular disease. *Lancet.* 2014;384:626-35.

Nordström, CK, Dwyer, KM, Merz, CNB, Shircore, A, Dwyer, JH. Leisure time physical activity and early atherosclerosis: the Los Angeles Atherosclerosis Study. *Am J Med.* 2003;115:19-25.

Nualnim, N, Barnes, JN, Tarumi, T, Renzi, CP, Tanaka, H. Comparison of central artery elasticity in swimmers, runners, and the sedentary. *Am J Cardiol.* 2011;107:783-7.

O'Donovan, G, Thomas, EL, McCarthy, JP, Fitzpatrick, J, Durighel, G, Mehta, S*, et al*. Fat distribution in men of different waist girth, fitness level and exercise habit. *Int J Obes (Lond).* 2009;33:1356-62.

Olchawa, B, Kingwell, BA, Hoang, A, Schneider, L, Miyazaki, O, Nestel, P*, et al*. Physical fitness and reverse cholesterol transport. *Arterioscler Thromb Vasc Biol.* 2004;24:1087-91.

Oliver, JJ and Webb, DJ. Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arterioscler Thromb Vasc Biol.* 2003;23:554-66.

Olsen, GN, Bolton, JW, Weiman, DS, Hornung, CA. Stair climbing as an exercise test to predict the postoperative complications of lung resection. Two years' experience. *Chest.* 1991;99:587-90.

Oni, ET, Agatston, AS, Blaha, MJ, Fialkow, J, Cury, R, Sposito, A*, et al*. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver; should we care? *Atherosclerosis.* 2013;230:258-67.

Oni, ET, Kalathiya, R, Aneni, EC, Martin, SS, Blaha, MJ, Feldman, T*, et al*. Relation of physical activity to prevalence of nonalcoholic Fatty liver disease independent of cardiometabolic risk. *Am J Cardiol.* 2015;115:34-9.

O'Rourke, MF and Hashimoto, J. Mechanical factors in arterial aging: a clinical perspective. *J Am Coll Cardiol.* 2007;50:1-13.

Pahkala, K, Heinonen, OJ, Simell, O, Viikari, JS, Rönnemaa, T, Niinikoski, H*, et al*. Association of physical activity with vascular endothelial function and intima-media thickness. *Circulation.* 2011;124:1956-63.

Pahkala, K, Laitinen, TT, Heinonen, OJ, Viikari, JS, Rönnemaa, T, Niinikoski, H*, et al*. Association of fitness with vascular intima-media thickness and elasticity in adolescence. *Pediatrics.* 2013;132:e77-84.

Palmefors, H, DuttaRoy, S, Rundqvist, B, Börjesson, M. The effect of physical activity or exercise on key biomarkers in atherosclerosis – A systematic review. *Atherosclerosis.* 2014;235:150-61.

Panagiotakos, DB, Pitsavos, C, Chrysohoou, C, Kavouras, S, Stefanadis, C, ATTICA Study. The associations between leisure-time physical activity and inflammatory and coagulation markers related to cardiovascular disease: the ATTICA Study. *Prev Med.* 2005;40:432-7.

Pandey, A, Patel, M, Gao, A, Willis, BL, Das, SR, Leonard, D*, et al*. Changes in mid-life fitness predicts heart failure risk at a later age independent of interval development of cardiac and noncardiac risk factors: the Cooper Center Longitudinal Study. *Am Heart J.* 2015;169:290,297.e1.

Park, E, Meininger, JC, Kang, DH, Gabriel, KP, Padhye, NS. Association of cardiorespiratory fitness and adiposity with inflammatory biomarkers in young adults. *Am J Hum Biol.* 2017;29:3.

Park, JH, Miyashita, M, Takahashi, M, Harada, K, Takaizumi, K, Kim, HS*, et al*. Oxidised low-density lipoprotein concentrations and physical activity status in older adults: the WASEDA active life study. *J Atheroscler Thromb.* 2011;18:568-73.

Patja, K, Jousilahti, P, Hu, G, Valle, T, Qiao, Q, Tuomilehto, J. Effects of smoking, obesity and physical activity on the risk of type 2 diabetes in middle-aged Finnish men and women. *J Intern Med.* 2005;258:356-62.

Pepys, MB and Hirschfield, GM. C-reactive protein: a critical update. *J Clin Invest.* 2003;111:1805-12.

Pesonen, E, Kaprio, J, Martimo, P, Ruismäki, A. Topography of intimal thickening in the left coronary artery of children compared with the topography of atherosclerosis. *Coron Artery Dis.* 1996;7:459-65.

Pichurko, BM. Exercising your patient: which test(s) and when? *Respir Care.* 2012;57:100-10.

Pignoli, P, Tremoli, E, Poli, A, Oreste, P, Paoletti, R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation.* 1986;74:1399-406.

Pitsavos, C, Panagiotakos, DB, Chrysohoou, C, Kavouras, S, Stefanadis, C. The associations between physical activity, inflammation, and coagulation markers, in people with metabolic syndrome: the ATTICA study. *Eur J Cardiovasc Prev Rehabil.* 2005;12:151-8.

Plaisance, EP and Grandjean, PW. Physical activity and high-sensitivity C-reactive protein. *Sports Med.* 2006;36:443-58.

Polak, JF, Person, SD, Wei, GS, Godreau, A, Jacobs, DR,Jr, Harrington, A*, et al*. Segment-specific associations of carotid intima-media thickness with cardiovascular risk factors: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Stroke.* 2010;41:9-15.

Porkka, KV, Raitakari, OT, Leino, A, Laitinen, S, Räsänen, L, Rönnemaa, T*, et al*. Trends in serum lipid levels during 1980-1992 in children and young adults. The Cardiovascular Risk in Young Finns Study. *Am J Epidemiol.* 1997;146:64-77.

Pronk, NP. Short term effects of exercise on plasma lipids and lipoproteins in humans. *Sports Med.* 1993;16:431-48.

Prospective Studies Collaboration, Lewington, S, Whitlock, G, Clarke, R, Sherliker, P, Emberson, J*, et al*. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet.* 2007;370:1829-39.

Quan, HL, Blizzard, CL, Sharman, JE, Magnussen, CG, Dwyer, T, Raitakari, O*, et al*. Resting heart rate and the association of physical fitness with carotid artery stiffness. *Am J Hypertens.* 2014;27:65-71.

Rader, DJ, Alexander, ET, Weibel, GL, Billheimer, J, Rothblat, GH. The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. *J Lipid Res.* 2009;50 Suppl:S189-94.

Raitakari, M, Mansikkaniemi, K, Marniemi, J, Viikari, JS, Raitakari, OT. Distribution and determinants of serum high-sensitive C-reactive protein in a population of young adults: The Cardiovascular Risk in Young Finns Study. *J Intern Med.* 2005;258:428-34.

Raitakari, O, Porkka, K, Taimela, S, Telama, R, Räsänen, L, Viikari, J. Effects of persistent physical activity and in activity on coronary risk factors in children and young adults. *Am J Epidemiol.* 1994;140:195-205.

Raitakari, O, Taimela, S, Porkka, K, Telama, R, Välimäki, I, Åkerblom, H*, et al*. Associations between physical activity and risk factors for coronary heart disease: The Cardiovascular Risk in Young Finns Study. *Med Sci Sports Exerc.* 1997;29:1055-61.

Raitakari, OT, Juonala, M, Kähönen, M, Taittonen, L, Laitinen, T, Mäki-Torkko, N*, et al*. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *J. Am. Med. Assoc.* 2003;290:2277-83.

Raitakari, OT, Juonala, M, Rönnemaa, T, Keltikangas-Järvinen, L, Räsänen, L, Pietikäinen, M*, et al*. Cohort profile: the cardiovascular risk in Young Finns Study. *Int J Epidemiol.* 2008;37:1220-6.

Raitakari, OT, Juonala, M, Viikari, JS. Obesity in childhood and vascular changes in adulthood: insights into the Cardiovascular Risk in Young Finns Study. *Int J Obes (Lond).* 2005;29 Suppl 2:S101-4.

Raitakari, OT, Porkka, KV, Räsänen, L, Viikari, JS. Relations of life-style with lipids, blood pressure and insulin in adolescents and young adults. The Cardiovascular Risk in Young Finns Study. *Atherosclerosis.* 1994;111:237-46.

Raitakari, OT, Taimela, S, Porkka, KV, Viikari, JS. Effect of leisure-time physical activity change on high-density lipoprotein cholesterol in adolescents and young adults. *Ann Med.* 1996;28:259-63.

Raman, M, Ahmed, I, Gillevet, PM, Probert, CS, Ratcliffe, NM, Smith, S*, et al*. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* 2013;11:868,75.e1-3.

Rana, JS, Arsenault, BJ, Despres, JP, Cote, M, Talmud, PJ, Ninio, E*, et al*. Inflammatory biomarkers, physical activity, waist circumference, and risk of future coronary heart disease in healthy men and women. *Eur Heart J.* 2011;32:336-44.

Rankinen, T, Church, TS, Rice, T, Bouchard, C, Blair, SN. Cardiorespiratory fitness, BMI, and risk of hypertension: the HYPGENE study. *Med Sci Sports Exerc.* 2007;39:1687-92.

Rankinen, T, Roth, SM, Bray, MS, Loos, R, Perusse, L, Wolfarth, B*, et al*. Advances in exercise, fitness, and performance genomics. *Med Sci Sports Exerc.* 2010;42:835-46.

Reed, J and Ones, DS. The effect of acute aerobic exercise on positive activated affect: A meta-analysis. *Psychol Sport Exerc.* 2006;7:477-514.

Rennie, KL, Hemingway, H, Kumari, M, Brunner, E, Malik, M, Marmot, M. Effects of moderate and vigorous physical activity on heart rate variability in a British study of civil servants. *Am J Epidemiol.* 2003;158:135-43.

Ridker, PM. High-sensitivity C-reactive protein, inflammation, and cardiovascular risk: from concept to clinical practice to clinical benefit. *Am Heart J.* 2004;148:S19-26.

Ridker, PM. LDL cholesterol: controversies and future therapeutic directions. *Lancet.* 2014;384:607-17.

Ridker, PM, Hennekens, CH, Buring, JE, Rifai, N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* 2000;342:836-43.

Ridker, PM, Rifai, N, Rose, L, Buring, JE, Cook, NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med.* 2002;347:1557-65.

Ried-Larsen, M, Grøntved, A, Østergaard, L, Cooper, AR, Froberg, K, Andersen, LB*, et al*. Associations between bicycling and carotid arterial stiffness in adolescents: The European Youth Hearts Study. *Scand J Med Sci Sports.* 2015;25:661-9.

Ring-Dimitriou, S, Paulweber, B, von Duvillard, SP, Stadlmann, M, LeMura, LM, Lang, J*, et al*. The effect of physical activity and physical fitness on plasma adiponectin in adults with predisposition to metabolic syndrome. *Eur J Appl Physiol.* 2006;98:472-81.

Rizzo, NS, Ruiz, JR, Oja, L, Veidebaum, T, Sjostrom, M. Associations between physical activity, body fat, and insulin resistance (homeostasis model assessment) in adolescents: the European Youth Heart Study. *Am J Clin Nutr.* 2008;87:586-92.

Rohatgi, A, Khera, A, Berry, JD, Givens, EG, Ayers, CR, Wedin, KE*, et al*. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med.* 2014;371:2383-93.

Rosa-Caldwell, ME, Lee, DE, Brown, JL, Brown, LA, Perry, RA,Jr, Greene, ES*, et al*. Moderate physical activity promotes basal hepatic autophagy in diet-induced obese mice. *Appl Physiol Nutr Metab.* 2017;42:148-56.

Ross, R, Blair, SN, Arena, R, Church, TS, Despres, JP, Franklin, BA*, et al*. Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. *Circulation.* 2016;134:e653-99.

Ross, R and Janssen, I. Physical activity, total and regional obesity: dose-response considerations. *Med Sci Sports Exerc.* 2001;33:521-7.

Rossi, A, Dikareva, A, Bacon, SL, Daskalopoulou, SS. The impact of physical activity on mortality in patients with high blood pressure: a systematic review. *J Hypertens.* 2012;30:1277-88.

Rönnemaa, T, Lehtonen, A, Järveläinen, H, Viikari, J. Plasma lipids and lipoproteins of young male athletes and the effect of their serum on cultured human aortic smooth muscle cells. *Scand J Sports Sci.* 1980;2:33-8.

Saadeh, S, Younossi, ZM, Remer, EM, Gramlich, T, Ong, JP, Hurley, M*, et al*. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology.* 2002;123:745-50.

Saarelainen, H, Valtonen, P, Punnonen, K, Laitinen, T, Raitakari, OT, Juonala, M*, et al*. Subtle changes in ADMA and l-arginine concentrations in normal pregnancies are unlikely to account for pregnancy-related increased flow-mediated dilatation. *Clin Physiol Funct Imaging.* 2008;28:120-4.

Saarikoski, LA, Juonala, M, Huupponen, R, Viikari, JS, Lehtimäki, T, Jokinen, E*, et al*. Low serum adiponectin levels in childhood and adolescence predict increased intima-media thickness in adulthood. The Cardiovascular Risk in Young Finns Study. *Ann Med.* 2017;49:42-50.

Saladini, F, Benetti, E, Mos, L, Mazzer, A, Casiglia, E, Palatini, P. Regular physical activity is associated with improved small artery distensibility in young to middle-age stage 1 hypertensives. *Vascular Medicine.* 2014;19:458-64.

Saleheen, D, Haycock, PC, Zhao, W, Rasheed, A, Taleb, A, Imran, A*, et al*. Apolipoprotein(a) isoform size, lipoprotein(a) concentration, and coronary artery disease: a mendelian randomisation analysis. *Lancet Diabetes & Endocrinology.* 2017;5:524-33.

Salomaa, V, Riley, W, Kark, JD, Nardo, C, Folsom, AR. Non-insulin-dependent diabetes mellitus and fasting glucose and insulin concentrations are associated with arterial stiffness indexes. The ARIC Study. Atherosclerosis Risk in Communities Study. *Circulation.* 1995;91:1432-43.

Sarnak, MJ, Levey, AS, Schoolwerth, AC, Coresh, J, Culleton, B, Hamm, LL*, et al*. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation.* 2003;108:2154-69.

Saverymuttu, SH, Joseph, AE, Maxwell, JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *BMJ (Clin Res Ed).* 1986;292:13-5.

Schepers, E, Speer, T, Bode-Boger, SM, Fliser, D, Kielstein, JT. Dimethylarginines ADMA and SDMA: the real water-soluble small toxins? *Semin Nephrol.* 2014;34:97-105.

Schlatmann, TJ and Becker, AE. Histologic changes in the normal aging aorta: implications for dissecting aortic aneurysm. *Am J Cardiol.* 1977;39:13-20.

Schmidt, MD, Cleland, VJ, Thomson, RJ, Dwyer, T, Venn, AJ. A comparison of subjective and objective measures of physical activity and fitness in identifying associations with cardiometabolic risk factors. *Ann Epidemiol.* 2008;18:378-86.

Schwenzer, NF, Springer, F, Schraml, C, Stefan, N, Machann, J, Schick, F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. *J Hepatol.* 2009;51:433-45.

Scriba, D, Aprath-Husmann, I, Blum, WF, Hauner, H. Catecholamines suppress leptin release from in vitro differentiated subcutaneous human adipocytes in primary culture via beta1- and beta2-adrenergic receptors. *Eur J Endocrinol.* 2000;143:439-45.

Senti, M, Elosua, R, Tomas, M, Sala, J, Masia, R, Ordovas, JM*, et al*. Physical activity modulates the combined effect of a common variant of the lipoprotein lipase gene and smoking on serum triglyceride levels and high-density lipoprotein cholesterol in men. *Hum Genet.* 2001;109:385-92.

Serlachius, A, Elovainio, M, Juonala, M, Shea, S, Sabin, M, Lehtimäki, T*, et al*. High perceived social support protects against the intergenerational transmission of obesity: The Cardiovascular Risk in Young Finns Study. *Prev Med.* 2016;90:79-85.

Shephard, RJ, Allen, C, Benade, AJ, Davies, CT, Di Prampero, PE, Hedman, R*, et al*. The maximum oxygen intake. An international reference standard of cardiorespiratory fitness. *Bull World Health Organ.* 1968;38:757-64.

Short, KR, Vittone, JL, Bigelow, ML, Proctor, DN, Rizza, RA, Coenen-Schimke, JM*, et al*. Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. *Diabetes.* 2003;52:1888-96.

Siddiqi, HK, Kiss, D, Rader, D. HDL-cholesterol and cardiovascular disease: rethinking our approach. *Curr Opin Cardiol.* 2015;30:536-42.

Singh, SJ, Morgan, MD, Scott, S, Walters, D, Hardman, AE. Development of a shuttle walking test of disability in patients with chronic airways obstruction. *Thorax.* 1992;47:1019-24.

Skilton, MR, Siitonen, N, Wurtz, P, Viikari, JS, Juonala, M, Seppälä, I*, et al*. High birth weight is associated with obesity and increased carotid wall thickness in young adults: the cardiovascular risk in young Finns study. *Arterioscler Thromb Vasc Biol.* 2014;34:1064-8.

Smart, NA, King, N, McFarlane, JR, Graham, PL, Dieberg, G. Effect of exercise training on liver function in adults who are overweight or exhibit fatty liver disease: a systematic review and meta-analysis. *Br J Sports Med.* 2016;

Solberg, LA and Strong, JP. Risk factors and atherosclerotic lesions. A review of autopsy studies. *Arteriosclerosis.* 1983;3:187-98.

Solomon, TP, Malin, SK, Karstoft, K, Knudsen, SH, Haus, JM, Laye, MJ*, et al*. Association between cardiorespiratory fitness and the determinants of glycemic control across the entire glucose tolerance continuum. *Diabetes Care.* 2015;38:921-9.

Steptoe, A and Ussher, M. Smoking, cortisol and nicotine. *Int J Psychophysiol.* 2006;59:228-35.

Strath, SJ, Bassett, DR,Jr, Swartz, AM. Comparison of the college alumnus questionnaire physical activity index with objective monitoring. *Ann Epidemiol.* 2004;14:409-15.

Stubbe, JH, Boomsma, DI, Vink, JM, Cornes, BK, Martin, NG, Skytthe, A*, et al*. Genetic influences on exercise participation in 37,051 twin pairs from seven countries. *PLoS One.* 2006;1:e22.

Sugawara, J, Komine, H, Hayashi, K, Yoshizawa, M, Otsuki, T, Shimojo, N*, et al*. Reduction in alpha-adrenergic receptor-mediated vascular tone contributes to improved arterial compliance with endurance training. *Int J Cardiol.* 2009;135:346.

Sung, KC, Ryu, S, Lee, JY, Kim, JY, Wild, SH, Byrne, CD. Effect of exercise on the development of new fatty liver and the resolution of existing fatty liver. *J Hepatol.* 2016;65:791-7.

Suomela, E, Oikonen, M, Virtanen, J, Parkkola, R, Jokinen, E, Laitinen, T*, et al*. Prevalence and determinants of fatty liver in normal-weight and overweight young adults. The Cardiovascular Risk in Young Finns Study. *Ann Med.* 2014;1-7.

Taimela, S, Lehtimäki, T, Porkka, KV, Räsänen, L, Viikari, JS. The effect of physical activity on serum total and low-density lipoprotein cholesterol concentrations varies with apolipoprotein E phenotype in male children and young adults: The Cardiovascular Risk in Young Finns Study. *Metabolism.* 1996;45:797-803.

Tammelin, T, Ekelund, U, Remes, J, Näyhä, S. Physical activity and sedentary behaviors among Finnish youth. *Med Sci Sports Exerc.* 2007;39:1067-74.

Tanaka, H, DeSouza, CA, Seals, DR. Absence of age-related increase in central arterial stiffness in physically active women. *Arterioscler Thromb Vasc Biol.* 1998;18:127-32.

Tanaka, H, Seals, DR, Monahan, KD, Clevenger, CM, DeSouza, CA, Dinenno, FA. Regular aerobic exercise and the age-related increase in carotid artery intima-media thickness in healthy men. *J Appl Physiol (1985).* 2002;92:1458-64.

Tanisawa, K, Ito, T, Sun, X, Cao, ZB, Sakamoto, S, Tanaka, M*, et al*. Polygenic risk for hypertriglyceridemia is attenuated in Japanese men with high fitness levels. *Physiol Genomics.* 2014;46:207-15.

Targher, G and Arcaro, G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. *Atherosclerosis.* 2007;191:235-40.

Taylor, KS, Heneghan, CJ, Farmer, AJ, Fuller, AM, Adler, AI, Aronson, JK*, et al*. All-cause and cardiovascular mortality in middle-aged people with type 2 diabetes compared with people without diabetes in a large U.K. primary care database. *Diabetes Care.* 2013;36:2366-71.

Telama, R and Yang, X. Decline of physical activity from youth to young adulthood in Finland. *Med Sci Sports Exerc.* 2000;32:1617-22.

Telama, R, Yang, X, Hirvensalo, M, Raitakari, O. Participation in Organized Youth Sport as a Predictor of Adult Physical Activity: A 21-Year Longitudinal Study. *Pediatric Exercise Science.* 2006;18:76-88.

Telama, R, Laakso, L, Yang, X. Physical activity and participation in sports of young people in Finland. *Scand J Med Sci Sports.* 1994;4:65-74.

Telama, R, Leskinen, E, Yang, X. Stability of habitual physical activity and sport participation: a longitudinal tracking study. *Scand J Med Sci Sports.* 1996;6:371-8.

Telama, R, Yang, X, Laakso, L, Viikari, J. Physical activity in childhood and adolescence as predictor of physical activity in young adulthood. *Am J Prev Med.* 1997;13:317-23.

Telama, R, Yang, X, Viikari, J, Välimäki, I, Wanne, O, Raitakari, O. Physical activity from childhood to adulthood: a 21-year tracking study. *Am J Prev Med.* 2005;28:267-73.

Thompson, PD, Buchner, D, Piña, IL, Balady, GJ, Williams, MA, Marcus, BH*, et al*. Exercise and Physical Activity in the Prevention and Treatment of Atherosclerotic Cardiovascular Disease: A Statement From the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation.* 2003;107:3109-16.

Timmons, JA, Knudsen, S, Rankinen, T, Koch, LG, Sarzynski, M, Jensen, T*, et al*. Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. *J Appl Physiol.* 2010;108:1487-96.

Tipton, CM. The history of "Exercise Is Medicine" in ancient civilizations. *Adv Physiol Educ.* 2014;38:109-17.

Toprak, A, Kandavar, R, Toprak, D, Chen, W, Srinivasan, S, Xu, JH*, et al*. C-reactive protein is an independent predictor for carotid artery intima-media thickness progression in asymptomatic younger adults (from the Bogalusa Heart Study). *BMC Cardiovasc Disord.* 2011;11:78,2261-11-78.

Toshimitsu, K, Matsuura, B, Ohkubo, I, Niiya, T, Furukawa, S, Hiasa, Y*, et al*. Dietary habits and nutrient intake in non-alcoholic steatohepatitis. *Nutrition.* 2007;23:46-52.

Trigona, B, Aggoun, Y, Maggio, A, Martin, XE, Marchand, LM, Beghetti, M*, et al*. Preclinical noninvasive markers of atherosclerosis in children and adolescents with type 1 diabetes are influenced by physical activity. *J Pediatr.* 2010;157:533-9.

Tsarouhas, K, Karatzaferi, C, Tsitsimpikou, C, Haliassos, A, Kouretas, D, Pavlidis, P*, et al*. Effects of walking on heart rate recovery, endothelium modulators and quality of life in patients with heart failure. *Eur J Cardiovasc Prev Rehabil.* 2011;18:594-600.

Tudor-Locke, C and Bassett, DR,Jr. How many steps/day are enough? Preliminary pedometer indices for public health. *Sports Med.* 2004;34:1-8.

Tudor-Locke, C, Williams, JE, Reis, JP, Pluto, D. Utility of pedometers for assessing physical activity: construct validity. *Sports Med.* 2004;34:281-91.

Tuomilehto, J, Marti, B, Salonen, JT, Virtala, E, Lahti, T, Puska, P. Leisure-time physical activity is inversely related to risk factors for coronary heart disease in middle-aged Finnish men. *Eur Heart J.* 1987;8:1047-55.

Uhlar, CM and Whitehead, AS. Serum amyloid A, the major vertebrate acute-phase reactant. *European Journal of Biochemistry.* 1999;265:501-23.

Vaitkevicius, PV, Fleg, JL, Engel, JH, O'Connor, FC, Wright, JG, Lakatta, LE*, et al*. Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation.* 1993;88:1456-62.

van de Laar, RJ, Ferreira, I, van Mechelen, W, Prins, MH, Twisk, JW, Stehouwer, CD. Habitual physical activity and peripheral arterial compliance in young adults: the Amsterdam growth and health longitudinal study. *Am J Hypertens.* 2011;24:200-8.

van de Laar, RJ, Ferreira, I, van Mechelen, W, Prins, MH, Twisk, JW, Stehouwer, CD. Lifetime vigorous but not light-to-moderate habitual physical activity impacts favorably on carotid stiffness in young adults: the Amsterdam growth and health longitudinal study. *Hypertension.* 2010;55:33-9.

Van Nostrand, D, Kjelsberg, MO, Humphrey, EW. Preresectional evaluation of risk from pneumonectomy. *Surg Gynecol Obstet.* 1968;127:306-12.

Vanhees, L, Lefevre, J, Philippaerts, R, Martens, M, Huygens, W, Troosters, T*, et al*. How to assess physical activity? How to assess physical fitness? *Eur J Cardiovasc Prev Rehabil.* 2005;12:102-14.

Vasankari, T, Lehtonen-Veromaa, M, Möttönen, T, Ahotupa, M, Irjala, K, Heinonen, O*, et al*. Reduced mildly oxidized LDL in young female athletes. *Atherosclerosis.* 2000;151:399-405.

Vasankari, TJ, Kujala, UM, Vasankari, TM, Ahotupa, M. Reduced oxidized LDL levels after a 10-month exercise program. *Med Sci Sports Exerc.* 1998;30:1496-501.

Venugopal, SK, Devaraj, S, Yuhanna, I, Shaul, P, Jialal, I. Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. *Circulation.* 2002;106:1439-41.

Verdaet, D, Dendale, P, De Bacquer, D, Delanghe, J, Block, P, De Backer, G. Association between leisure time physical activity and markers of chronic inflammation related to coronary heart disease. *Atherosclerosis.* 2004;176:303-10.

Verhoye, E, Langlois, MR, Asklepios Investigators. Circulating oxidized low-density lipoprotein: a biomarker of atherosclerosis and cardiovascular risk? *Clin Chem Lab Med.* 2009;47:128-37.

Viikari, J, Rönnemaa, T, Seppänen, A, Marniemi, J, Porkka, K, Räsänen, L*, et al*. Serum lipids and lipoproteins in children, adolescents and young adults in 1980-1986. *Ann Med.* 1991;23:53-9.

Viinikka, L, Vuori, J, Ylikorkala, O. Lipid peroxides, prostacyclin, and thromboxane A2 in runners during acute exercise. *Med Sci Sports Exerc.* 1984;16:275-7.

Vogt, P. 1993, Dictionary of statistics and methodology. A nontechnical guide for the social sciences. Newbury Park, Sage Publications.

Vos, LE, Oren, A, Uiterwaal, C, Gorissen, WH, Grobbee, DE, Bots, ML. Adolescent blood pressure and blood pressure tracking into young adulthood are related to subclinical atherosclerosis: the Atherosclerosis Risk in Young Adults (ARYA) study. *Am J Hypertens.* 2003;16:549-55.

Vos, MB and Lavine, JE. Dietary fructose in nonalcoholic fatty liver disease. *Hepatology.* 2013;57:2525-31.

Vuorimaa, T, Ahotupa, M, Irjala, K, Vasankari, T. Acute prolonged exercise reduces moderately oxidized LDL in healthy men. *Int J Sports Med.* 2005;26:420-5.

Välimäki, I, Hursti, M-, Pihlakoski, L, Viikari, J. Exercise performance and serum lipids in relation to physical activity in schoolchildren. *Int J Sports Med.* 1980;1:132-6.

Wanne, O, Viikari, J, Telama, R, Åkerblom, HK, Pesonen, E, Uhari, M*, et al*. Physical activity and serum lipids in 8-year-old Finnish school boys. *Scand J Sports Sci.* 1983;5:10-4.

Wanne, O, Viikari, J, Välimäki, I. 1984, "Physical performance and serum lipids in 14 to 16-year-old trained, normally active and physically inactive children." in *Children and Sport. Pediatric Work Physiology X.* Springer Verlag, Berlin Heidelberg, pp 241-6.

Ward, AM, Takahashi, O, Stevens, R, Heneghan, C. Home measurement of blood pressure and cardiovascular disease: systematic review and meta-analysis of prospective studies. *J Hypertens.* 2012;30:449-56.

Weninger, WJ, Muller, GB, Reiter, C, Meng, S, Rabl, SU. Intimal hyperplasia of the infant parasellar carotid artery: a potential developmental factor in atherosclerosis and SIDS. *Circ Res.* 1999;85:970-5.

Wilkinson, IB and McEniery, CM. Arterial stiffness, endothelial function and novel pharmacological approaches. *Clin Exp Pharmacol Physiol.* 2004;31:795-9.

Willeit, P, Freitag, DF, Laukkanen, JA, Chowdhury, S, Gobin, R, Mayr, M*, et al*. Asymmetric dimethylarginine and cardiovascular risk: systematic review and meta-analysis of 22 prospective studies. *J Am Heart Assoc.* 2015;4:e001833.

Wisloff, U, Najjar, SM, Ellingsen, O, Haram, PM, Swoap, S, Al-Share, Q*, et al*. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science.* 2005;307:418-20.

World Health Organization. Cardiovascular diseases (CVDs). 2016.

World Health Organization. Global Recommendations on Physical Activity for Health. 2010.

Yanagisawa, M, Kurihara, H, Kimura, S, Tomobe, Y, Kobayashi, M, Mitsui, Y*, et al*. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature.* 1988;332:411-5.

Yancey, AK, Wold, CM, McCarthy, WJ, Weber, MD, Lee, B, Simon, PA*, et al*. Physical inactivity and overweight among Los Angeles County adults. *Am J Prev Med.* 2004;27:146-52.

Yang, X, Telama, R, Hirvensalo, M, Mattsson, N, Viikari, JS, Raitakari, OT. The longitudinal effects of physical activity history on metabolic syndrome. *Med Sci Sports Exerc.* 2008;40:1424-31.

Yang, X, Telama, R, Leskinen, E, Mansikkaniemi, K, Viikari, J, Raitakari, OT. Testing a model of physical activity and obesity tracking from youth to adulthood: the cardiovascular risk in young Finns study. *Int J Obes (Lond).* 2007;31:521-7.

Yang, Y, Shi, Y, Wiklund, P, Tan, X, Wu, N, Zhang, X*, et al*. The Association between Cardiorespiratory Fitness and Gut Microbiota Composition in Premenopausal Women. *Nutrients.* 2017;9:10.3390/nu9080792.

Yataco, AR, Fleisher, LA, Katzel, LI. Heart rate variability and cardiovascular fitness in senior athletes. *Am J Cardiol.* 1997;80:1389-91.

Yki-Järvinen, H. Diagnosis of non-alcoholic fatty liver disease (NAFLD). *Diabetologia.* 2016;59:1104-11.

Zieske, AW, Malcom, GT, Strong, JP. Natural history and risk factors of atherosclerosis in children and youth: the PDAY study. *Pediatr Pathol Mol Med.* 2002;21:213-37.

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