Turun yliopisto
University of Turku

ADIPONECTIN AND LEPTIN AND THEIR ASSOCIATIONS WITH CARDIOVASCULAR RISK FACTORS AND MARKERS OF SUBCLINICAL ATHEROSCLEROSIS IN YOUNG ADULTS

The Cardiovascular Risk in Young Finns Study

Liisa Saarikoski
(née Viikari)

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1271 | Medica - Odontologica | Turku 2017

University of Turku

Faculty of Medicine
Department of Internal Medicine
University of Turku Doctoral Programme of Clinical Investigation
Research Centre of Applied and Preventive Cardiovascular Medicine
Department of Pharmacology, Drug Development and Therapeutics
Turku University Hospital

Supervised by

Professor Olli Raitakari, MD, PhD
Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku
Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital
Turku, Finland

Professor Risto Huupponen, MD, PhD
Department of Pharmacology, Drug Development and Therapeutics
University of Turku
Unit of Clinical Pharmacology, Turku University Hospital, Turku, Finland

Professor Emeritus Jorma Viikari, MD, PhD
Department of Medicine,
University of Turku, Turku, Finland

Reviewed by

Adjunct professor Päivi Keskinen, MD, PhD
Tampere Centre for Child Health Research, University of Tampere
Department of Pediatrics, Tampere University Hospital, Tampere, Finland

Adjunct professor Hannu Vanhanen, MD, PhD
Department of Medicine
University of Helsinki, Helsinki Finland

Opponent

Adjunct professor, Chief physician Leo Niskanen, MD, PhD
Endocrinology
Helsinki University Hospital and University of Helsinki, Helsinki, Finland
University of Eastern Finland, Kuopio, Finland

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-6734-6 (PRINT)
ISBN 978-951-29-6735-3 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama Oy - Turku, Finland 2017

To my father

ABSTRACT

Liisa Saarikoski (née Viikari). Adiponectin and leptin and their associations with cardiovascular risk factors and markers of subclinical atherosclerosis in young adults. The Cardiovascular Risk in Young Finns Study. From the Faculty of Medicine, Department of Internal Medicine, University of Turku Doctoral Programme of Clinical Investigation, Research Centre of Applied and Preventive Cardiovascular Medicine and the Department of Pharmacology, Drug Development and Therapeutics, University of Turku, Turku, Finland. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Finland, 2017.

Background: Effective prevention of atherosclerosis requires identification of individuals at high risk already in childhood. Adipose tissue produces various proteins that participate in the regulation of metabolism and have been suggested to influence the cardiovascular system directly and via inflammation.

Aims: The aims of this study were to examine serum levels of adipose tissue-derived proteins adiponectin and leptin in Finnish children, adolescents and adults and to study their associations with metabolic syndrome, carotid intima-media thickness and flow-mediated dilatation of the brachial artery. Moreover, the aim was to study if adiponectin levels in childhood and adolescence predict carotid atherosclerosis in adulthood.

Participants and methods: The present 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. Serum adiponectin concentrations were measured from samples taken in 1980, 2001 and 2007 and leptin concentrations from samples taken in 1980 and 2001. Ultrasonic evaluation of carotid artery intima-media thickness and brachial artery flow-mediated dilatation were performed in 2001 and 2007.

Results: Serum adiponectin levels decreased from childhood to adulthood in both males and females whereas serum leptin levels increased in females and decreased slightly in males. In adulthood, decreased serum adiponectin levels were associated with incident metabolic syndrome, increased carotid artery intima-media thickness and attenuated flow-mediated dilatation of the brachial artery. Serum adiponectin levels in childhood were inversely associated with carotid atherosclerosis in adulthood. There were no independent associations between leptin levels and surrogate markers of atherosclerosis.

Conclusions: Serum adiponectin, but not leptin, levels may be a useful biomarker in cardiovascular risk assessment.

Keywords: Adipose tissue, adiponectin, leptin, atherosclerosis, risk factor

TIIVISTELMÄ

Liisa Saarikoski (o.s. Viikari). Adiponektiini ja leptiini nuorilla suomalaisilla aikuisilla ja niiden yhteys sydän- ja verisuonitautien riskitekijöihin sekä varhaisiin verisuonimuutoksiin. Lasten Sepelvaltimotaudin Riskitekijät (LASERI) -tutkimus. Turun yliopisto, Lääketieteellinen tiedekunta, Sisätautioppi, Turun yliopiston kliininen tohtoriohjelma, Sydäntutkimuskeskus, Farmakologia, lääkekehitys ja lääkehoito. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Suomi, 2017.

Tausta: Valtimonkovettumataudin ehkäisemiseksi olisi tärkeää tunnistaa jo lapsuudessa henkilöt, joille tulisi tarjota ennaltaehkäisevää ohjausta. Rasvakudoksen tuottamilla valkuaisaineilla on suuri merkitys monissa elimistön toiminnoissa ja niillä on esitetty olevan suoria ja välillisiä vaikutuksia myös verenkiertoelimistöön.

Tavoite: Tämän tutkimuksen tavoitteena oli kuvata rasvakudoksen tuottamien adiponektiinin ja leptiinin seerumipitoisuudet suomalaisilla lapsilla, nuorilla ja aikuisilla sekä tutkia niiden yhteyksiä metaboliseen oireyhtymään, ultraäänellä mitattuun kaulavaltimon seinämäpaksuuteen ja olkavaltimon sisäkalvon toimintaan. Lisäksi tavoitteena oli selvittää, ennustaako lapsuus- ja nuoruusiässä mitattu seerumin adiponektiinipitoisuus aikuisiällä kaulavaltimon seinämän paksuuntumista.

Menetelmät: Tämä väitöskirjatutkimus on osa Lasten Sepelvaltimotaudin Riskitekijät -tutkimusta, johon osallistui 3596 lasta (iältään 3-18 vuotta) vuonna 1980. Seerumin adiponektiinipitoisuuksia on määritetty vuosien 1980, 2001 ja 2007 näytteistä ja leptiinipitoisuuksia vuosien 1980 ja 2001 näytteistä. Kaulavaltimon ja olkavaltimon ultraäänitutkimukset tehtiin tutkittaville vuosina 2001 ja 2007.

Tulokset: Seerumin adiponektiinipitoisuudet pienenivät lapsuudesta aikuisuuteen tytöillä ja pojilla. Leptiinin pitoisuus seerumissa suurentui tytöillä lapsuusiästä aikuisikään, kun taas pojilla pitoisuus hieman pieneni. Aikuisilla seerumin pieni adiponektiinipitoisuus oli yhteydessä metabolisen oireyhtymän ilmaantuvuuteen, suurempaan kaulavaltimon sisäseinämän paksuuteen ja pienentyneeseen olkavaltimon laajentumiskykyyn. Pieni adiponektiinipitoisuus lapsuus- ja nuoruusiässä ennusti suurentunutta riskiä kaulavaltimon seinämän paksuuntumiselle aikuisena. Seerumin leptiinipitoisuudella ei ollut itsenäistä yhteyttä valtimomuutoksiin.

Johtopäätökset: Seerumin adiponektiinipitoisuuden määrittäminen saattaa auttaa valtimonkovettumataudin riskin arvioinnissa.

Avainsanat: Rasvakudos, adiponektiini, leptiini, valtimonkovettumatauti, riskitekijä

TABLE OF CONTENTS

ABBREVIATIONS

ApoE	Apolipoprotein E
AUC	Area under the receiver operating characteristic curve
BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
FMD	Flow-mediated dilatation
HDL	High density lipoprotein
HMW	High molecular weight
HOMA-B	Homeostasis model assessment for beta-cell function
HOMA-IR	Homeostasis model assessment for insulin resistance
IMT	Intima-media thickness
KO	Knock-out
LDL	Low density lipoprotein
LMW	Low molecular weight
MetS	Metabolic syndrome
MMW	Middle molecular weight
OC	Oral contraceptive
OR	Odds ratio
PCSK9	Proprotein convertase subtilisin/kexin type 9
SD	Standard deviation
SE	Standard error
SNPs	Single nucleotide polymorphism
STRIP	the Special Turku coronary Risk factor Intervention Project

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to within the text by the Roman numerals I-IV. In addition, previously unpublished data are presented.

I Viikari LA, Huupponen RK, Viikari JSA, Marniemi J, Eklund C, Hurme M, Lehtimäki T, Kivimäki M, Raitakari OT. Relationship between leptin and C-reactive protein in young Finnish adults. J Clin Endocrinol Metab. 2007;92:4753-8.

II Saarikoski LA*, Huupponen RK, Viikari JSA, Marniemi J, Juonala M, Kähönen M, Raitakari OT. Adiponectin is related with carotid artery intima-media thickness and brachial flow-mediated dilatation in young adults - the Cardiovascular Risk in Young Finns Study. Ann Med. 2010;42:603-11.

III Saarikoski LA*, Juonala M, Huupponen R, Viikari JSA, Lehtimäki T, Jokinen E, Hutri-Kähönen N, Taittonen L, Laitinen T, Raitakari OT. 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.

IV Juonala M, Saarikoski LA*, Viikari JSA, Oikonen M, Lehtimäki T, Lyytikäinen LP, Huupponen R, Magnussen CG, Koskinen J, Laitinen T, Taittonen L, Kähönen M, Kivimäki M, Raitakari OT. A longitudinal analysis on associations of adiponectin levels with metabolic syndrome and carotid artery intima-media thickness. The Cardiovascular Risk in Young Finns Study. Atherosclerosis. 2011;217:234-9.

* née Viikari

1 INTRODUCTION

Changes in nutrition and physical activity both at work and leisure during the second half of the 20th century have led to a substantial increase in obesity and cardiovascular diseases worldwide (Gaziano 2010). In developed countries over a third of adults and a fifth of children were overweight in 2013 (Ng *et al.* 2014). Cardiovascular diseases are still among the leading causes of death worldwide (Ford *et al.* 2007) and at present its leading risk factors are high blood pressure, obesity, diabetes and high serum cholesterol level (Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration 2014).

In the past 20 years, it has been established that adipose tissue is an active endocrine organ and not just passive energy storage (Booth *et al.* 2016). Adipose tissue secretes numerous different adipokines that participate in the regulation of satiety, glucose metabolism and inflammation (Booth *et al.* 2016). Dysregulation of adipokine secretion in obesity leads to increased secretion of proinflammatory adipokines and decreased secretion of anti-inflammatory adipokines that is thought to participate in the pathophysiology of obesity-related diseases (Maury and Brichard 2010).

In the mid 1990s, two important adipokines, leptin and adiponectin were discovered. Leptin participates in the regulation of satiety end energy expenditure in the central nervous system, and has been shown to have various effects in peripheral tissues, including proinflammatory and prothrombotic effects (Blüher and Mantzoros 2015). Adiponectin was identified as a potential regulator of energy homeostasis, having positive effects on insulin sensitivity as well as anti-inflammatory effects (Wang and Scherer 2016).

In 1978 an epidemiological study of risk factors for coronary heart disease was planned for Finnish children and adolescents (the Cardiovascular Risk in Young Finns Study). The main study started in 1980 with 3,596 subjects and the participants have been followed since for more than 30 years.

This study examined the serum levels of leptin and adiponectin in the Cardiovascular Risk in Young Finns Study and determined the extent to which they track, and their associations with conventional cardiovascular risk factors and subclinical atherosclerosis.

2 REVIEW OF THE LITERATURE

2.1 Adipose tissue as an endocrine organ

2.1.1 General aspects of adipose tissue

Previously, adipose tissue was mostly considered as passive energy storage. Now, it is recognized as a complex endocrine organ that participates in the regulation of energy balance, the immune system and the pathophysiology of obesity related diseases such as cardiovascular diseases, type 2 diabetes (Booth *et al.* 2016), and carcinogenesis (Cabia *et al.* 2016).

The predominant adipose tissue in humans is the white adipose tissue situated subcutaneously and as visceral fat. Brown adipose tissue was previously known to maintain normal body temperature in newborns and it was believed to regress with age (Thoonen *et al.* 2016). However, it has been shown to exist in healthy adults (Virtanen *et al.* 2009) and it is now considered to have a role in oxidization of glucose and fatty acids to produce heat for thermoregulation also in adults (Thoonen *et al.* 2016).

White adipose tissue consists of many cell types, mostly of adipocytes, but also fibroblasts, endothelial cells and immune cells. The main function of adipose tissue is the storage of excessive calories as triglycerides and the release of fatty acids during fasting (Blüher and Mantzoros 2015). Different cell types secrete various proteins with autocrine, paracrine and endocrine actions. These proteins are called adipokines, and their dysregulation in obesity contributes to inflammation and insulin resistance (Maury and Brichard 2010).

The first adipose tissue-derived protein recognized was complement factor D, adipsin, involved in innate immunity (Cook *et al.* 1987). The real trigger for the following vigorous research on adipose tissue was the discovery of leptin in 1994 (Zhang *et al.* 1994). During the past two decades, there has been a substantive effort to identify adipose tissue derived proteins and to gain understanding of their functions. A decade after the identification of leptin more than 50 adipokines had been identified (Trayhurn and Wood 2004). Since this time, more than 600 adipokines have been identified and the search is ongoing (Blüher and Mantzoros 2015).

2.1.2 Leptin

The existence of an unknown circulating factor responsible for the obese/ diabetic state of the *ob/ob* mouse was first suggested in 1973 (Coleman 1973, Coleman and Hummel 1973). This factor, now known as leptin, was identified in 1994 as the *obese* (*ob*) gene product (Zhang *et al.* 1994) participating in the regulation of appetite and energy expenditure (Halaas *et al.* 1995). Soon after its discovery, leptin overexpression was shown in adipose tissue of obese humans (Lonnqvist *et al.* 1995).

Elevated serum leptin levels were identified in obese adults (Considine *et al.* 1996) and children (Hassink *et al.* 1996) compared with normal-weight subjects. Leptin levels are higher in females than in males (Saad *et al.* 1997). In 1997, a mutation in human leptin gene causing leptin deficiency and morbid obesity was identified confirming the physiological role of leptin in the regulation of body weight in humans (Montague *et al.* 1997). This finding was further highlighted by treatment with human recombinant methionyl leptin resulting in reduced energy-intake and weight-loss (Farooqi *et al.* 1999).

In addition to the rare mutations in leptin gene, other genes may influence leptin levels by regulating its production and metabolism. Up to 30-50% of the variation in leptin levels is estimated to be explained by genetic factors, and recently 4 loci outside the leptin gene were identified to associate with leptin levels independent of adiposity in a genome-wide meta-analysis (Kilpeläinen *et al.* 2016).

The actions of leptin are mediated by its receptors in the central nervous system and peripheral tissues (Tartaglia *et al.* 1995, Muoio and Lynis Dohm 2002). Mutations in the leptin receptor gene have been shown to cause the severe metabolic disturbances of the obese *db/db* mice (Chen *et al.* 1996). In humans, mutations in the leptin receptor gene have been shown to cause severe obesity and hypopituitarism emphasizing the physiological importance of leptin (Clement *et al.* 1998).

Leptin regulates appetite and energy expenditure and has physiological importance in energy-deficient states, such as anorexia nervosa and exercise-induced amenorrea, by participating in the regulation of hypothalamic-pituitary-peripheral endocrine axis (Chan and Mantzoros 2005). Leptin is also thought to have a role in pubertal development. In both girls and boys, there is a rise in leptin levels preceding rises of gonadotrophin levels (Garcia-Mayor *et al.* 1997). In leptin deficient humans, absent or delayed pubertal development has progressed with leptin replacement therapy (Chan and Mantzoros 2005).

At first, leptin was hoped to be the solution for the treatment of obesity in general. However, leptin therapy does not significantly affect appetite or body weight in obese individuals without leptin deficiency. This is generally considered to result from leptin resistance (Blüher and Mantzoros 2015). In generalized lipodystrophy, a state associated with hypoleptinemia, leptin replacement therapy markedly restores severe metabolic disturbances such as impaired glycemic control, hypertriglyceridemia and hepatic steatosis and decreases energy intake (Savage and O'Rahilly 2002). Several clinical trials on the effect of human recombinant methionyl leptin therapy in various lipodystrophies, obesity, non-alcoholic fatty liver disease and type 1 diabetes are ongoing (Chou and Perry 2013).

2.1.3 Adiponectin

Adiponectin, the most abundant protein secreted by the adipocytes, was identified in 1995 in mice (Scherer *et al.* 1995) and in 1996 in humans (Maeda *et al.* 1996). Adiponectin exists in three complexes: low molecular form (low molecular weight, LMW, trimer), middle molecular form (middle molecular weight, MMW, hexamer) and high molecular form (high molecular weight, HMW, 12-18mer), which may have different biological effects (Wang and Scherer 2016). Serum concentrations of adiponectin are reduced in obesity and higher in females than in males (Arita *et al.* 1999). In both females and males, adiponectin levels decrease during puberty (Böttner *et al.* 2004, Andersen *et al.* 2007).

The actions of adiponectin are mediated through its receptors AdipoR1 and AdipoR2 expressed in adipose tissue, muscle, liver and other tissues (Yamauchi *et al.* 2003). Adiponectin has insulin sensitizing, anti-inflammatory and anti-apoptotic effects on a number of different cell types (Turer and Scherer 2012). It inhibits gluconeogenesis, increases glucose uptake, stimulates fatty acid oxidation and reduces the triglyceride content in liver and muscle (Yamauchi *et al.* 2007, Maury and Brichard 2010). An additional receptor for adiponectin, T-cadherin, that is expressed in endothelial cells, vascular smooth muscle cells, and cardiac myocytes, specifically binds to MMW and HMW forms of adiponectin and may mediate its suggested cardioprotective effects (Wang and Scherer 2016).

Mutations in or near the ADIPOQ gene encoding adiponectin explain 4% of the variation in adiponectin levels (Lee *et al.* 2016). Additionally, several genes in other loci contribute to adiponectin levels (Dastani *et al.* 2012).

In a two-year weight-loss intervention study, adiponectin levels significantly increased over time, and associated with reduction in waist circumference and positive changes in lipid profile independent of weight change (Ma *et al.* 2016). In another study, adiponectin levels increased after a very-low-calorie diet, induced marked weight loss, and remained elevated after a six-month weight maintenance period (Heinonen *et al.* 2009).

AdipoRon, an agonist for adiponectin receptors, has been shown to ameliorate insulin resistance and glucose metabolism in mice (Okada-Iwabu *et al.* 2013). It remains to be seen if influencing adiponectin levels or enhancing its actions in humans will prove beneficial in the treatment of diseases associated with obesity and insulin resistance (Blüher and Mantzoros 2015).

2.2 Atherosclerosis

2.2.1 Development of atherosclerosis

Atherosclerosis is a process starting from the accumulation of cholesterol esters and fibrous elements in the intimal layers of arteries, finally leading to clinical complications such as acute myocardial infarction and stroke.

Evidence of atherosclerotic plaques was found over 100 years ago in autopsies performed on ancient Egyptian mummies and Peruvian mummies (Thomas *et al.* 2014). Computed tomography scannings of 137 Egyptian, Peruvian, Native American, and Aleutian mummies found evidence of atherosclerosis in all populations, and atherosclerotic lesions in 34% of the examined mummies (Thompson *et al.* 2013). Additionally, computed tomography studies have proven the presence of atherosclerotic lesions in European "Ötzi the Iceman" who lived over 5,000 years ago (Murphy *et al.* 2003), suggesting that humans have been susceptible to the development of atherosclerosis in very different living environments (Thomas *et al.* 2014).

Coronary atherosclerotic lesions were a common (77%) finding in autopsies performed on 300 soldiers (age 18-48 years) killed in the Korean War in the 1950s that varied from fibrous thickening to large plaques (Enos *et al.* 1953). The publication of these findings, in part, facilitated the initiation of large epidemiological studies on atherosclerosis and highlighted the importance of preventive actions already in childhood and adolescence. This idea was further supported by autopsy findings from 105 Vietnam War casualties (age 18-37 years) showing some degree of atherosclerosis in 45% of the soldiers

(McNamara *et al.* 1971). The prevalence of atherosclerosis in United States Armed Forces was evaluated again in the years 2001-2011 in a comprehensive autopsy study including 3,832 cases (age 18-59) (Webber *et al.* 2012). The overall prevalence of atherosclerosis was 12%, substantially lower than in the historical studies. In this study, age, lower educational level, overweight (body mass index, BMI, 25-29.9) or obesity (BMI≥30) and prior diagnoses of dyslipidemia, hypertension or obesity were associated with atherosclerosis.

Human atherosclerosis begins very early in life, with fatty streaks reported to exist already in fetal aortas (Napoli *et al.* 1997), and intimal thickening of the coronary arteries found in autopsies on children who had died before the age of one year (Pesonen *et al.* 1975). In autopsy studies on children and young adults who had participated in the Bogalusa Heart Study and died accidentally or due to homicide at young age (2-39 years), the prevalence of fatty streaks was 50% and the prevalence of raised fibrous plaques 8% in the coronary arteries of children and adolescents. The prevalence of fatty streaks increased to 85% and raised fibrous plaques to 69% in young adults, and correlated with previously measured risk factor levels, supporting the need of cardiovascular risk evaluation and prevention already in childhood (Berenson *et al.* 1998).

The now generally accepted "response to injury" -theory was introduced in the 1970s by Ross et al. (Ross *et al.* 1977). They stated that the development of atherosclerotic lesions initiates with endothelial injury caused by various factors such as mechanical stress, hyperlipidemia or infection, leading to platelet adhesion and aggregation and subsequently proliferation of smooth muscle cells and formation of new connective tissue as well as intracellular and extracellular lipid deposition.

The Committee on Vascular Lesions of the Council of Atherosclerosis, American Heart Association has provided a classification of atherosclerotic lesions (Stary *et al.* 1994, Stary *et al.* 1995):

- Type I lesion describes intimal thickening due to accumulation of lipids and macrophages.
- Type II lesion contains more macrophage foam cells and lipid laden smooth muscle cells. Fatty streaks are included in type II lesion.
- In type III lesions, there are microscopically visible extracellular lipid droplets and particle collections among the layers of intimal smooth muscle cells.

- Type IV lesions, atheromas, are potentially symptom-producing lesions characterized by an extracellular lipid core.

- Type V lesions, fibroatheromas, have a lipid core and thick layers of fibrous connective tissue (Va). Some type V lesions are largely calcified (type Vb), and some consist mainly of fibrous connective tissue and little or no accumulated lipid or calcium (type Vc). These lesions cause narrowing of affected arteries.

- The complicated type VI lesions refer to type IV and V lesions with disruptions of the lesion surface (type VIa), hematoma or hemorrhage (type VIb), and thrombotic deposits (type VIc). Type VIabc indicates the presence of all three features.

2.2.2 Subclinical atherosclerosis

Ultrasound imaging can be used to estimate the development of atherosclerosis in its subclinical stages. The three most widely used methods are measurements of intima-media thickness (IMT) of the common carotid artery, brachial flow-mediated dilatation (FMD) and carotid artery distensibility.

Common carotid IMT is considered a structural marker of atherosclerosis that can be measured reliably in vivo by ultrasound (Pignoli *et al.* 1986). It is associated with vascular risk factors cross-sectionally in adults (Poli *et al.* 1988, Haapanen *et al.* 1989) and risk factor levels in childhood are associated with IMT in adulthood (Davis *et al.* 2001, Li *et al.* 2003, Raitakari *et al.* 2003). IMT predicts the likelihood of cardiovascular events in population studies (Bots *et al.* 1997, Chambless *et al.* 1997, O'Leary *et al.* 1999). Although the association of IMT with future cardiovascular events has been shown in many middle-aged and elderly populations, the addition of IMT to traditional risk prediction models did not significantly improve model performance in a meta-analysis of 15 studies (van den Oord *et al.* 2013).

Endothelial dysfunction occurs in early stages of atherosclerosis and may predict cardiovascular end-points (Ross 1993). FMD of the brachial artery is a measurable marker of systemic endothelial function (Celermajer *et al.* 1992) that is mainly mediated by nitric oxide released by arterial endothelial cells (Joannides *et al.* 1995). Impaired brachial FMD is related to obesity in children (Tounian *et al.* 2001); associated with the prevalence of coronary atherosclerosis (Neunteufl *et al.* 1997); and predicts cardiovascular events (Chan *et al.* 2003).

The elasticity of large arteries decreases with age due to degeneration of elastic fibers and increase in collagen content in the artery wall (Hanon *et al.* 2001). Carotid artery distensibility refers to the ability of the carotid arteries to expand as a response to pulse pressure caused by cardiac contraction and relaxation (Oliver and Webb 2003). A decrease in the carotid artery distensibility is an early change in atherosclerosis that is associated with cardiovascular risk factors (Tounian *et al.* 2001) and may independently predict cardiovascular events in high-risk individuals (Blacher *et al.* 1998).

2.2.3 Risk factors for atherosclerosis

In the 1850s, Virchow suggested the inflammatory nature of atherosclerosis and discovered the presence of cholesterol in the atheroma (Hort 1994, Barton 2013). In 1913 Anitschkow published evidence of severe atherosclerosis in rabbits that consumed a high-cholesterol diet, suggesting high levels of blood cholesterol caused atherosclerosis (Steinberg 2013). However, his theory was not widely accepted at that time, and further interest in the causal role of cholesterol in human atherosclerosis began to re-emerge in the 1950s.

The Framingham Heart Study was the first epidemiological study conducted to determine risk factors for cardiovascular diseases, and remains ongoing today (Dawber *et al.* 1951, Tsao and Vasan 2015). In the first published article from the Framingham Heart Study (Dawber *et al.* 1951), the authors summarized the understanding of the epidemiology of cardiovascular diseases and the research needs at that time as follows:

- "Of the epidemiology of hypertensive or arteriosclerotic cardiovascular disease almost nothing is known, although these two account for the great bulk of deaths from cardiovascular disease."
- "Clearly, what is required is the epidemiological study of these diseases based on populations of normal composition, including both the sick and the well as they are found in the community."
- "As a working hypothesis it is assumed that these diseases do not each have a single cause (as is the case in most infectious diseases), but that they are the result of multiple causes which work slowly within the individual."

At the time of the four-year follow-up of the Framingham Heart Study, data showed that hypertension, overweight and hypercholesterolemia were associated with increased risk for incident coronary heart disease in middle-aged men

(Dawber *et al.* 1957). The six-year follow-up added a low educational level and smoking to these risk factors (Dawber *et al.* 1959).

Since the initiation of the Framingham Heart Study, many epidemiological studies have added to the knowledge on cardiovascular risk factors. Large follow-up studies on children and young adults have provided data on childhood risk factors, their tracking and clustering, as well as their association with early indicators of atherosclerosis in adulthood. These studies listed in **Table 1** have together formed the International Childhood Cardiovascular Cohort Consortium (i3C) (Dwyer *et al.* 2013). The i3C Consortium enables pooling of data to increase power, such as cardiovascular disease events in adulthood, and for the collection and analysis of genetic data. The epidemiological evidence shows that childhood risk factors are associated with subclinical atherosclerosis in adulthood independent of risk factors in adulthood (Mahoney *et al.* 1996, Davis *et al.* 2001, Li *et al.* 2003, Raitakari *et al.* 2003).

Table 1. Summary of the studies included in the International Childhood Cardiovascular Cohort (i3C) Consortium.

Study	Country	Study years in childhood and adolescence	Participants in childhood and adolescence	References
Muscatine Study	USA	1970-1981	n=11,377 aged 5-18 years	(Lauer *et al.* 1975, Lauer *et al.* 1988)
Bogalusa Heart Study	USA	1973-1994	n=12,164 aged 4-17 years	(Voors *et al.* 1977, Freedman *et al.* 1987)
Princeton Lipid Research Clinics Study	USA	1973	n=6,775 aged 6-18 years	(Morrison *et al.* 1977)
Minneapolis Childhood Cohort Studies	USA	1978-1989	n=1,207 aged 6-9 to 17-20 years	(Prineas *et al.* 1980, Sinaiko *et al.* 1999)
Cardiovascular Risk in Young Finns Study	Finland	1980	n=3,596 aged 3-18 years	(Akerblom *et al.* 1985b)
Childhood Determinants of Adult Health Study	Australia	1985	n=8,498 aged 7-15years	(Venn *et al.* 2007)
National Heart, Lung, and Blood Institute Growth and Health Study	USA	1987-1996	n=2,379 aged 9-19 years	(Thompson *et al.* 2007)

Prospective studies on middle-aged populations, such as the Seven Countries Study (Keys *et al.* 1966), the Uppsala Longitudinal Study of Adult Men (Lithell *et al.* 1984), the Copenhagen City Heart Study (Andersen and Jensen 2010), the Nurses Health Study (Colditz and Hankinson 2005) and the Health Professionals Follow-up Study (Rimm *et al.* 1991) have been able to demonstrate geographical differences in risk factor levels and cardiovascular mortality as well as connections between risk factor levels and cardiovascular events and mortality. In a large, international, standardized, case-control study, the INTERHEART study, it was shown that the major risk factors for acute myocardial infarction are similar in both sexes, at all ages, and in all regions (Yusuf *et al.* 2004). The two most important risk factors worldwide were abnormal lipids measured as raised apolipoprotein B/apolipoprotein A1 ratio and cigarette smoking whereas the importance of other risk factors (hypertension, diabetes, abdominal obesity, and psychosocial factors) and protective factors (daily consumption of fruit and vegetables, alcohol consumption, and physical activity) varied (Yusuf *et al.* 2004).

Several intervention studies have been conducted in order to decrease cardiovascular risk. In the Oslo Study in the 1970s (Hjermann 1983), a five-year intervention consisting of dietary and antismoking advice combined with regular follow-up visits every 6 months led to a 47% decrease in first myocardial infarction. At the same time in Finland, the North Karelia Project (Puska *et al.* 1979, Salonen *et al.* 1979) was launched due to the extremely high cardiovascular mortality in Eastern Finland. The aim of the study was to decrease the cardiovascular mortality rate by influencing diet and lifestyle in order to lower blood pressure and cholesterol levels and decrease smoking. The study expanded nationwide after the first five years, and since its beginning, the cardiovascular mortality rate in North Karelia has decreased about 85% (Puska *et al.* 2016). In the Finnish Helsinki Businessmen Study (Strandberg *et al.* 2016), 1,222 men aged 44-55 years who had a high-risk profile of cardiovascular disease based on health check-ups performed in 1964-1973 were randomized for a 5-year intervention consisting of intensive lifestyle modification and contemporary drug treatment for hypertension or dyslipidemia. During the trial, the intervention group had a 46% reduction of total cardiovascular risk and the incidence of stroke was reduced compared with the control group. The long-time follow-up has also shown that elevated risk factor levels in midlife associate with lower quality of life, function and cognition in old age (Strandberg *et al.* 2015).

High serum concentration of low density lipoprotein (LDL) cholesterol is a key factor in atherosclerosis. Its importance is most obvious in familial hypercholesterolemia, a hereditary disorder associated with severe atherosclerosis at young age. The hereditary nature and the clinical presentation with xanthomas, hypercholesterolemia, coronary heart disease and cardiovascular

mortality at young age were described already in 1938 by Norwegian physician Müller (Besseling *et al.* 2015). In 1973, patients with familial hypercholesterolemia were shown to have increased activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase leading to increased cholesterol synthesis (Goldstein and Brown 1973), with the disease mechanism identified as a defect in the LDL-receptor in 1974 (Goldstein and Brown 2009).

Medical approaches to influence cardiovascular risk factor levels have aimed to reduce serum total and LDL cholesterol levels. Reduction in incidence of coronary heart disease has been shown for cholestyramine in Lipid Research Clinics Coronary Primary Prevention Trial (Rifkind 1984) and gemfibrozil in the Helsinki Heart Study (Frick *et al.* 1987). In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study, fenofibrate treatment was associated with reduced incidence of total cardiovascular events but did not reduce cardiovascular mortality in type 2 diabetic patients (Keech *et al.* 2005).

The most widely used cholesterol-lowering group of drugs are the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, the statins. Their efficacy in reducing cardiovascular events and mortality has been shown in many studies (LaRosa *et al.* 1999). In the landmark study of statin therapy, the Scandinavian Simvastatin Survival Study (4S), simvastatin treatment reduced the risk of major coronary event by 34% and the risk of coronary mortality by 42% in secondary prevention (Pedersen *et al.* 1998). Since then, the safety and efficacy of different statins has been established in extensive studies (Collins *et al.* 2016).

Treatments combining statins with other cholesterol-lowering drugs have also been studied. In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, combination treatment with simvastatin and fenofibrate did not reduce the rate of cardiovascular events in patients with type 2 diabetes compared to monotherapy with simvastatin (ACCORD Study Group *et al.* 2010). In the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), combination treatment with simvastatin and ezetimibe, a drug reducing intestinal absorption of cholesterol, resulted in lower LDL cholesterol levels and reduced risk of cardiovascular events (Cannon *et al.* 2015).

The use of the most recently introduced LDL cholesterol-lowering drugs, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors evolocumab (Sabatine *et al.* 2015) and alirocumab (Robinson *et al.* 2015) have been shown to lower LDL cholesterol levels efficiently alone or used in combination with statins. The use of these PCSK9 inhibitors seems to decrease the risk of cardiovascular events, but further studies of their efficacy and safety are needed (Robinson *et al.* 2015, Sabatine *et al.* 2015).

Hypertension is one of the undisputable risk factors for cardiovascular diseases. Combined data from numerous prospective studies has shown that higher blood pressure level is associated with increased cardiovascular and cerebrovascular mortality in all age groups, even at blood pressure levels within the normal range (Lewington *et al.* 2002). The target for lowering blood pressure varies from <130/80 to <140/90, most guidelines recommend lower targets for subjects with diabetes, chronic kidney disease, and coronary heart disease (Rosendorff *et al.* 2015). The recently published data from the Systolic Blood Pressure Intervention Trial (SPRINT) supports setting the target of systolic blood pressure even in elderly subjects to as low as <120 mmHg, resulting in reduced risk of myocardial infarction, stroke, heart failure and cardiovascular death (Wright *et al.* 2015).

2.2.4 Cardiovascular risk evaluation and cardiovascular mortality

In 1981, Hopkins and Williams already listed 246 established or suggested cardiovascular risk factors (Hopkins and Williams 1981). Studies on each risk factor have helped in understanding the pathophysiological mechanisms of cardiovascular diseases. However, the clinical potential of a risk factor is based on its usefulness, which can be evaluated by three questions: 1) Can the clinician measure it? 2) Does it add new information? 3) Does it help the clinician to manage patients? (Morrow and de Lemos 2007).

Established classical risk factors for cardiovascular diseases include older age, male sex, high total and LDL cholesterol, low high-density lipoprotein (HDL) cholesterol, elevated blood pressure, smoking, impaired glycemic control including diabetes, and family history of cardiovascular diseases at young age (The National Institutes of Health Consensus Development Panel 1985, D'Agostino *et al.* 2008). These risk factors are used in various cardiovascular risk assessment calculators in clinical practice worldwide, such as Framingham risk calculators (D'Agostino *et al.* 2008), German risk calculator from Prospective Cardiovascular Münster Study for Northern European populations (Assmann *et al.* 2002) and Finnish FINRISKI risk calculator (Vartiainen *et al.* 2007).

To evaluate the need of intensive drug treatment, calculators for 10-year risk of fatal cardiovascular events, like in the Systematic COronary Risk Evaluation (SCORE) project (Conroy *et al.* 2003), are useful. However, in order to evaluate the life-long risk of cardiovascular diseases, other kinds of approaches are needed (Marma *et al.* 2010). In large cohort studies from USA, life-time risk of cardiovascular diseases has been reported to be as high as 60% for men and 55% for women at the age of 45 years (Wilkins *et al.* 2012). In clinical practice, it is important to understand the differences between risk calculators using various

risk factors and different end-points, because the same calculator can have different utility across different age groups and between sexes (Ketola *et al.* 2010).

The importance of life-long exposure to a cardiovascular risk factor is well demonstrated by familial hypercholesterolemia. In familial hypercholesterolemia, the exposure to elevated LDL cholesterol level begins in the fetal period leading to cardiovascular morbidity in early adulthood if not treated early with dietary counseling and early iniated statin therapy (Wiegman *et al.* 2015). On the other hand, mutations that lead to decreased LDL cholesterol levels are associated with decreased risk for cardiovascular disease (Cohen *et al.* 2006, Ference *et al.* 2016). Compared to risk reduction with statin therapy started later in life, genetically low LDL cholesterol leads to a three-fold risk reduction for each mmol/L lower LDL cholesterol level (Ference *et al.* 2012). Nutritional and lifestyle counseling started in early childhood has been shown to be safe and to result in positive changes in risk factor levels in the Special Turku coronary Risk factor Intervention Project (STRIP) launched in 1989 (Simell *et al.* 2009). It can be assumed that a healthy lifestyle adopted in childhood that persists in adulthood leads to a decreased cardiovascular risk due to decreased life-long exposure similarly as a genetically low risk factor level.

Although mortality from cardiovascular diseases has decreased in many countries, they remain the leading cause of death in the world (31% in 2013). From 1980 through 2000, the age-adjusted death rate for coronary heart disease decreased in the United States from 543 to 267 deaths per 100,000 among men and from 263 to 134 per 100,000 among women (Ford *et al.* 2007). In IMPACT analysis (Capewell *et al.* 1999), approximately 44% of the decrease was attributed to the improvements in risk factor levels (Ford *et al.* 2007). In Finland, from 1969-1972 to 2012, coronary heart disease mortality decreased from 643 to 118 per 100,000 working aged men and from 114 to 17 deaths per 100,000 working aged women. Results from the national FINRISK study show that positive changes in risk factor levels (decreased smoking prevalence, serum total cholesterol and systolic blood pressure) explained nearly all of the reduction in observed coronary heart disease mortality during the first ten years of the study (Jousilahti *et al.* 2016). In the last ten years of the study, changes in these three main risk factors explain two thirds of the reduction. In other countries, changes in nutrition and life-style affect risk factors negatively. For example, in China the age-adjusted coronary heart disease mortality increased between 1984 an 1999 by 50% in men and 26% in women. Most of the increase was explained by the observed 1 mmol/L rise in total cholesterol level (Critchley *et al.* 2004).

Research on cardiovascular risk factors is, in part, shifting from studies on associations of one or more risk factors with cardiovascular disease towards more complex approaches using Mendelian randomization (Davey Smith and Ebrahim 2005), genetic risk scores, whole exome or whole genome sequencing, epigenetics, transcriptomics, metabolomics and lipidomics (Hoefer *et al.* 2015, Niiranen and Vasan 2016). Data obtained by these approaches may improve individual risk assessment in the future.

2.2.5 Metabolic syndrome and atherosclerosis

Metabolic syndrome (MetS) is a concept describing clustering of risk factors for cardiovascular diseases and type 2 diabetes (Alberti *et al.* 2009). It was introduced in 1988 by Reaven as a cluster of resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinemia, increased very-low-density lipoprotein triglyceride, decreased HDL cholesterol, and hypertension (Reaven 1988).

Many organizations have proposed definitions for MetS with some variation in their criteria for the five key components: abnormal glucose or insulin metabolism, central obesity, hypertension, elevated triglycerides and decreased HDL cholesterol. Some definitions have included also other components. The different definitions are summarized in **Table 2**.

A harmonized definition of MetS was published in 2009 as a collaboration between the International Diabetes Federation, the National Heart, Lung and Blood Institute, the American Heart Association, the World Heart Federation, the International Atherosclerosis Society and the International Association for the Study of Obesity in order to unify the criteria used in both clinical practice and research (Alberti *et al.* 2009). According to these criteria, there is no single obligatory component for MetS, but three abnormal findings out of five would qualify a person for the MetS. The five components are elevated waist circumference, elevated triglycerides, reduced HDL cholesterol, elevated blood pressure and elevated fasting glucose. Medications for any of the components are considered as alternate indicators. For waist circumference, there are different suggested sex-specific cut points for different ethnic groups.

Table 2. Definitions of the metabolic syndrome.

	MetS definition	Obesity	Hypertension	Glucose metabolism	Dyslipidemia
WHO[1]	Type 2 diabetes, impaired glucose tolerance or insulin resistance AND 2 other features *	Waist-to-hip ratio >0.90 (men) >0.85 (women) and/or BMI >30 kg/m^2	≥160/90 mmHg	Impaired glucose tolerance, type 2 diabetes or insulin resistance by euglycaemic clamp	HDL cholesterol ≤0.9 mmol/L (men) ≤1.0 mmol/L (women) Triglycerides ≥1.7 mmol/L
EGIR[2]	Hyperinsulinemia AND 2 other features	Waist circumference ≥94 cm (men) ≥80 cm (women)	≥140/90 mmHg**	Fasting plasma glucose ≥6.1 mmol/L	HDL cholesterol <1.0 mmol/L Triglycerides ≥2.0 mmol/L
Updated NCEP ATPIII / AHA and NHLBI[3]	At least 3 features (all equal)	Waist circumference ≥102 cm (men) ≥88 cm (women)	≥130/85 mmHg**	Fasting plasma glucose ≥5.6 mmol/L **	HDL cholesterol ≤1.03 mmol/L (men) ** ≤1.29 mmol/L (women) ** Triglycerides ≥1.7 mmol/L **
ACE and AACE[4]	Clinical judgement ***		≥130/85 mmHg	Fasting plasma glucose ≥6.1 mmol/L or Impaired glucose tolerance in oral glucose tolerance test	HDL cholesterol ≤1.03 mmol/L (men) ** ≤1.29 mmol/L (women) ** Triglycerides ≥1.7 mmol/L **
IDF[5]	Central obesity mandatory component AND 2 other features	Waist circumference ≥94 cm (men) ≥80 cm (women)	≥130/85 mmHg**	Fasting plasma glucose >5.6 mmol/L or Type 2 diabetes	HDL cholesterol ≤1.03 mmol/L (men) ** ≤1.29 mmol/L (women) ** Triglycerides ≥1.7 mmol/L **
The harmonized definition[6]	At least 3 features (all equal)	Waist circumference ≥102 cm (men) ≥88 cm (women) or ≥94 cm (men) ≥80 cm (women) also population- and country-specific cut points	≥130/85 mmHg**	Fasting plasma glucose ≥5.6 mmol/L **	HDL cholesterol ≤1.0 mmol/L (men)** ≤1.3 mmol/L (women)** Triglycerides ≥1.7 mmol/L**

1) World Health Organization (Alberti and Zimmet 1998)
2) European Group for the Study on Insulin Resistance (Balkau and Charles 1999)
3) National Cholesterol Education Program Adult Treatment Panel III / American Heart Association and the National Heart, Lung and Blood Institute (Grundy *et al.* 2005)
4) American College of Endocrinology and American Association of Clinical Endocrinologists (Einhorn *et al.* 2003)
5) International Diabetes Federation (Alberti *et al.* 2005)
6) A collaboration between the International Diabetes Federation, the National Heart, Lung and Blood Institute, the American Heart Association, the World Heart Federation, the International Atherosclerosis Society and the International Association for the Study of Obesity (Alberti *et al.* 2009)
* Additional criteria: microalbuminuria (urine albumin excretion rate ≥ 20 μg/min or albumin/creatinine ratio ≥ 20 mg/g)
** Or drug treatment
*** Additional criteria: e.g. abnormal uric acid metabolism, prothrombotic factors, markers of inflammation, endothelial dysfunction, non-Caucasian ethnicity and family history of type 2 diabetes and cardiovascular diseases.

The presence of MetS has been reported to associate with increased cardiovascular disease morbidity and mortality (Isomaa *et al.* 2001, Lakka *et al.* 2002, Ford 2005, Santaniemi *et al.* 2014). In the Botnia Study, the presence of MetS according to the WHO criteria was associated with a three-fold risk of coronary heart disease and stroke, and over five-fold risk of cardiovascular death in Finnish and Swedish middle-aged males and females (Isomaa *et al.* 2001). Also in the Kuopio Ischaemic Heart Disease Risk Factor Study on Finnish middle-aged men, the presence of MetS at baseline was associated with a three-fold risk of coronary heart disease and over two-fold risk of cardiovascular death (Lakka *et al.* 2002).

MetS has also been reported to associate with increased incidence of type 2 diabetes (Sattar *et al.* 2008). However, it has been questioned if MetS provides any greater risk for cardiovascular diseases or type 2 diabetes compared with its individual components (Borch-Johnsen and Wareham 2010). In apparently healthy subjects, MetS is less effective than the Framingham Risk Score to predict cardiovascular diseases and no better in predicting type 2 diabetes than fasting plasma glucose alone (Reaven 2011). In a collaboration of the Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study, MetS in childhood was reported to predict increased risk for subclinical atherosclerosis and type 2 diabetes in adulthood, but was shown to be no better than indices of overweight and obesity (Magnussen *et al.* 2010).

The current understanding of the MetS is that although the concept has been helpful in adding the awareness of risk factor clustering, it should not be considered as a true syndrome or a clinical diagnosis. The treatment of MetS is no different from the treatment of the individual components: healthy diet, regular physical activity, smoking cessation and pharmacological treatment when necessary (Simmons *et al.* 2010, Reaven 2011).

2.2.6 The role of inflammation in atherosclerosis

The classification of atherosclerotic lesions described in chapter 2.2.1 focuses on the structure of the lesion. However, the progress from lipid accumulation to a complicated lesion is highly dependent on functional processes within the lesion. The presence of macrophages and lymphocytes in atherosclerotic lesions was recognized by Anitschkow 100 years ago but further knowledge of the crucial role of immunity and inflammation in the pathogenesis of atherosclerosis has been accumulating only for the past 30 years (Hansson 2009).

Inflammatory substances, dyslipidemia and mechanical stress from hypertension promote adhesion of leukocytes to endothelial cells, causing changes in

endothelial permeability. The subsequent accumulation of LDL particles and monocytes in the arterial wall leads to the formation of lipid-laden macrophages called foam cells. The inflammatory process in the artery wall involves numerous cytokines, chemokines, growth factors and enzymes secreted by monocytes and T-lymphocytes. Together these factors promote the migration of smooth muscle cells from the media to the intima and the proliferation of smooth muscle cells, as well as formation of fibrous tissue in the vessel wall, vascularisation, and ultimately, plaque stability (Ross 1999, Libby *et al.* 2011).

C-reactive protein (CRP) is a protein synthesized mainly by hepatocytes in response to inflammation, infection or tissue damage. The synthesis following such stimulus is rapid, and after the stimulus ceases, the plasma levels of CRP decrease rapidly to a stable CRP concentration (Pepys and Hirschfield 2003). Increased plasma CRP concentrations have been associated with cardiovascular events in many studies (Ridker *et al.* 2003, Danesh *et al.* 2004). However, genetic variation of plasma CRP levels was not associated with coronary heart disease in a large Mendelian randomisation meta-analysis, suggesting that CRP is unlikely to be a causal factor in coronary heart disease (Wensley *et al.* 2011). In the population of the Cardiovascular Risk in Young Finns Study, serum CRP levels in childhood did not predict carotid IMT in adulthood (Juonala *et al.* 2006). As an easily measurable marker of inflammation, plasma CRP level might still be useful in risk evaluation (Ridker 2016).

In addition to their cholesterol-lowering effect, statins affect the inflammatory processes in the atheroma from leukocyte adhesion to plaque stability (Schönbeck and Libby 2004). There is now increased interest to develop anti-inflammatory treatments for atherosclerosis. In studies on atherosclerotic mice, immunomodulation by inhibition of CD40 signalling (Mach *et al.* 1998) and intravenous immunoglobuline infusion (Nicoletti *et al.* 1998) have been shown to reduce atherosclerosis. In humans, anti-inflammatory and immunomodulating drugs are widely used in the treatment of numerous other diseases. Of those drugs, methotrexate and colchicine have been shown to reduce atherosclerosis in animal models and to associate with reduced risk of myocardial infarction in epidemiological studies (Bäck and Hansson 2015). Also tumor necrosis factor α -inhibitors used for the treatment of other diseases in humans have been associated with lower risk of cardiovascular events (Jacobsson *et al.* 2005). Currently, there are ongoing clinical trials on the use of methotrexate and interleukin-1β-neutralizing monoclonal antibody canakinumab for secondary prevention of myocardial infarction (Bäck and Hansson 2015). In clinical practice, anti-inflammatory treatment for atherosclerosis is already used in the form of preventing restenosis after revascularization with immonosuppressive drug-eluting stents (Nikam *et al.* 2014).

2.3 Adipokines and cardiovascular diseases

Adipose tissue dysregulation in obesity is thought to explain the connection between obesity and cardiovascular diseases (Van Gaal *et al.* 2006). Adipokines play a role in the development of atherosclerosis both directly, by influencing endothelial function, vascular homeostasis and atherogenesis, and indirectly via insulin resistance (Maury and Brichard 2010, Ntaios *et al.* 2013, Carbone *et al.* 2015). The effects of some adipokines on the cardiovascular system, insulin sensitivity and inflammation are shown in **Figure 1**. The role of adipokines in the prediction of cardiovascular events or pharmacological treatment of cardiovascular diseases is controversial (Blüher and Mantzoros 2015, Ouchi 2016).

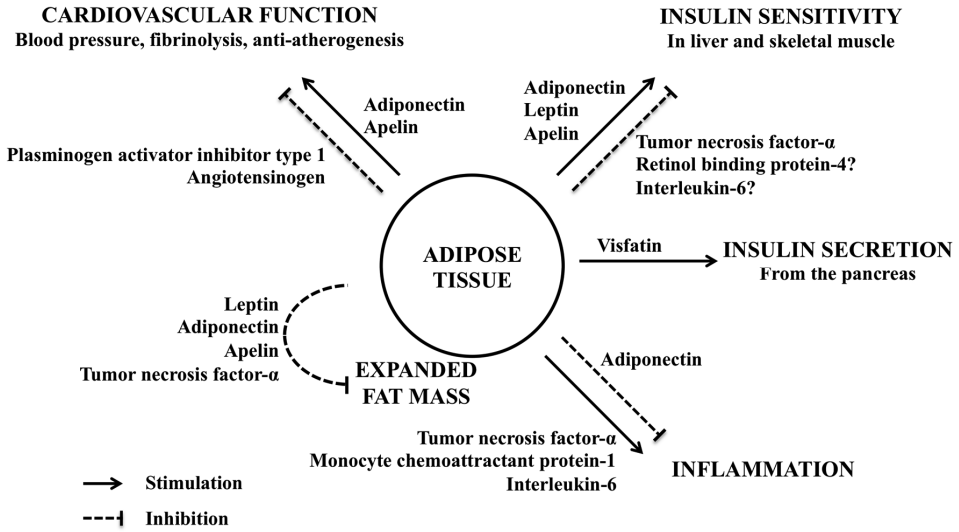

Figure 1. Adipokines involved in the pathogenesis of the metabolic syndrome (modified according to Maury and Brichard, 2010).

2.3.1 Adiponectin, leptin, and atherosclerosis in experimental and animal studies

Evidence from preclinical studies suggests that adiponectin is involved in many of the key events in the development of atherosclerosis and has cardioprotective effects. *In vitro*, physiological concentrations of adiponectin suppressed the transformation of human monocyte-derived macrophages to foam cells (Ouchi *et al.* 2001). Preincubation with human recombinant adiponectin inhibited tumor necrosis factor α -induced monocyte adhesion to human aortic endothelial cells and adhesion molecule expression (Ouchi *et al.* 1999). Treatment of human

umbilical vein and bovine aortic endothelial cell monolayers with the globular domain of adiponectin protected against angiotensin II and tumor necrosis factor α -induced hyperpermeability measured by transendothelial electric resistance and diffusion of albumin (Xu *et al.* 2008). Treatment with bacterially produced full-length adiponectin increased nitric oxide production in bovine aortic endothelial cells (Chen *et al.* 2003). Also, treatment with recombinant globular adiponectin suppressed oxidized LDL stimulated cell proliferation in bovine aortic endothelial cells and ameliorated the suppression of endothelial nitric oxide production (Motoshima *et al.* 2004).

In vivo, surgical induction of ischemia and reperfusion caused increased myocardial infarction and myocardial apoptosis in adiponectin-knockout (KO) mice compared with wild-type mice. Pretreatment of both wild-type and adiponectin-KO mice with adenoviral vectors expressing adiponectin significantly decreased infarct area after ischemia-reperfusion (Shibata *et al.* 2005). Similar difference between adiponectin-KO mice and wild-type mice has been shown also after cerebral ischemia and reperfusion, the actions of adiponectin being mediated through endothelial nitric oxide synthase (Nishimura *et al.* 2008). In apolipoprotein E (ApoE) KO mice, overexpression of adiponectin attenuated the progression of atherosclerotic lesion (Okamoto *et al.* 2002) and compared to ApoE KO mice, ApoE/adiponectin double-KO mice showed accelerated atherogenesis (Okamoto *et al.* 2008).

Leptin has been suggested to have many proatherosclerotic actions *in vitro*. Leptin has been reported to promote angiogenesis in human umbilical vein endothelial cells (Bouloumie *et al.* 1998) and in bovine capillary endothelial cells (Cao *et al.* 2001). Short form of the leptin receptor has been shown to be expressed in rat aortic vascular smooth muscle cells and leptin treatment to stimulate both proliferation and migration of the vascular smooth muscle cells (Oda *et al.* 2001). In human macrophages, leptin increases lipoprotein lipase secretion (Maingrette and Renier 2003). In human umbilical vein endothelial cells, leptin has been reported to induce oxidative stress that participates in the regulation of inflammatory processes in the vascular wall (Bouloumie *et al.* 1999).

In vivo, the data are more controversial. In rats, continuos infusion of leptin resulted in increased mean arterial blood pressure and elevated heart rate (Shek *et al.* 1998). In leptin-deficient *ob/ob* mice, the mean arterial pressure was significantly lower compared with lean wild-type mice, suggesting leptin might have a role in the regulation of obesity-related hypertension (Mark *et al.* 1999).

In studies on leptin-deficient *ob/ob* and leptin receptor-deficient *db/db* mice, even on atherogenic, high-fat diet, the mice did not develop pronounced arterial

lesions after induced carotid artery injury compared with wild-type mice. Daily administration of leptin in the *ob/ob* mice but not *db/db* mice increased the size of the atherosclerotic lesion (Schäfer *et al.* 2004). In concordance, intraperitoneal administration of leptin resulted in increased atherosclerosis and shortened time to occlusive thrombosis after vascular injury in ApoE-KO mice (Bodary *et al.* 2005). Compared with ApoE-KO mice, leptin/ApoE double-KO mice have decreased progression of fatty streaks to fibrous plaques (Chiba *et al.* 2008)

Leptin administration has also been reported to have antiatherogenic effecs. Three studies on obese, atherosclerosis prone, leptin deficient mouse models (Lloyd *et al.* 2008, Jun *et al.* 2012, Hoffmann *et al.* 2016) have reported that administration of leptin in the subphysiological to physiological range inhibits the progression of atherosclerosis and reduces atherosclerotic lesions. The antiatherogenic effects in these studies were explained by decrease in total cholesterol levels in plasma as well as improved glucose metabolism and increased levels of adiponectin.

The promotion of atherosclerosis with supraphysiological doses of leptin seen in the earlier studies (Schäfer *et al.* 2004, Bodary *et al.* 2005) compared to the decreased atherosclerosis seen with subphysiological to physiological doses (Lloyd *et al.* 2008, Jun *et al.* 2012, Hoffmann *et al.* 2016) suggests that the effect of leptin on atherogenesis may be dose-dependent.

2.3.2 Adiponectin, leptin, and cardiovascular risk factors including the metabolic syndrome

Circulating adiponectin levels are decreased in obesity (Arita *et al.* 1999), whereas leptin levels increase with obesity (Considine *et al.* 1996, Hassink *et al.* 1996). Both adiponectin and leptin levels are higher in females compared with males (Cnop *et al.* 2003).

In both males and females, leptin has been reported to correlate positively with total and LDL cholesterol and triglyceride levels, and negatively with HDL cholesterol (Reis *et al.* 2008) whereas adiponectin has been reported to correlate positively with HDL cholesterol and negatively with triglycerides in adolescents (Winer *et al.* 2006) and adults (Cnop *et al.* 2003).

Similarly, leptin levels have been reported to correlate positively with systolic and diastolic blood pressure (Reis *et al.* 2008), whereas a negative correlation has been reported between adiponectin and blood pressure (Ohashi *et al.* 2011).

Moreover, low adiponectin levels are associated with insulin resistance (Cnop *et al.* 2003) and increased risk of type 2 diabetes (Kadowaki *et al.* 2006, Li *et al.* 2009, Matsuzawa *et al.* 2011). Since the components of the MetS are elevated waist circumference, elevated triglycerides, reduced HDL cholesterol, elevated blood pressure and elevated fasting glucose, it is not surprising that low plasma adiponectin levels have been reported to associate with the presence of MetS (Santaniemi *et al.* 2006). Leptin levels have been associated with fasting insulin levels but not with insulin sensitivity in healthy children and adolescents (Arslanian *et al.* 1998). In lean and obese adults, insulin resistance correlated with intra-abdominal fat accumulation but not independently with leptin levels (Cnop *et al.* 2002).

In concordance with these associations, medication used to influence cardiovascular risk factors (thiazolidinediones for type 2 diabetes, statins for dyslipidemia and angiotensin II receptor blockers for hypertension) have been reported to increase adiponectin levels (Hossain *et al.* 2015). Moreover, statins have been reported to decrease leptin levels (Dubey and Hesong 2006).

In a large meta-analysis of genome-wide association studies, a risk score calculated for single nucleotide polymorphisms (SNPs) decreasing adiponectin levels was associated with increased risk of type 2 diabetes, increased triglycerides, increased waist-to-hip ratio, increased glucose two hours post oral glucose tolerance testing, increased fasting insulin, and with lower HDL cholesterol concentrations, and decreased BMI (Dastani *et al.* 2012). However, a large Mendelian randomization study using genetic variants of the ADIPOQ gene found no evidence of an association of adiponectin-lowering alleles with insulin sensitivity or type 2 diabetes (Yaghootkar *et al.* 2013).

2.3.3 Adiponectin, leptin, and inflammation

Many of the hundreds of substances secreted by adipose tissue are involved in inflammatory processes. These include cytokines such as tumor necrosis factor α, interleukin-1β and interleukin-6, acute phase proteins such as plasminogen activator inhibitor-1 and haptoglobin as well as inflammation-related proteins such as adiponectin and monocyte chemoattractant protein 1 (Trayhurn and Wood 2004). Obesity is associated with adipose tissue dysregulation resulting in increased production of proinflammatory substances and decreased production of anti-inflammatory substances (Maury and Brichard 2010).

In experimental studies, adiponectin has anti-inflammatory functions locally in the adipose tissue, where it inhibits tumor necrosis factor α production (Maeda *et*

al. 2002). In other tissues, adiponectin reduces the expression of cell adhesion molecules by attenuating the tumor necrosis factor α induced nuclear factor-κ B signaling in endothelial cells as well as suppresses tumor necrosis factor α secretion from macrophages (Ouchi and Walsh 2008). Impaired removal of apoptotic cells increases systemic inflammation (Savill *et al.* 2002). Adiponectin has been shown to promote phagocytosis of apoptotic cells by macrophages through binding to the cell surfaces of both macrophages and apoptotic cells (Takemura *et al.* 2007).

Evidence from experimental studies indicates a proinflammatory role for leptin. Leptin induces proliferation of human monocytes and production of cytokines tumor necrosis factor α and interleukin-6 *in vitro* (Santos-Alvarez *et al.* 1999), which could increase the production of CRP in the liver. The binding of leptin to the long form of leptin receptor activates pathways similar to those activated after cytokine administration (Frühbeck 2006). Leptin has also been shown to enhance the stimulation of human T lymphocytes (Martin-Romero *et al.* 2000). In addition, the genetic variants of the leptin receptor have been observed to associate with plasma interleukin-6 levels (Zhang *et al.* 2007).

In humans, plasma adiponectin levels have been reported to associate inversely with plasma CRP levels in obese children and adolescents (Winer *et al.* 2006) and obese postmenopausal women (Engeli *et al.* 2003). Leptin levels have been reported to associate positively with plasma CRP in young healthy males (Kazumi *et al.* 2003), middle-aged males and females (Shamsuzzaman *et al.* 2004), and elderly males and females (Ble *et al.* 2005).

2.3.4 Adiponectin, leptin, and surrogate markers of atherosclerosis in humans

The association between adiponectin and carotid IMT has been reported in all age groups. In pediatric populations, inverse correlation between adiponectin and IMT has been reported for obese adolescents (Pilz *et al.* 2005, Beauloye *et al.* 2007) and in the STRIP study in over 500 healthy adolescents (Jaakkola *et al.* 2015). Inverse associations between adiponectin and IMT have been reported in several middle-aged populations (Iglseder *et al.* 2005, Behre *et al.* 2006, Dullaart *et al.* 2007, Gardener *et al.* 2012). However, the relationship between adiponectin and IMT may be different in males and females. A Swedish study reported increased adiponectin levels to be associated with decreased IMT in middle-aged males but not in females (Nilsson *et al.* 2006). In line, results from a longitudinal international multicenter study showed an independent association with adiponectin and baseline mean bifurcation IMT and progression of mean carotid IMT in males but not in females. Accordingly, a gene score of adiponectin-

raising alleles was inversely associated with baseline mean bifurcation IMT in males (Persson *et al.* 2015). On the other hand, an inverse association between adiponectin and IMT has also been reported in a population of 100 healthy females aged 24-59 years (Lo *et al.* 2006). Additionally, an inverse association with baseline adiponectin and IMT has been reported in non-diabetic postmenopausal females, with age-adjusted adiponectin in the lowest quartile related to progression of IMT after 12 months (Störk *et al.* 2007).

In some studies, leptin levels were directly associated with IMT in females aged 18 to 75 years (Velarde *et al.* 2015). In a study among healthy males, leptin levels but not adiponectin levels were associated with IMT, and after adjusting for conventional cardiovascular risk factors, leptin:adiponectin ratio was an independent predictor of IMT (Norata *et al.* 2007). However, in some studies, leptin levels were not independently associated with IMT (Dullaart *et al.* 2007, Störk *et al.* 2007).

Low adiponectin has also been associated with attenuated endothelial function in some (Shimabukuro *et al.* 2003, Yilmaz *et al.* 2008), but not all (Singhal *et al.* 2005, Golledge *et al.* 2008) studies. In overweight adults, leptin levels have been reported to associate directly with brachial FMD (Morioka *et al.* 2014).

Some studies on adiponectin and arterial elasticity have suggested that hypoadiponectinemia may be associated with increased arterial stiffness measured by carotid artery distensibility (Störk *et al.* 2007) or by assessment of pulse wave velocity (Mahmud and Feely 2005, Tsioufis *et al.* 2007, Nguyen *et al.* 2008). In healthy adolescents, increased leptin levels have been independently associated with impaired brachial artery distensibility (Singhal *et al.* 2002).

2.3.5 Adiponectin, leptin, and clinical manifestations of cardiovascular disease

The data from the experimental studies as well as the associations of adiponectin and leptin with cardiovascular risk factors and surrogate markers of atherosclerosis suggest that adiponectin might have beneficial effects on cardiovascular disease whereas leptin might have negative effects. Therefore, the associations of adiponectin and leptin with clinical endpoints such as the prevalence of coronary artery disease and myocardial infarction have been studied in cross-sectional and prospective studies.

In middle-aged males, low plasma adiponectin levels have been reported to associate with prevalence of coronary artery disease (Kumada *et al.* 2003). In the

prospective Health Professionals Study, high adiponectin levels were independently associated with low risk of myocardial infarction in males (Pischon *et al.* 2004). Similarly, in the Framingham offspring Study, high adiponectin levels predicted lower risk of incident coronary heart disease in males, whereas in females the association was not significant after adjustment for all risk factors (Ai *et al.* 2011). However, in other prospective studies, adiponectin did not predict cardiovascular events (Lawlor *et al.* 2005, Lindsay *et al.* 2005). In a Finnish study, adiponectin levels did not predict cardiovascular events in middle-aged males and females after a median follow-up of 18 years (Santaniemi *et al.* 2014).

In 2013, three meta-analyses on the association of adiponectin with cardiovascular events were published. Hao et al. analyzed 17 prospective studies on the association of adiponectin with coronary heart disease and stroke (Hao *et al.* 2013). They found increased adiponectin to be related with an elevated risk of ischemic stroke, but no clear relationship between adiponectin and the risk of coronary heart disease. On the other hand, Zhang et al. included 12 prospective studies and found higher levels of adiponectin to associate with lower risk of coronary heart disease (Zhang *et al.* 2013). The meta-analysis by Kanhai et al. included 14 studies on coronary heart disease and 2 studies on stroke (Kanhai *et al.* 2013). In contrast to the previous meta-analyses, Kanhai et al. found no association between adiponectin and risk for future coronary heart disease or stroke events. Thus, although it has been more or less consistently reported that low adiponectin is associated with preclinical atherosclerosis in young populations, the connection between adiponectin levels and cardiovascular events in middle-aged and elderly populations remains controversial.

Taking into account the antiatherogenic and anti-inflammatory actions of adiponectin, the associations of adiponectin with all cause and cardiovascular mortality are somewhat puzzling. In a longitudinal Danish cohort study of cardiovascular disease and risk factors, high adiponectin levels were independently associated with all-cause mortality and with cardiovascular events despite positive association with a preferable risk factor profile (Lindberg *et al.* 2013). Also in Finnish adults with type 1 diabetes high adiponectin levels have been reported to associate with all-cause and cardiovascular mortality (Forsblom *et al.* 2011). Additionally, high adiponectin levels have been reported to associate with increased mortality in subjects with chronic heart failure (Kistorp *et al.* 2005) and in hemodialysis patients (Rhee *et al.* 2015). The mechanisms behind these associations are unknown. Adiponectin levels are markedly increased in subjects with end-stage renal disease possibly due to decreased renal clearance (Yahya *et al.* 2016). It is possible that the increase in adiponectin levels is a response to wasting. That is, increased energy expenditure and weight loss

associated with chronic heart failure and chronic renal failure (Kistorp *et al.* 2005, Rhee *et al.* 2015). The possibility of adiponectin resistance has also been speculated (Lindberg *et al.* 2013).

Increased plasma leptin levels have been associated with coronary artery calcification in middle-aged males and females with type 2 diabetes (Reilly *et al.* 2004) as well as progression of coronary artery calcification independent of other cardiovascular risk factors (Maahs *et al.* 2005). In middle-aged males participating in the West of Scotland Coronary Prevention Study, higher baseline leptin levels were associated with increased risk for incident coronary events (Wallace *et al.* 2001). However, in a recently published prospective cohort study with median follow-up of 7.6 years, leptin levels were not associated with incident cardiovascular events in males or females (mean age 64.5 years at baseline) (Martin *et al.* 2015). In a meta-analysis of eight case-control studies, Chai et al. found that although the association between leptin levels and coronary heart disease was not statistically significant, there was a need for larger, sex-specific prospective studies (Chai *et al.* 2014).

3 AIMS OF THE STUDY

The purpose of this study was to investigate the associations of adiponectin and leptin with cardiovascular risk factors and surrogate markers of atherosclerosis in Finnish children, adolescents and adults.

The specific aims of this thesis were to:

1. study serum adiponectin and leptin levels in Finnish children, adolescents and young adults;

2. study tracking of serum adiponectin and leptin levels from childhood to adulthood;

3. study the associations of serum adiponectin and leptin levels with cardiovascular risk factor levels including inflammation and MetS;

4. study the cross-sectional associations of serum adiponectin and leptin levels with carotid artery IMT, brachial artery FMD and carotid artery distensibility in adulthood;

5. study the longitudinal associations of serum adiponectin levels in childhood and adolescence with carotid artery IMT and brachial artery FMD in adulthood.

4 MATERIALS AND METHODS

4.1 The Cardiovascular Risk in Young Finns Study

The Cardiovascular Risk in Young Finns Study is an ongoing multicenter study on atherosclerosis precursors in Finnish children, adolescents and young adults. Originally, it was designed to study cardiovascular risk factor levels, differences between eastern and western Finns and rural and urban participants, changes in risk factor levels in childhood and adolescence, and tracking and clustering of risk factors (Åkerblom *et al.* 1985a).

Two pilot studies were performed in 1978 (n=264, age 8 years) and 1979 (n=634, age groups 3, 12 and 17 years) to validate the study methods. The first large cross-sectional survey was conducted in 1980 in all five university cities with medical schools in Finland (Helsinki, Kuopio, Oulu, Tampere and Turku) and in their rural vicinities. The subjects were randomly selected from the national register from the study areas. The total sample size was 4,320, of which 3,596 (83,2%) participated. Six birth cohorts with ages of 3, 6, 9, 12, 15 and 18 years at the time of inclusion into the study were formed. Follow-up studies of the cohorts were carried out in 1983, 1986, 1989, 1992, 2001, 2007, and 2010-2012 (Åkerblom *et al.* 1985b, Raitakari *et al.* 2003, Raitakari *et al.* 2008, Juonala *et al.* 2013) (**Figure 2**).

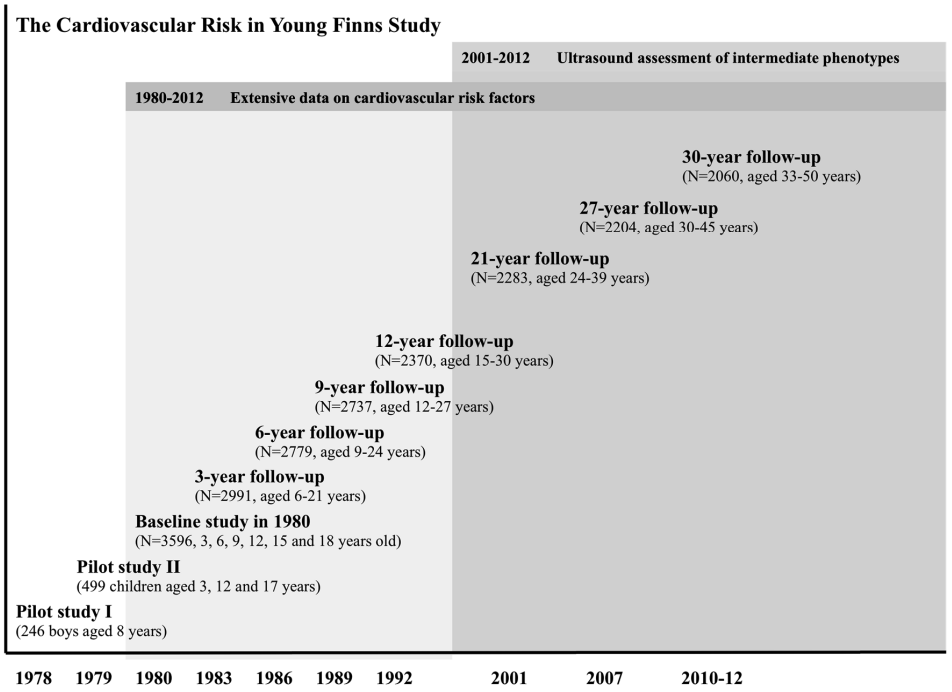

Figure 2. The design of the Cardiovascular Risk in Young Finns Study. (Modified from http://youngfinnsstudy.utu.fi/studydesign.html)

4.2 Study design and subjects

In total, 3,596 subjects participated in 1980, 2,283 in 2001 and 2,204 in 2007. 1,828 participants attended both 2001 and 2007 follow-up studies. Summary of the study design is shown in **Figure 3**.

In this thesis, serum adiponectin and leptin levels and age trends are shown for all subjects who had measurements made from serum samples taken in 1980 (n=1722 for adiponectin and n=264 for leptin), 2001 (n=2280 for adiponectin and n=2279 for leptin) and 2007 (n=2154 for adiponectin). Moreover, age- and sex-specific 21-year tracking of serum adiponectin levels from childhood to adulthood was analyzed in 1,715 subjects, 27-year tracking in 1,680 subjects and 6-year tracking in 1,786 subjects. Serum leptin levels in both childhood and adulthood were available from only 208 subjects. Therefore, tracking of serum leptin levels from childhood to adulthood was analyzed separately for females and males but not for different age groups.

The associations of serum adiponectin and leptin levels with conventional cardiovascular risk factors were examined both in childhood and adulthood (2001).

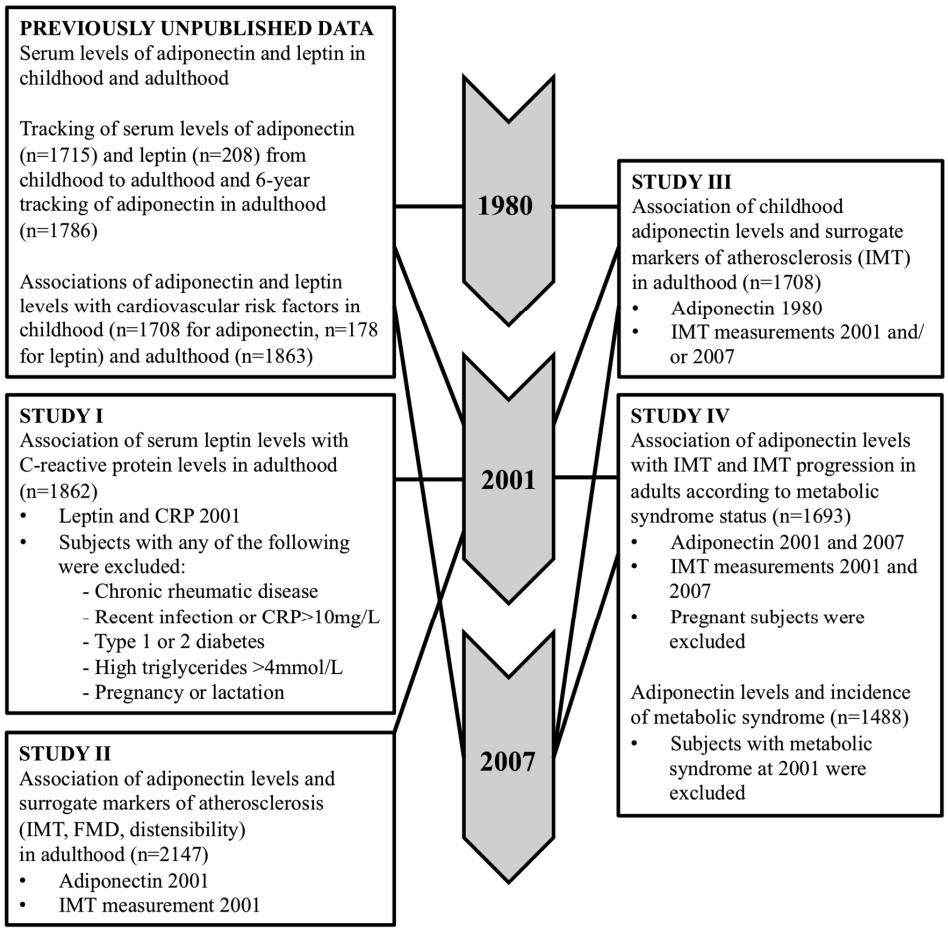

Figure 3. Description of substudies and their aims, numbers of participants and inclusion and exclusion criteria

Study I examined the association of serum leptin and CRP in adults aged 24-39 years. Since both leptin and CRP concentrations are increased in obesity, the aim was to study if the association was independent of the degree of obesity. To examine causality, the association of genetic causes of CRP elevation and leptin levels was analyzed. The study included 1,862 subjects (971 females and 891 males) from the 2001 follow-up. Genetic analyses were based on a sample of 1706 (889 females and 817 males) subjects with full data on five genetic variants of the CRP gene. Subjects with chronic rheumatic disease, history of recent infection, diabetes, as well as pregnant or lactating women were excluded from the main analyses. In addition, subjects with triglycerides above 4 mmol/L were excluded, because the Friedewald formula (Friedewald *et al.* 1972) could not be applied. The remaining subjects with serum CRP concentration above the limit of normal (10 mg/L) were also excluded, since the elevation of CRP above 10 mg/L

was considered more likely to be due to an acute cause or an undiagnosed disease, instead of low-grade chronic inflammation.

In this thesis, the association between serum adiponectin and serum CRP levels in adulthood (study year 2001) was analyzed in the study population of Study I. Moreover, differences in serum adiponectin, leptin and CRP levels in females using or not using oral contraceptives as well as in females with levonorgestrel-releasing intrauterine device were analyzed.

Study II was a cross-sectional study on the association of serum adiponectin levels with cardiovascular risk factors and surrogate markers of atherosclerosis. The results were based on the follow-up visit in 2001 when subjects were aged 24 to 39 years. Complete data were available on 2,147 subjects (1,174 females and 973 males) included in the study.

In Study III, the longitudinal association of serum adiponectin levels in childhood with carotid IMT in adulthood was examined. The study included those participants who had adiponectin measurements performed from stored baseline (1980) serum samples and participated in the carotid ultrasound studies in the 2001 and/or 2007 follow-ups. A total of 1,708 subjects (972 females and 736 males) were included in the analyses.

Study IV examined if serum adiponectin levels in adults predict incident MetS after six years of follow-up and the associations of adiponectin and carotid atherosclerosis according to MetS status. The study included those participants who had adiponectin data from the 2001 follow-up study and carotid ultrasound data from the 2001 and 2007 follow-ups and who were not pregnant. Therefore, a total of 1,693 subjects (915 females and 778 males) were included in the study. The analyses examining MetS incidence were based on 1,488 subjects (829 females and 659 males), as 205 participants with MetS at baseline were excluded.

4.3 Biochemical analyses

The blood samples were drawn after a 12-hour fast mostly between 7am and 11am. The basic analyses were performed in the same laboratory (the laboratory of the Rehabilitation Research Centre of the Social Insurance Institution, Turku, in 1980 and in its followers the laboratory of the Research and Development Unit of the Social Insurance Institution, Turku, in 2001 and the laboratory of the Population Research of the National Institute for Health and Welfare, Turku, in 2007). Adiponectin and leptin measurements were performed in the laboratory of

the Department of Pharmacology, Drug Development and Therapeutics, University of Turku, Turku. In 1980, insulin measurements were performed in the Research Laboratory of the Department of Pediatrics, University of Oulu, Oulu. CRP genotyping was performed in the laboratory of the Department of Clinical Chemistry, University of Tampere, Tampere, Finland.

4.3.1 Adiponectin and leptin measurements

Serum adiponectin and leptin concentrations were measured from serum samples taken in different study years. The samples analyzed, their storage and analysis year are shown in **Table 3**.

Serum adiponectin concentrations were analyzed with a radioimmunoassay (Human Adiponectin RIA kit, Linco Research, Inc, MO, USA). The interassay coefficient of variation was 5.5-11.9%.

Serum leptin concentrations were also analyzed with a radioimmunoassay (Human Leptin RIA kit, Linco Research, Inc, MO, USA). The interassay coefficient of variation was 7-9%.

Table 3. Sample year, storage temperature, and analysis year of the adiponectin and leptin measurements in the Cardiovascular Risk in Young Finns Study.

	Sample year	Stored in	Analysis year
Adiponectin	1980	-20°C	2013
	2001	-70°C	2005-2006
	2007	-70°C	2008-2009
Leptin	1980	-20°C	1999
	2001	-70°C	2002

4.3.2 Lipid measurements

In 1980, serum total cholesterol and triglyceride concentrations were measured enzymatically with a clinical chemistry analyzer (OLLI 3000; Olli Ltd, Kivenlahti, Finland) and HDL cholesterol concentrations from the serum supernatant after precipitation of very low density lipoproteins and LDL cholesterol with dextran sulphate 500 000 (Porkka *et al.* 1994).

In 2001, serum cholesterol and triglyceride concentrations were also determined enzymatically with a clinical chemistry analyzer (AU400; Olympus Optical Ltd, Mishima, Japan) and HDL cholesterol was analyzed after precipitation of very

low density lipoproteins and LDL cholesterol with dextrane sulphate 500 000. Apolipoprotein B was analyzed immunoturbidometrically (Orion Diagnostica, Espoo, Finland).

LDL cholesterol was calculated using the Friedewald formula for participants with triglycerides <4 mmol/l. Non-HDL cholesterol was calculated as total cholesterol – HDL cholesterol.

Due to changes in determination methods and reagents, lipid levels from 1980 and triglycerides from 2007 were corrected to correspond to the samples taken in 2001 using correction factor equations determined with linear regression analysis using standardized principal components adjustments (Juonala *et al.* 2004).

Total Cholesterol = 1.091 * total cholesterol 1980 - 0.271 mmol/L

HDL cholesterol = 1.068 * HDL cholesterol 1980 - 0.0277 mmol/L

Triglycerides = 1.00756 * triglycerides 1980 + 0.0582 mmol/L

Triglycerides = (triglycerides 2007 + 0.03226) / 0.9811

4.3.3 Insulin and glucose measurements

Childhood serum insulin concentrations were measured with immunoassay (Herbert *et al.* 1965). In 2001 and 2007, plasma glucose concentrations were analyzed enzymatically with a clinical chemistry analyzer (AU400; Olympus Optical Ltd, Mishima, Japan), and serum insulin concentrations were measured by microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot).

Due to changes in methods or reagents from 2001 to 2007, glucose and insulin levels were corrected by using the following correction factor equations.

Glucose = (glucose 2007 - 0.0235) / 0.9471

Insulin = insulin 2007 * 1.3728 -0.8795

Homeostasis model assessment (HOMA) was used to assess beta-cell function (HOMA-B, (fasting serum insulin mU/L * 20) / (fasting plasma glucose mmol/L - 3.49)) and insulin resistance (HOMA-IR, (fasting serum insulin mU/L * fasting plasma glucose mmol/L / 22.5)) (Matthews *et al.* 1985).

4.3.4 C-reactive protein measurements

Serum CRP concentrations (study year 2001) were analyzed by a high-sensitive latex turbidometric immunoassay (Wako Chemicals GmbH, Neuss, Germany). The detection limit of the assay was 0.06 mg/L, and the coefficient of variation of repeated measurements was 3.3%. In 2005, serum samples taken in 1980 and stored at -20°C were analyzed using the same method as in 2001.

4.4 Physical examination and questionnaires

During each visit, height and weight were measured and BMI (weight/height^2) was calculated. In adulthood, waist circumference was measured midway between the lowest rib at the mid-axillary line and iliac crest. Hip circumference was measured at the greater trochanter and waist to hip ratio calculated.

Systolic and diastolic blood pressures were measured in sitting position after 5 minutes' rest. Blood pressure was measured with a standard mercury sphygmomanometer in 1980 and with a random-zero sphygmomanometer (Hawksley & Sons Ltd, Lancin, UK) in 2001 and 2007. In 1980, blood pressure from 3-year-old children was measured with an ultrasound device (Arteriosonde 1020, Roche, Branchburg, New Jersey, USA). The average of 3 measurements was used in statistical analyses.

The subjects filled in a questionnaire on smoking habits (in 1980 among those aged 12 years or older), physical activity during leisure time and commuting to work, medication, and family history of ischemic heart disease.

In Studies I and II, subjects were classified as non-smokers if they had never smoked; otherwise they were classified as smokers in the analyses. In studies III and IV, daily smokers were classified as smokers. Pack-years was calculated as years of smoking*cigarettes per day/20.

A metabolic equivalent index for physical activity was calculated from frequency*intensity*time spent for physical activity during spare time and commuting to work. The coefficients for the variables were estimated from existing tables (Ainsworth *et al.* 1993), where one MET is the consumption of one kcal of a person per weight kilogram per hour in rest.

Family history for ischemic heart disease was considered positive if the subject's mother or father had before the age of 55 years either suffered from a myocardial infarction, had a coronary angioplasty or coronary bypass surgery.

Use of medication including oral contraceptives and levonorgestrel-releasing intrauterine device was inquired.

4.5 C-reactive protein genotyping

Five SNPs in the CRP gene [CRP-717A>G (rs 2794521); CRP-286C>T>A (rs3091244); CRP+1059G>C (rs1800947); CRP+1444T>C (rs1130864); and CRP+1846G>A (rs1205)] were genotyped using the ABI Prism 7900HT Sequence Detection System for both PCR and allelic discrimination (Applied Biosystems, Foster City, CA). For SNP CRP+1059G>C a commercial kit from Applied Biosystem was used (Assay On Demand, C_177490_10 CRP). The other SNPs were genotyped using Assays By Design from Applied Biosystems under standard conditions, with the exception of the triallelic tagSNP (Carlson *et al.* 2005) that was genotyped as described previously, except for the genotype calling which was done manually from the PCR run component tab.

4.6 Metabolic syndrome

The presence of MetS in 2001 and 2007 was determined by the harmonized definition for MetS (Alberti *et al.* 2009), see **Table 4**. In Study II, subjects with diabetes (6 females and 6 males with type 1 diabetes, 1 male with type 2 diabetes) were excluded from the analysis for MetS whereas in study IV, subjects with type 2 diabetes were included in the group with MetS at baseline. In Study IV, those with prevalent MetS or MetS component at 2007 follow-up but not in 2001 were defined as having incident MetS or incident MetS component (abdominal obesity, high triglycerides, low HDL cholesterol, hypertension, hyperglycemia). Participants in the reference group had no MetS at baseline or at follow-up.

Table 4. The harmonized definition of metabolic syndrome (MetS).

Risk factors (at least three required for MetS)	Males	Females
Elevated waist circumference	≥102 cm	≥88
Raised triglycerides (or treatment for it)	>1.7 mmol/l	>1.7 mmol/l
Reduced HDL cholesterol (or treatment for it)	<1.0 mmol/l	<1.3 mmol/l
Raised blood pressure (or treatment for it)		
- Systolic blood pressure	≥130 mmHg	≥130 mmHg
- Diastolic blood pressure	≥85 mmHg	≥85 mmHg
Raised fasting plasma glucose (or treatment for it)	≥5.6 mmol/l	≥5.6 mmol/l

4.7 Ultrasound studies

At each study centre, carotid ultrasound studies were performed with Sequoia 512 ultrasound mainframes (Acuson, CA, USA) using a 13.0-MHz, linear-array transducer.

To derive mean and maximum carotid IMT, the image was focused on the posterior (far) wall of the left common carotid artery. At least three measurements were taken 10 mm proximal to the bifurcation. The between-visit coefficient of variation of IMT measurements was 6.4% and the intra-observer coefficient of variation was 3.4%.

Carotid IMT was also measured from the carotid artery bifurcation (bulb) and evident plaque lesions were documented, defined as distinct area of the carotid vessel wall protruding into the lumen >50% of the adjacent intima-media layer. All plaques were observed in the carotid bulb. In study III, as a marker of pre-clinical atherosclerosis, a binary outcome variable was defined as carotid IMT≥95th percentile at the carotid artery bifurcation or plaque evident in carotid scans either in 2001 or 2007. The digitally stored scans were manually analyzed by one reader blinded to subjects' details.

In Study II, the mean carotid IMT in 2001 was used as the outcome variable. In Study III, the mean value of maximal carotid IMT levels in 2001 and 2007 was used as a continuous outcome variable. In study IV, the mean carotid IMT in 2001 and 2007 as well as the progression of mean IMT were used as outcome variables.

To assess brachial FMD, the left brachial artery diameter was measured both at rest and during reactive hyperemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 minutes, followed by release. Three measurements of arterial diameter were performed at end-diastole at a fixed distance from an anatomic marker at rest and at 40, 60 and 80 seconds after cuff release. The vessel diameter in scans after reactive hyperemia was expressed as the percentage relative to the resting scan. The average of three measurements at each time point was used to derive the maximum FMD (the greatest value between 40 and 80 seconds). All ultrasound scans were analyzed by a single reader blinded to the subject's details. The between-visit coefficient of variation was 3.2% for brachial artery diameter and 26.0% for FMD.

To assess carotid artery elasticity indices, the best quality cardiac cycle was selected from the carotid ultrasound images. The common carotid diameter 10 mm proximal to the bifurcation was measured at least twice at end-diastole and

end-systole and the means were calculated. Ultrasound and concomitant brachial blood pressure measurements were used to calculate the carotid artery distensibility (referred to as carotid artery compliance (CAC) in Study II) as ([systolic diameter - diastolic diameter] / diastolic diameter) / (systolic blood pressure - diastolic blood pressure). Distensibility measures the ability of the arteries to expand as a response to pulse pressure caused by cardiac contraction and relaxation. The between-visit coefficient of variation was 2.7% for diastolic carotid diameter and 16.3% for carotid artery distensibility.

4.8 Statistical analyses

Data are presented as mean±standard deviation (SD), mean±standard error (SE) or median (interquartile range). Comparisons between the groups were performed using t-test or χ^2-test as appropriate. Due to deviation from normal distribution, nonparametric Wilcoxon rank-sum test was used for comparisons of serum adiponectin, leptin, insulin, triglyceride and CRP concentrations and metabolic equivalent index, HOMA-IR and HOMA-B between the sexes (Studies I and II) as well as between females using or not using oral contraceptives. Differences between the sexes were further analyzed with analysis of covariance adjusting for BMI and waist-hip ratio (Study I). Pairwise correlations of adiponectin and leptin with other parameters were evaluated with Spearman's rank order and Pearson correlation coefficients. Due to skewed distributions, logarithmic transformations were used for adiponectin, leptin, CRP, insulin, triglycerides, metabolic equivalent index, HOMA-IR and HOMA-B in Pearson correlation analyses.

Due to previously reported changes in levels of both adiponectin and leptin as well as cardiovascular risk factors during childhood and adolescence, partial correlation coefficients adjusted for age were calculated for the correlations between adipokines and other risk factors in 1980.

Spearman's correlation coefficients were calculated to estimate the tracking of serum adiponectin levels from childhood to adulthood separately for females and males as well as each age group. The analysis was done separately for females and males owing to difference in adiponectin levels between sexes and previous reports of sex-specific associations between adiponectin and cardiovascular disease (Pischon *et al.* 2004, Ai *et al.* 2011). 6-year tracking in adulthood was also analyzed using this approach. 21-year tracking of leptin levels from childhood to adulthood was also analyzed. However, due to small sample size the analysis was performed separately for females and males but not for different age groups. Partial correlation coefficients adjusted for age were calculated.

Differences in correlation coefficients for adiponectin between females and males were tested using normal probability test for difference between Z-transformed correlation coefficients.

In Study I, multiple regression analyses were performed to identify variables contributing significantly to the variation of CRP concentration in serum. Serum leptin, insulin, CRP and triglyceride levels were logarithmically transformed prior to the analysis. In addition, age, BMI, waist circumference, serum LDL cholesterol and HDL cholesterol, systolic blood pressure, diastolic blood pressure and smoking status were included in the model. Stepwise multiple regression analyses were performed to analyse the most significant variables contributing to the variation of CRP concentration. First, all variables were entered into the model simultaneously. In each following step, the variable having the least significant p-value was excluded from the model. Finally, all variables with $p \leq 0.15$ remained in the model. Standardized beta estimate was used to determine which variable had the strongest effect on serum CRP concentration. The analyses were performed separately for males and females and for females using or not using oral contraceptives. Additionally, the relationship between adiponectin and CRP levels in adulthood was analyzed by calculating Spearman's correlation coefficients and by adding adiponectin to the final multivariable regression models of Study I.

Instrumental variable methods were used to test for reverse causality between CRP and leptin levels (Greenland 2000). According to this approach, functional genetic variants in the CRP gene (eg. haplotypes) represent a good instrument for CRP levels that is largely free from confounding and reverse causation bias. If there is a reverse causality between CRP and leptin (CRP → leptin), then the instrument should be related to leptin levels to the extent predicted by the magnitude of its association with circulating CRP levels. In this analysis, the two-stage age- and sex-adjusted least squares to fit the instrumental variables models included an examination of F-statistics from the first-stage regressions to evaluate the strength of the instrument (values greater than 10 indicate sufficient strength to ensure the validity of instrumental variable methods), and a comparison of the instrumental variable estimates to those from ordinary linear regression using the Durbin form of the Durbin-Wu-Hausman statistic (main analyses). In agreement with previous studies, a model that assumes each of a participant's two haplotypes contributes additively to his/her value of CRP was used. This approach excluded subjects with rare haplotypes (prevalence <1%) (n=51) and expressed associations per doubling of the CRP level.

In Study II, variables contributing significantly to the variation of mean common carotid IMT, carotid artery distensibility or FMD were analyzed using

multivariable regression analyses. Due to skewed distributions, adiponectin, leptin, CRP, insulin and triglyceride concentrations were logarithmically transformed prior to analysis. Variables that correlated significantly with IMT, distensibility, or FMD in univariable analyses were included as independent determinants in the multivariable models.

In Study III, multivariable modeling was used to study if childhood adiponectin is an independent determinant of IMT in adulthood. The model was adjusted for age, sex, childhood non-HDL cholesterol, BMI, and systolic blood pressure. Logistic regression modeling was used with the binary outcome variable. Interaction terms of age*adiponectin and sex*adiponectin were included in the model to test if the association between adiponectin and IMT was modified by age or sex. Since interaction term p values were >0.6, the analyses were performed sexes combined. The incremental value of adding risk variables to predict the binary outcome (plaque and/or high bulb IMT) was examined based on multivariable logistic regression models. The ability of several models to predict binary IMT risk was estimated using C statistics by calculating the area under the receiver operating characteristic curve (AUC) and the integrated discrimination index. Model calibration was tested by the Hosmer-Lemeshow X^2 test (Pencina *et al.* 2008). Linear regression analysis was used with the continuous IMT outcome variable. The distribution of childhood adiponectin levels was close to normal and therefore, the analyses were performed without logarithmic transformations.

In Study IV, odds ratios (OR) of incident MetS or MetS components according to baseline serum adiponectin levels were assessed using multivariable logistic regression models. First, an unadjusted univariable analysis was performed. Then the model was adjusted for age and sex and additionally for baseline levels of BMI, serum LDL cholesterol, leptin, insulin levels and CRP level, smoking and family history of coronary artery disease. In the final model, additional adjustment for waist circumference (in place of BMI), serum HDL cholesterol, and triglycerides, systolic blood pressure, and plasma glucose was performed. To test if adiponectin levels had a similar influence on IMT in those with or without MetS, correlation coefficients between adiponectin and IMT were calculated in both groups. Then, linear regression models were constructed to test for adiponectin*MetS interaction. These models included age, sex, family history of coronary artery disease, smoking, MetS, serum adiponectin, leptin, insulin, CRP, triglycerides, LDL cholesterol and HDL cholesterol levels, waist circumference, systolic blood pressure, plasma glucose levels and adiponectin*MetS interaction term as the independent variables explaining IMT. To examine if sex modifies the associations between adiponectin and MetS/IMT, a sex*adiponectin interaction term was included in the regression models. As no significant

interactions were observed in these analyses, the results are shown combined for sex. Values for adiponectin, triglycerides, and CRP were logarithmically transformed due to skewness. However, to ease the interpretation of the results, OR values are shown for non-transformed adiponectin values.

Statistical analyses were performed with SAS System, versions 8.2, 9.1, 9.2 and 9.4 (SAS Institute Inc., Cary, NC, USA) and statistical significance was inferred at a 2-tailed P-value <0.05. Genetic analyses were performed with instrumental variable regression analysis in Stata, version 8.0 (Stata Institute, Texas, USA).

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. The participants provided informed consent in 2001 and 2007 and their parents provided it in 1980.

5 RESULTS

5.1 Characteristics of participants

Participant characteristics in studies I, II and IV are shown in the original publications (Study I, Table 1; Study II, Table I; Study IV, Table 1). Participant characteristics in Study III at baseline are shown here (**Table 5**).

Table 5. Characteristics of participants in Study III at baseline 1980.

	All n=1708	Females n=972	Males n=736	P for difference
Age (years)	11.0±4.9	11.1±4.9	11.0±4.9	0.8
Age groups 3 ,6 ,9 ,12 ,15 ,18 yrs	(2.8-18.9)	(2.8-18.9)	(2.8-18.8)	
Adiponectin (µg/mL)	13.2±5.4	13.9±5.2	12.3±5.6	<0.0001
Leptin (ng/mL)	4.4±2.8 (n=178)	5.4±3.0 (n=92)	3.3±2.1 (n=86)	<0.0001
Body mass index (kg/m$^{2)}$	18.0±3.1)	17.9±3.0	18.0±3.2	0.4
Systolic blood pressure (mmHg)	113±12	112±11	114±13	0.0003
Total cholesterol (mmol/L)	5.3±0.9	5.4±0.9	5.2±0.9	<0.0001
Non-HDL cholesterol (mmol/L)	3.7±0.8	3.8±0.8	3.7±0.8	0.0002
Insulin (mU/L)	9.9±6.0	10.5±6.2	9.1±5.6	<0.0001
Smoking in childhood				
12 year-olds	0.3% (1/337)	0% (0/194)	0.7% (1/143)	0.4
15 year-olds	8.2% (27/329)	9.0% (17/189)	7.0% (10/141)	0.6
18 year-olds	24.5% (70/286)	19.5% (32/164)	31.1% (38/122)	0.03

Data are mean±SD for continuous variables or percent (n/N) for categorical variables.

5.2 Tracking of adiponectin and leptin levels

Serum adiponectin and leptin levels in childhood and adulthood for all subjects and separately for females and males are shown in **Table 6**. Females had higher adiponectin and leptin levels in all study years, p<0.0001 in all comparisons. Adiponectin and leptin levels in different sex and age groups are shown in **Table 7**. In childhood and adolescence, adiponectin concentrations decreased with age in both females and males, p for trend <0.0001, whereas leptin levels increased with age in females (p for trend <0.0001). In males, leptin levels tended to decrease, but this was not statistically significant (p for trend 0.11) (**Figure 4**).

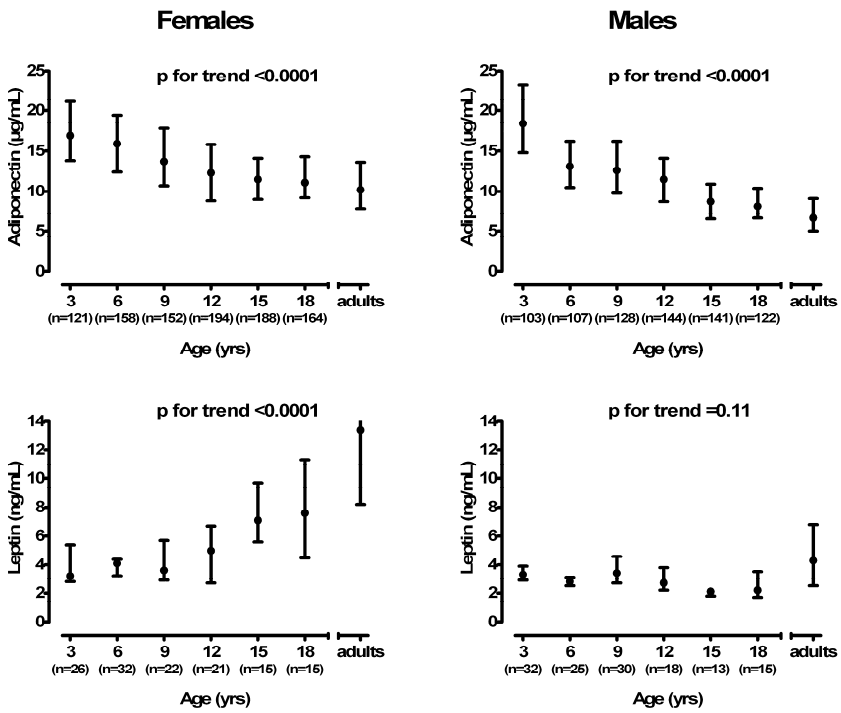

Figure 4. Serum adiponectin and leptin levels (median±interquartile range) according to age. Values for age groups 3-18 years are from study year 1980 and adult values from study year 2001 (age 24-39 years).

In adulthood, there was no age trend for adiponectin levels in males whereas there was a statistically significant decreasing trend in females in 2001, p=0.005 but not in 2007. Leptin levels seemed to increase with age in males in 2001, p=0.0004, whereas there was no age trend in females.

Childhood adiponectin levels correlated with adiponectin levels in adulthood (1980 vs 2001, r=0.53 for all subjects, p<0.0001, n=1715; 1980 vs 2007, r=0.51, p<0.0001, n=1680). 6-year tracking correlation in adulthood was r=0.77, p<0.0001, n=1786. Among 12- to 18-year-olds 21- and 27-year tracking was essentially similar among boys and girls and among different age cohorts. Among younger age groups, 27-year tracking was significantly better among girls (age-adjusted r-values 0.57 for girls vs 0.41 for boys, P=0.004) (**Table 8**).

Leptin levels in 1980 correlated with leptin levels in 2001 (r=0.50, p<0.0001, n=208 for all subjects; r=0.28, p=0.004, n=107 for females; r=0.29, p=0.004, n=101 for males). The sample size was too small to study correlations separately for different age groups. The age-adjusted correlation coefficient was 0.21 for females, p=0.03, and 0.35 for males, p=0.0004.

Table 6. Serum adiponectin (μg/mL) and leptin (ng/mL) levels median (interquartile range) according to study year.

	All	Females	Males	P for difference
Adiponectin 1980	12.4 (9.2-16.3) n=1,722	13.2 (10.0-16.9) n=977	11.4 (8.4-15.1) n=745	<0.0001
Adiponectin 2001	8.6 (6.1-11.8) n=2,280	10.2 (7.8-13.6) n=1,255	6.6 (5.0-9.1) n=1,025	<0.0001
Adiponectin 2007	8.9 (6.4-13.0) n=2,154	11.3 (8.3-15.0) n=1,183	7.0 (5.1-9.2) n=971	<0.0001
Leptin 1980	3.3 (2.6-5.0) n=264	4.3 (3.1-6.6) n=131	2.9 (2.2-3.7) n=133	<0.0001
Leptin 2001	8.1 (4.2-14.9) n=2,279	13.4 (8.2-20.5) n=1,255	4.3 (2.5-6.8) n=1,024	<0.0001

Table 7. Serum adiponectin (μg/mL) and leptin (ng/mL) levels median (interquartile range) according to study year, sex and age.

	Adiponectin 1980		Adiponectin 2001		Adiponectin 2007		Leptin 1980		Leptin 2001	
	Age		Age		Age		Age		Age	
Females	3	16.9 (13.8-21.1) n=121	24	10.7 (8.1-13.9) n=176	30	11.6 (8.5-14.8) n=168	3	3.2 (2.8-5.4) n=26	24	13.0 (7.9-21.3) n=176
	6	15.9 (12.4-19.4) n=158	27	10.6 (8.1-15.1) n=215	33	11.6 (8.9-15.2) n=203	6	4.1 (3.2-4.4) n=32	27	13.8 (8.4-19.8) n=215
	9	13.7 (10.7-17.8) n=152	30	9.7 (7.4-13.3) n=206	36	10.8 (7.7-15.6) n=187	9	3.6 (2.9-5.7) n=22	30	13.6 (8.8-22.8) n=206
	12	12.3 (8.8-15.8) n=194	33	10.3 (7.8-13.7) n=236	39	10.8 (7.5-14.7) n=232	12	5.0 (2.7-6.7) n=21	33	13.8 (8.6-20.9) n=236
	15	11.5 (9.0-14.1) n=188	36	9.7 (7.4-12.8) n=219	42	11.3 (8.0-15.5) n=210	15	7.1 (5.6-9.7) n=15	36	13.1 (7.8-20.7) n=219
	18	11.1 (9.2-14.3) n=164	39	10.0 (7.9-13.2) n=203	45	11.5 (8.9-14.5) n=183	18	7.6 (4.5-11.3) n=15	39	12.2 (7.6-18.3) n=203
Males	3	18.4 (14.8-23.2) n=103	24	6.9 (5.4-9.8) n=156	30	7.2 (5.3-8.6) n=159	3	3.3 (2.9-3.9) n=32	24	3.1 (1.9-5.5) n=156
	6	13.1 (10.5-16.2) n=107	27	7.0 (5.4-9.1) n=151	33	7.1 (4.9-9.9) n=145	6	2.8 (2.5-3.1) n=25	27	3.9 (2.4-6.3) n=150
	9	12.6 (9.8-16.2) n=128	30	6.8 (5.2-9.4) n=191	36	7.2 (5.5-9.4) n=172	9	3.4 (2.7-4.6) n=30	30	4.5 (2.6-7.9) n=191
	12	11.5 (8.7-14.1) n=144	33	6.6 (5.0-9.0) n=177	39	6.7 (5.2-9.3) n=172	12	2.7 (2.2-3.8) n=18	33	4.5 (3.0-7.0) n=177
	15	8.7 (6.5-10.9) n=141	36	6.1 (4.5-8.7) n=184	42	6.8 (4.6-8.8) n=172	15	2.1 (1.8-2.3) n=13	36	4.5 (2.7-7.0) n=184
	18	8.1 (6.6-10.4) n=122	39	6.8 (5.2-9.0) n=166	45	7.0 (5.2-9.7) n=151	18	2.2 (1.7-3.5) n=15	39	4.7 (2.7-7.3) n=166

Table 8. Sex- and age-specific Spearman's correlation coefficients for 21- , 27- and 6-year tracking of serum adiponectin levels.

Sex	Age in 1980	21-year tracking 1980-2001	27-year tracking 1980-2007	6-year tracking 2001-2007
Females	3	0.57	0.59	0.70
		n=121	n=118	n=130
	6	0.59	0.53	0.70
		n=158	n=156	n=169
	9	0.54	0.62	0.71
		n=149	n=147	n=153
	12	0.58	0.59	0.73
		n=194	n=192	n=202
	15	0.61	0.65	0.73
		n=188	n=179	n=184
	18	0.64	0.66	0.70
		n=164	n=158	n=163
	All	0.56	0.56	0.72
		n=974	n=950	n=1,001
Males	3	0.46	0.43	0.69
		n=102	n=102	n=113
	6	0.49	0.45	0.61
		n=106	n=106	n=111
	9	0.44	0.40	0.72
		n=128	n=124	n=141
	12	0.55	0.63	0.73
		n=143	n=142	n=147
	15	0.62	0.61	0.76
		n=140	n=135	n=142
	18	0.60	0.57	0.68
		n=122	n=121	n=131
	All	0.46	0.43	0.70
		n=741	n=730	n=785

5.3 Adiponectin, leptin, and cardiovascular risk factors

Because levels of serum adiponectin and leptin change during childhood and adolescence, the correlations of adiponectin and leptin with other risk factors in childhood were age-adjusted.

The age-adjusted associations of adiponectin and leptin levels with cardiovascular risk factors in childhood and adolescence (1980) are shown in **Table 9** for all subjects and in **Table 10** separately for females and males. In childhood, adiponectin levels correlated positively only with total and non-HDL cholesterol. When sexes were analyzed separately, the association was statistically significant only in females. Leptin levels correlated positively with obesity, systolic blood pressure, triglycerides, insulin, and adiponectin. When analyzed separately, the correlations of leptin levels with systolic blood pressure and insulin remained significant only in females. In males, there was a negative correlation of leptin levels with LDL cholesterol.

The associations of adiponectin and leptin levels with cardiovascular risk factors in adulthood (2001) are shown in **Table 11** for all subjects and in **Table 12** separately for males and females. In adulthood, serum adiponectin levels correlated positively with HDL cholesterol and negatively with obesity indices, blood pressure, total and non-HDL cholesterol, triglycerides and insulin levels. These correlations were consistent across both sexes. In both females and males, leptin levels were positively associated with obesity indices, CRP, blood pressure, total and non-HDL cholesterol, triglycerides and insulin, and negatively associated with HDL cholesterol and adiponectin levels. When all subjects were analyzed together, associations between leptin levels and waist to hip ratio as well as systolic blood pressure became negative and the association between leptin levels with HDL cholesterol and adiponectin levels became positive owing to differences in the levels of these risk factors between sexes.

Table 9. Age-adjusted associations of serum adiponectin and leptin levels with cardiovascular risk factors in childhood and adolescence (1980).

Variable	Adiponectin n=1708	p	Leptin n=178	p
Height	-0.06	0.47	-0.15	0.04
Weight	-0.06	0.42	0.20	0.007
BMI	-0.007	0.92	0.41	<0.0001
Systolic blood pressure	0.01	0.86	0.16	0.04
Total cholesterol	0.17	0.03	-0.00	0.99
HDL cholesterol	0.26	0.0004	0.14	0.06
LDL cholesterol	0.06	0.42	-0.09	0.24
Non-HDL cholesterol	0.06	0.39	-0.05	0.49
Triglycerides	0.01	0.92	0.16	0.04
Insulin	-0.06	0.42	0.37	<0.0001
Adiponectin			0.27	0.0002

Table 10. Sex-specific age-adjusted associations of serum adiponectin and leptin levels with cardiovascular risk factors in childhood and adolescence (1980).

	Adiponectin Females n=972		Adiponectin Males n=736		Leptin Females n=92		Leptin Males n=86	
Variable	r	p	r	p	r	p	r	p
Height	0.18	0.08	-0.13	0.22	0.004	0.97	0.06	0.57
Weight	0.03	0.76	-0.09	0.40	0.46	<0.0001	0.29	0.007
BMI	-0.01	0.90	-0.03	0.77	0.62	<0.0001	0.33	0.002
Systolic blood pressure	0.02	0.82	0.02	0.87	0.21	<0.05	0.11	0.32
Total cholesterol	0.27	0.01	0.07	0.50	0.07	0.52	-0.20	0.06
HDL cholesterol	0.33	0.002	0.17	0.13	0.13	0.20	0.11	0.34
LDL cholesterol	0.12	0.23	0.00	0.99	-0.02	0.88	-0.26	0.02
Non-HDL cholesterol	0.12	0.26	0.01	0.92	0.02	0.88	-0.26	0.02
Triglycerides	-0.04	0.68	-0.03	0.76	0.19	0.07	-0.11	0.32
Insulin	-0.11	0.32	-0.10	0.36	0.50	<0.0001	0.13	0.23
Adiponectin					0.17	0.10	0.18	0.10

Table 11. Associations of serum adiponectin and leptin levels with cardiovascular risk factors in adulthood (2001).

Variable	Adiponectin n=1863	p	Leptin n=1863	p
CRP	-0.06	0.005	0.42	<0.0001
Age	-0.06	0.006	0.04	0.1
BMI	-0.33	<0.0001	0.39	<0.0001
Waist	-0.46	<0.0001	0.13	<0.0001
Hip	-0.21	<0.0001	0.49	<0.0001
Waist to hip ratio	-0.50	<0.0001	**-0.19**	<0.0001
Systolic blood pressure	-0.25	<0.0001	**-0.05**	0.03
Diastolic blood pressure	-0.18	<0.0001	0.07	0.004
Total cholesterol	-0.05	0.02	0.12	<0.0001
HDL cholesterol	0.48	<0.0001	**0.10**	<0.0001
LDL cholesterol	-0.13	<0.0001	0.03	0.1
Non-HDL cholesterol	-0.21	<0.0001	0.08	0.0008
Triglycerides	-0.28	<0.0001	0.16	<0.0001
Insulin	-0.22	<0.0001	0.46	<0.0001
Adiponectin			**0.18**	<0.0001

The bolded correlations are conflicting when males and females are analyzed together compared to analyzing separately.

Table 12. Sex-specific associations of serum adiponectin and leptin levels with cardiovascular risk factors in adulthood (2001)

	Adiponectin Females n=971		Adiponectin Males n=892		Leptin Females n=971		Leptin Males n=892	
Variable	r	p	r	p	r	p	r	p
CRP	-0.14	<0.0001	-0.12	0.0004	0.47	<0.0001	0.46	<0.0001
Age	-0.10	0.001	-0.06	0.1	-0.03	0.4	0.14	<0.0001
BMI	-0.29	<0.0001	-0.25	<0.0001	0.73	<0.0001	0.72	<0.0001
Waist	-0.36	<0.0001	-0.24	<0.0001	0.69	<0.0001	0.74	<0.0001
Hip	-0.22	<0.0001	-0.17	<0.0001	0.73	<0.0001	0.69	<0.0001
Waist to hip ratio	-0.34	<0.0001	-0.23	<0.0001	**0.37**	<0.0001	**0.52**	<0.0001
Systolic blood pressure	-0.10	0.003	-0.12	0.0003	**0.22**	<0.0001	**0.25**	<0.0001
Diastolic blood pressure	-0.08	0.01	-0.14	<0.0001	0.21	<0.0001	0.30	<0.0001
Total cholesterol	-0.01	0.9	0.01	0.8	0.22	<0.0001	0.29	<0.0001
HDL cholesterol	0.38	<0.0001	0.38	<0.0001	**-0.14**	<0.0001	**-0.24**	<0.0001
LDL cholesterol	-0.09	0.005	-0.02	0.5	0.19	<0.0001	0.23	<0.0001
Non-HDL cholesterol	-0.13	<0.0001	-0.10	0.002	0.27	<0.0001	0.35	<0.0001
Triglycerides	-0.19	<0.0001	-0.24	<0.0001	0.36	<0.0001	0.48	<0.0001
Insulin	-0.25	<0.0001	-0.26	<0.0001	0.59	<0.0001	0.60	<0.0001

The bolded correlations are conflicting when males and females are analyzed together compared to analyzing separately.

5.4 Adiponectin, leptin, and inflammation

In univariable correlation analysis, serum CRP levels were associated with leptin ($r=0.47$, $p<0.0001$ for women; $r=0.46$, $p<0.0001$ for men) and with BMI ($r=0.41$, $p<0.0001$ for women; $r=0.44$, $p<0.0001$ for men). There was a weak inverse correlation between CRP and adiponectin levels ($r=-0.14$, $p<0.0001$ for females; $r=-0.12$, $p<0.0001$ for males). Due to previously reported influence of oral contraceptive use on CRP levels, scatterplots for ln(CRP) according to ln(leptin) are shown separately for females using or not using oral contraceptives, as well as for males in **Figure 5**.

There was a strong positive correlation between BMI and leptin ($r=0.72$, $p<0.0001$ for both women and men). Partial correlation coefficients for leptin and CRP adjusted for age and BMI were $r=0.25$, $p<0.0001$ for women and $r=0.23$, $p<0.0001$ for men. A graphic evaluation of mean CRP levels stratified by tertiles of leptin and BMI suggests a linear association between leptin and CRP independent of obesity (study I, Figure 1).

Twenty-seven percent (n=262) of females used oral contraceptives (OC) and 11 percent (n=103) had a levonorgestrel-releasing intrauterine device. There were no differences in median concentrations of CRP, adiponectin or leptin between females with or without levonorgestrel-releasing intrauterine device and therefore they were analyzed as one group (n=709). Median concentrations of serum CRP were significantly higher in females using oral contraceptives compared with non-users (1.64 (0.12-9.75) vs. 0.55 (0.05-9.90) mg/L, respectively, $p<0.0001$). Median concentrations of serum adiponectin were also significantly higher in females using oral contraceptives compared with non-users (11.6 (9.1-15.2) vs. 9.9 (7.8-13.4) μg/mL, respectively, $p<0.0001$). Contraceptive use did not affect serum leptin concentrations (12.85 (2.1-52.4) vs. 12.5 (1.5-63.3) ng/mL, respectively, $p=0.5$) (**Figure 6**).

In 1980, one 15-year-old female and 19 females in the oldest age group used oral contraceptives. Among those aged 18-years currently using oral contraceptives, CRP concentration was available from 16 subjects, adiponectin concentration from 12 subjects and leptin concentration from only one subject. In females using oral contraceptives, adiponectin levels were similar compared to non-users (11.8 (9.6-14.3) vs. 11.1 (9.1-14.3) μg/mL, respectively, $p=0.7$) whereas CRP levels were higher (1.06 (0.68-2.51) vs. 0.25 (0.14-0.63) mg/L, respectively, $p<0.0001$).

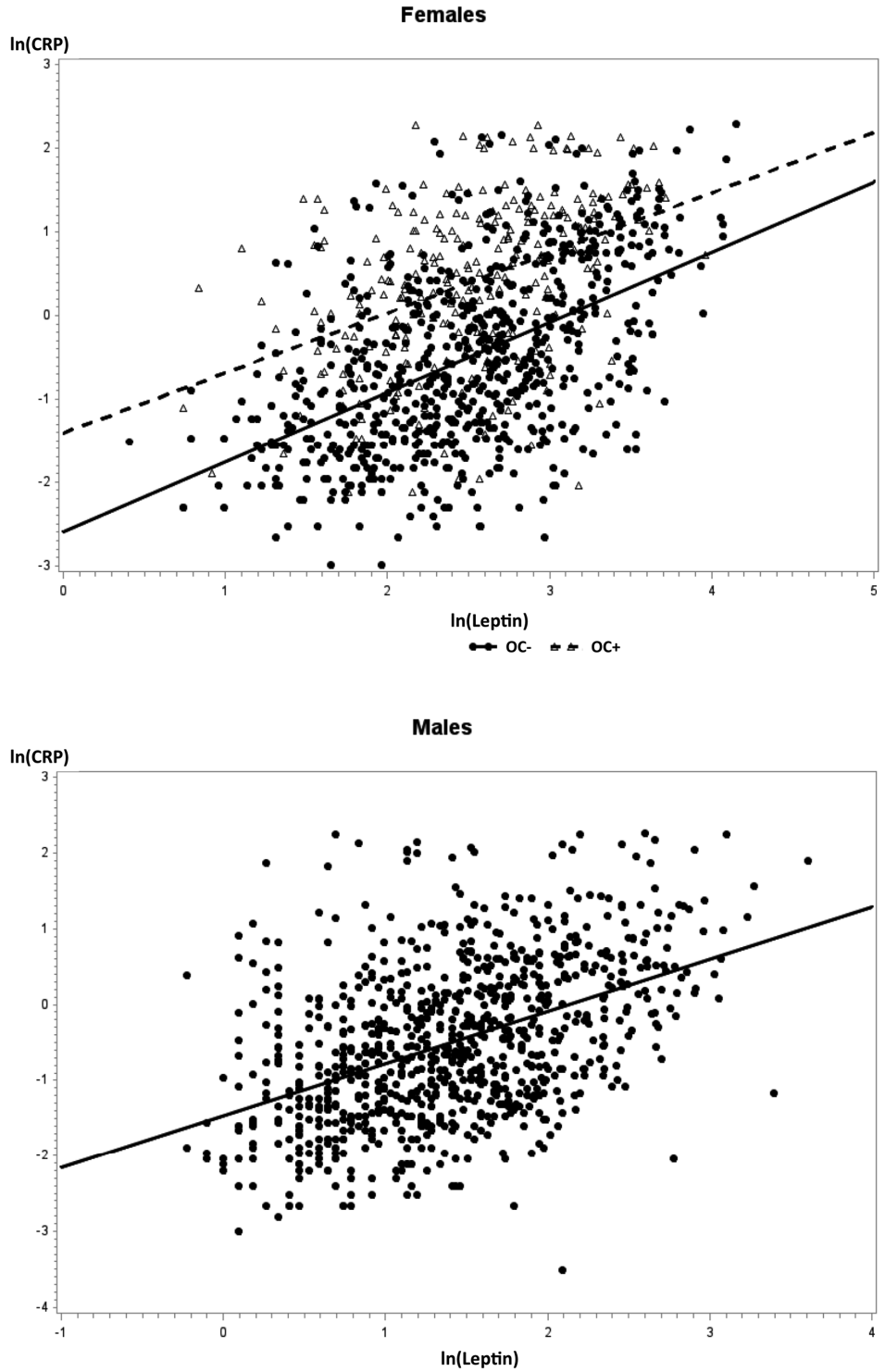

Figure 5. Scatterplots of ln(serum CRP) according to ln(serum leptin) in females and males. Female subjects are grouped by oral contraceptive (OC) use.

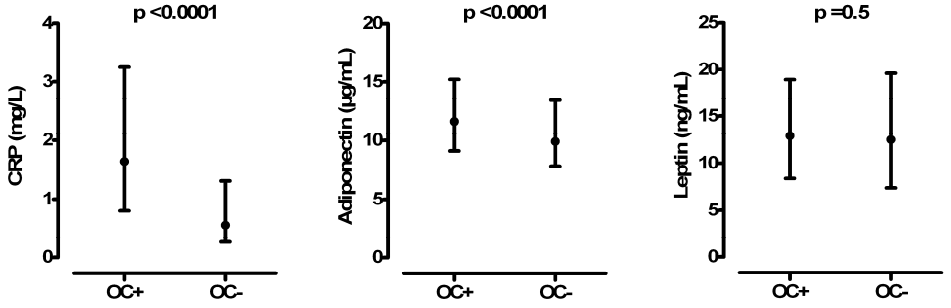

Figure 6. Serum CRP, adiponectin and leptin levels in adulthood according to oral contraceptive (OC) use (OC+ n=262, OC- n=709).

To analyze if the association between CRP and leptin levels was independent, a multiple regression analysis was performed separately for females and males. In this analysis, leptin was independently associated with CRP after adjustment for age, BMI, waist circumference, insulin, triglycerides, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, smoking status, and oral contraceptive use in females. In the stepwise multiple regression analysis, leptin was the main correlate of CRP in males, and the second most important correlate of CRP in females ($p<0.0001$ for both males and females) after oral contraceptive use (study I, Table 3). When the analysis was repeated separately for females using or not using oral contraceptives, leptin was the main determinant of CRP before BMI and triglycerides. Adding adiponectin to the final multiple regression analysis of Study I did not change the association between leptin and CRP levels (**Table 13**), whereas the weak association between adiponectin and CRP remained significant for females but not for males.

In males, there was an unexpected association between high CRP and low insulin level in the multivariable analysis. The relationship between CRP and insulin was positive as expected when insulin, BMI, and various other variables were included in the model. The relationship became negative when leptin was entered into the model, suggesting a strong correlation between leptin and insulin ($r=0.61$, $p<0.0001$).

To assure that the association between leptin and CRP was not secondary to obesity, the multivariable analysis was repeated in normal weight individuals with BMI from 18.5 to 25 kg/m^2 (n=435 females not using oral contraceptives, n=185 females using oral contraceptives, n=431 males). Leptin remained the main determinant of CRP in all groups. In addition, leptin was the main determinant of CRP in analyses using pack-years or number of cigarettes smoked per day in 2001 instead of smoking status.

Table 13. Variables explaining ln(serum CRP) in sex-specific multivariable models.

Variable	Beta±SE	Standardized Beta	P-value
Females (n=971)			
Use of oral contraceptives	0.90±0.07	0.36	<0.0001
ln(leptin)	0.48±0.06	0.27	<0.0001
BMI	0.05±0.01	0.21	<0.0001
ln(triglycerides)	0.25±0.08	0.09	0.001
ln(adiponectin)	-0.18±0.07	-0.07	0.01
Model R^2=39%			
Males (n=892)			
ln(leptin)	0.49±0.07	0.32	<0.0001
Waist circumference	0.02±.004	0.23	<0.0001
Smoking status (1=no, 2=yes)	0.22±0.06	0.11	0.0003
ln(insulin)	-0.17±0.07	-0.09	0.02
HDL cholesterol	-0.26±0.12	-0.07	0.04
ln(adiponectin)	-0.04±0.08	-0.02	0.6
Model R^2=26%			

In the genetic analysis (n=1655) to study causality, F-statistics suggested sufficient strength for the haplotype instrument [F(df=4, 1648)=12.05, p<0.0001]. There was a statistically significant difference in the magnitude of the age- and sex-adjusted association between CRP and leptin obtained from the ordinary least squares regression analysis and the instrumental variables analysis (p=0.001). The coefficient per doubling of CRP level was 0.19±0.01 (p<0.0001) in the first analysis and 0.02±0.06 (p=0.76) in the latter. The observed null association between the haplotype instrument for CRP levels and leptin suggests that CRP was not a causal agent for leptin.

5.5 Adiponectin levels and incidence of metabolic syndrome

Adiponectin participates in the regulation of insulin sensitivity. To study if adiponectin levels predict the incidence of MetS, a multivariable logistic regression analysis was performed. Adiponectin levels in 2001 were inversely associated with the incidence of MetS from 2001 to 2007 (OR 0.86 (CI 0.83-0.90), p<0.0001). This association remained significant after adjustment with age, sex, MetS components, CRP, leptin, insulin, smoking and family history of coronary artery disease (OR 0.94 (CI 0.90-0.99), p=0.04). In the analyses performed separately for each MetS component, adiponectin levels in 2001 were associated with the incidence of hypertension, hyperglycemia, hypertriglyceridemia, and low HDL cholesterol. In the fully adjusted models, adiponectin levels were significantly associated with 6-year incidence of hyperglycemia (Study IV, Tables 2 and 3).

5.6 Adiponectin, leptin, and vascular ultrasound findings

5.6.1 Cross-sectional and longitudinal relationships in adulthood

In adulthood (study II), serum adiponectin levels correlated inversely with carotid IMT ($r=-0.16$, $p<0.0001$), and directly with FMD ($r=0.12$, $p<0.0001$) and carotid artery distensibility ($r=0.20$, $p<0.0001$). There was no correlation between serum leptin levels and IMT or carotid artery distensibility. Leptin levels correlated directly with FMD ($r=0.22$, $p<0.0001$).

To further analyze if the association between adiponectin levels with IMT was independent or explained by other risk factors, a multivariable regression analysis adjusting for age, sex, BMI, CRP, total cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, glucose, and smoking status was performed. The association between adiponectin levels and IMT remained significant (Study II, Table III). The association remained statistically significant when the analysis was repeated using waist circumference, waist-to-hip ratio, or both waist and hip circumferences instead of BMI; LDL cholesterol instead of total cholesterol; HOMA indices instead of glucose; and pack-years instead of smoking status. The results of the multivariable model remained similar also when MetS was included in the model. In addition, performing the multivariable analysis separately for sexes did not affect the independent association of adiponectin levels with IMT. There was no sex*adiponectin interaction for IMT ($p=0.57$).

To analyze if the association between adiponectin levels and FMD was independent, multivariable regression adjusting for sex, leptin, CRP, BMI, systolic blood pressure, HDL cholesterol, and insulin was performed. The association between adiponectin levels and FMD remained significant (Study II, Table IV). The association remained statistically significant also when the analysis was repeated using waist circumference, waist-to-hip ratio, or both waist and hip circumferences instead of BMI and using HOMA indices instead of insulin. Performing the multivariable analysis separately for sexes showed that the association of adiponectin levels with FMD was similar between sexes. There was no sex*adiponectin interaction for FMD ($p=0.73$). The association between leptin levels and FMD remained significant in a multivariable analysis adjusting for sex and BMI ($p=0.04$), but attenuated to non-significant when adiponectin was included in the model.

In **Figure 7**, IMT and FMD are shown according to adiponectin quintiles. The graphic evaluation supports a linear relationship between adiponectin levels and IMT as well as between adiponectin and FMD.

Adiponectin levels did not remain independently associated with carotid artery distensibility in multivariable regression models that adjusted for conventional risk variables and sex. Using stepwise modeling, the relationship between adiponectin and carotid artery distensibility attenuated to non-significant after simultaneously adjusting for waist circumference and systolic blood pressure.

In Study IV, adiponectin levels in 2001 were cross-sectionally associated with carotid IMT in 2001 and 2007, both in those with and without MetS in 2001 ($p<0.01$). Baseline adiponectin levels were not associated with IMT progression ($r=-0.04$, $P=0.11$) whereas the 6-year change in adiponectin levels correlated inversely with IMT progression ($r=-0.07$, $p=0.002$).

A significant adiponectin*MetS interaction was observed when IMT in 2001 (r=-0.11 (Mets(-)) vs. r=-0.17 (MetS(+)), p for interaction 0.047) and IMT in 2007 (r=-0.12 (Mets(-)) vs. r=-0.21(MetS(+)), p for interaction 0.005) were used as outcomes (Study IV, Figures 1 and 2). This suggests the inverse association between adiponectin and IMT is stronger among those with MetS. All models were adjusted for age, sex, BMI, LDL cholesterol, CRP, leptin, insulin, smoking, HDL cholesterol, waist circumference, triglycerides, systolic blood pressure, glucose and family history of coronary artery disease.

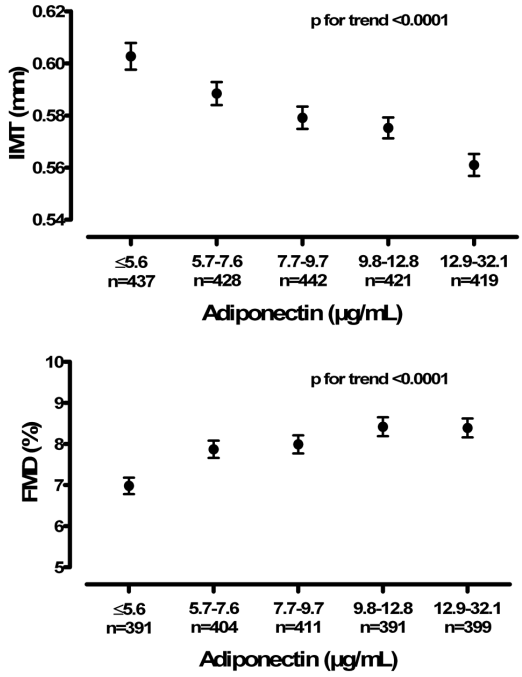

Figure 7. Levels of IMT and FMD (mean±SE) according to serum adiponectin quintiles in adulthood.

5.6.2 Longitudinal relationship from childhood to adulthood

In study III, adiponectin levels in childhood were inversely associated with preclinical atherosclerosis defined as carotid IMT≥95th percentile at the bifurcation and/or plaque lesion in 2001 or 2007 (n=160). The age and sex adjusted OR was 0.70 (confidence interval, CI 0.55-0.88, p=0.003) for one SD increase in childhood adiponectin level. This relationship remained similar (OR 0.68, CI 0.53-0.86, p=0.001) after adjustment for childhood BMI, systolic blood pressure and non-HDL cholesterol levels (**Figure 8**). The result remained similar when additionally adjusted for insulin levels in childhood, early life smoking and adiponectin levels in adulthood. No evidence was found for either age or sex interactions (interaction term p-values >0.6) suggesting that the association between adiponectin and adult IMT was not modified by age or sex. The result remained similar also when sex and age specific adiponectin quartiles were used in the analysis instead of adiponectin level.

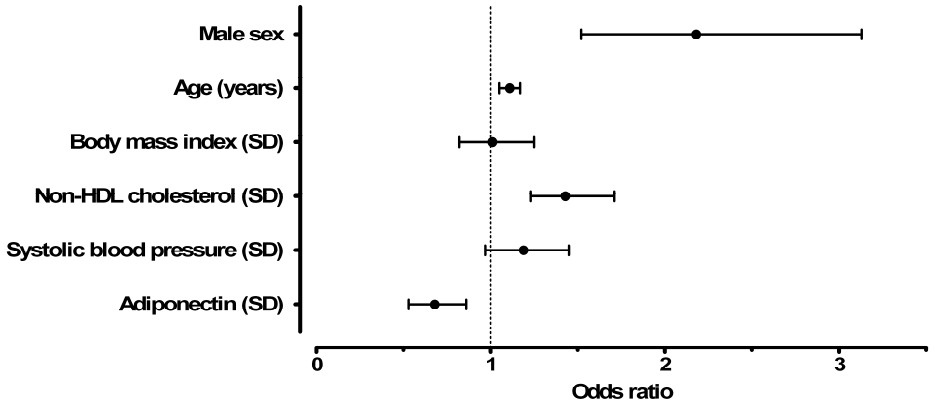

Figure 8. Multivariable odds ratios and their 95% confidence intervals for associations between childhood risk factors (in 1980) and carotid plaque and/or carotid bulb IMT≥95th percentile in adulthood.

When examining the incremental predictive utility, the model that additionally included childhood adiponectin levels showed a significantly improved AUC from 0.733 (95% CI 0.694-0.771) to 0.748 (95% CI 0.710-0.786), p=0.02 compared with an approach using only conventional risk factors (**Figure 9**). Similarly, the integrated discrimination index improved significantly (p<0.0001). The Hosmer-Lemeshow goodness of fit test p-value was non-significant for both models (p>0.4) indicating that there was no evidence for poor fit.

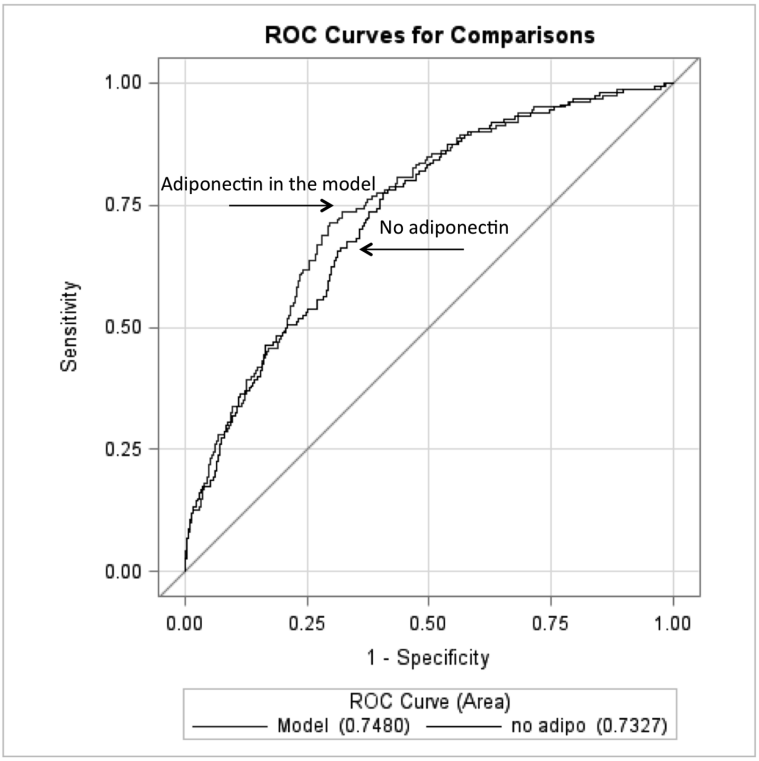

Figure 9. The incremental predictive ability of a model including serum adiponectin levels in addition to conventional childhood risk factors in predicting carotid plaque and/or carotid bulb IMT ≥95th percentile in adulthood.

In subgroups according to adiponectin level below or above sex and age specific median in childhood and/or adulthood, the percentage of subjects with plaque and/or high bulb IMT was highest in those subjects with adiponectin level below median in both childhood and adulthood (Study III, Figure 1).

In bivariate analyses childhood adiponectin levels were significantly associated with continuous adult IMT (beta=-3.9, p<0.0001). The association reduced, but remained statistically significant after adjustment for age and sex (beta=-1.1, p=0.009). In multivariable analyses adjusted for age, sex and conventional childhood risk factors, the effect of childhood adiponectin was not further reduced and remained statistically significant (**Table 14**). The result remained similar when sex and age specific adiponectin quartiles were used in the analysis instead of adiponectin level. The result remained similar when additionally adjusted for insulin levels in childhood and childhood smoking. The association became non-significant when additionally adjusted for adiponectin levels in adulthood (adiponectin 2001 in the model, beta=-0.19 for childhood adiponectin, p=0.70, beta=-1.98 for adulthood adiponectin, p=0.0009; adiponectin 2007 in the

model, beta=-0.40 for childhood adiponectin, p=0.41, beta=-1.26 for adulthood adiponectin, p=0.01).

Childhood leptin levels were not associated with either the binary carotid bifurcation IMT or the continuous common carotid IMT variable.

Table 14. Multivariable associations between childhood risk factors and adult carotid IMT.

	Model 1		Model 2		Model 3	
Variable	Beta±SE	P-value	Beta±SE	P-value	Beta±SE	P-value
Adiponectin (μg/mL)	-3.9±0.4	<0.0001	-1.1±0.4	0.009	-1.1±0.4	<0.01
Male sex			26.1±4.1	<0.0001	24.6±4.1	<0.0001
Age (yrs)			5.9±0.5	<0.0001	3.7±0.6	<0.0001
Body mass index (kg/m^2)					2.7±0.9	
Non-HDL cholesterol (mmol/L)					7.1±2.4	0.003
Systolic blood pressure (mmHg)					1.0±0.2	<0.0001
Model R^2	5.7%		15.3%		18.1%	

Beta = change in IMT (μm) for one unit change in explanatory variable.

6 DISCUSSION

6.1 Subjects

The subjects in this thesis are participants of the Cardiovascular Risk in Young Finns Study started in 1980. The original design of the study was planned to provide information of risk factor levels in prepubertal and peripubertal children as well as postpubertal adolescents, both in males and females. The baseline study and the follow-up studies at regular intervals have created a setting for both cross-sectional and longitudinal analyses from childhood to adulthood.

Loss to follow-up is a problem in all epidemiological studies. The original participation rate of the invited subjects in 1980 was 83.2% and the final sample was concluded to be representative of the invited sample (Åkerblom *et al.* 1985b). In 2001, 63.5% of the original study cohort participated in the study. The participants were more often females and older than those who dropped out, but there were no differences in baseline risk factor levels between the participants and the non-participants (Juonala *et al.* 2004). In 2007, the participation rate was 61.3%, and the BMI in female participants was slightly higher at baseline compared to non-participants (Juonala *et al.* 2010).

The participation rate in the Cardiovascular Risk in Young Finns Study has been good compared with other longitudinal cohort studies. For example in the Muscatine Study, of the subjects examined at school-age, 67% of the eligible subjects participated in the re-examination at the age of 20-30 years (Lauer *et al.* 1988). In the Minneapolis Children's Blood Pressure Study, 46% of the invited sample of young school children participated in the longitudinal follow-up and 56% of those subjects participated in the study at mean age of 23 years (Sinaiko *et al.* 1999).

The wide range of data from anthropometric measurements, questionnaires on lifestyle factors, measurements of risk factor levels, and ultrasonic evaluation of subclinical atherosclerosis to storage of blood samples for additional analyses has provided many possibilities for comprehensive evaluation of childhood risk factors and cardiovascular disease later in life. In recent years, analyses of genetic variants have further widened the possibilities of the study.

6.2 Blood samples

For these studies, measurements of serum leptin and adiponectin concentrations were performed in the laboratory of the Department of Pharmacology and Clinical Pharmacology, University of Turku, Turku. All the analyses were performed by experienced laboratory technicians with commercial radioimmunoassay kits from the same manufacturer (Linco Research, Inc, MO, USA).

Serum samples from the baseline study were stored in -20°C, whereas the serum samples from follow-up studies in 2001 and 2007 were stored in -70°C. Leptin concentrations from the baseline samples were measured from 264 subjects already in 1999. From the 2001 study samples, leptin concentrations were measured in 2003-2004 and adiponectin concentrations in 2005-2006. Adiponectin concentrations were measured in 2008-2009 from the 2007 study samples and in 2013 from the stored baseline (1980) samples.

Baseline samples had been stored for 19 years at -20°C prior to childhood leptin measurement, and for 33 years prior to childhood adiponectin measurement. During long-term storage, protein levels may have been reduced as a result of proteolysis and aggregation. However, the leptin levels in the 3- and 6-year-old groups were very similar to those reported earlier in 2- and 5-year-old children participating in the STRIP study (Hakanen *et al.* 2004). Also, the leptin levels in the peripubertal children aged 9-12 years correspond to those reported from a Spanish population (Garcia-Mayor *et al.* 1997). Similarly, the adiponectin levels during puberty are in concordance with data from Denmark (Andersen *et al.* 2007) and the STRIP study (Jaakkola *et al.* 2015). Moreover, the adiponectin levels from 1980 in the older age groups are very similar to those of the younger age groups in 2001. Therefore, although the stability of leptin and adiponectin in serum samples stored long-term is unknown, it seems unlikely that the results would have been substantially influenced by sample degradation.

The interassay coefficients of variation were 7-9% for leptin and 5.5-11.9% for adiponectin, which are in concordance with those supplied by the manufacturer.

6.3 Results

6.3.1 Tracking of adiponectin and leptin levels

Serum adiponectin levels decreased during childhood and adolescence in both males and females, which is in concordance with adiponectin levels reported for 200 German children aged 8-18 years (Böttner *et al.* 2004) and those reported for 859 Danish children and adolescents aged 6-20 years (Andersen *et al.* 2007). In young adults aged 24-45 years, adiponectin levels were higher in females compared with males. There was a small decreasing trend in adult females in 2001 but not in 2007. There was no age trend for adiponectin levels in adult males. The level difference between sexes was seen in late puberty among 15 to 18 year-olds. A similar sex difference in puberty was observed in the German and Danish studies as well as in the STRIP study (Böttner *et al.* 2004, Andersen *et al.* 2007, Jaakkola *et al.* 2015). In the German study by Böttner et al., the decrease of adiponectin levels in boys during puberty was associated with increasing levels of testosterone and dehydroepiandrosterone sulphate.

Tracking of adiponectin levels from childhood to adulthood was relatively strong (r=0.51 in females, r=0.46 in males). It was similar to previously reported tracking correlations for total, HDL and LDL cholesterol levels (Webber *et al.* 1991, Porkka *et al.* 1994, Juhola *et al.* 2011) and higher than previously reported tracking of blood pressure and triglycerides (Juhola *et al.* 2011). In adulthood, the 6-year tracking of adiponectin was very strong (r=0.72 in females, r=0.70 in males). In part, strong tracking may be explained by hereditability of adiponectin levels observed in twins and family studies (Dastani *et al.* 2012).

Serum leptin levels increased in females during childhood and adolescence in concordance with leptin levels reported for 343 Spanish girls aged 5-15 years (Garcia-Mayor *et al.* 1997) and were further increased in young adults. In young adults aged 24-39 years, leptin levels remained stable in females. In 446 Spanish boys aged 5-15 years, leptin levels have been reported to decrease during puberty (Garcia-Mayor *et al.* 1997). In the current study, there was a decreasing trend in leptin levels during childhood and adolescence, but it was not statistically significant. This could be due to small sample size. In a previous study, testosterone levels were inversely associated with leptin levels in elderly men, and administration of testosterone enanthate to healthy young men suppressed serum leptin levels (Luukkaa *et al.* 1998). Also in the Spanish study, a decrease in leptin levels in males was preceded by a raise in

testosterone levels (Garcia-Mayor *et al.* 1997). In the Young Finns cohort, testosterone levels have not been measured and therefore the association between testosterone and leptin levels could not be studied. In adult males aged 24-39 years, leptin levels increased with age in concordance with increasing BMI.

Tracking of leptin levels from childhood to adulthood was relatively strong (r=0.50) when males and females were analyzed together, but only moderate (r=0.28 for females, r=0.29 for males) when the sexes were analyzed separately. Plotting the tracking correlations separately for males and females in the same graph demonstrates that males and females are two separate populations in this context and should not be analyzed together **(Figure 10**).

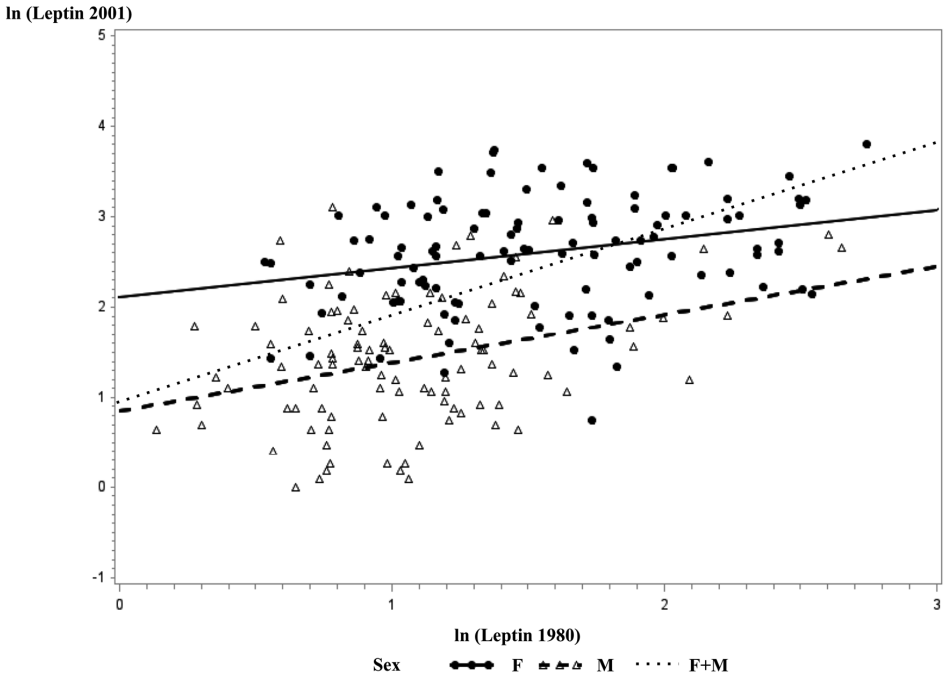

Figure 10. Correlation of serum leptin levels in childhood (1980) and in adulthood (2001) in females (F) and males (M).

6.3.2 Adiponectin, leptin and cardiovascular risk factor levels including metabolic syndrome

In adulthood, serum adiponectin levels correlated negatively with obesity markers such as BMI and waist-to-hip ratio, fasting insulin level and serum triglycerides in both males and females. Weaker negative correlations were seen

with systolic and diastolic blood pressure as well as serum non-HDL cholesterol. Adiponectin levels correlated positively with serum HDL cholesterol levels. These correlations are in line with other reports (Cnop *et al.* 2003, Iglseder *et al.* 2005, Kojima *et al.* 2005).

Adiponectin levels decrease markedly during childhood, and therefore correlations of childhood adiponectin levels with risk factor levels were adjusted for age. After the adjustment, adiponectin levels correlated positively only with total and HDL cholesterol levels and only in females. This is not surprising as other risk factor levels also change in their own distinctive patterns during childhood. For example, insulin levels increase from infancy until late puberty and decrease toward adulthood (Grant 1967, Rönnemaa *et al.* 1991). Total cholesterol levels increase in childhood until puberty, when a temporary decrease is seen, and thereafter increase again toward adult levels (Viikari *et al.* 1991). The positive correlation between adiponectin and HDL cholesterol levels is in concordance with a study on adolescents (Rubin *et al.* 2011).

In analyses performed separately for males and females, serum leptin levels correlated positively with obesity indices and insulin, non-HDL cholesterol and triglyceride levels. Negative correlations were seen between leptin levels and adiponectin as well as HDL cholesterol levels. However, when analyzed together, the correlations of leptin levels with adiponectin and HDL cholesterol levels turned positive and those with systolic blood pressure levels and waist-to-hip ratio negative. As with the tracking correlations of leptin levels from childhood to adulthood, plotting the correlations separately for males and females in the same graphs demonstrated again that males and females need to be analyzed as separate populations when examining the associations of leptin levels (**Figures 11** to **14**).

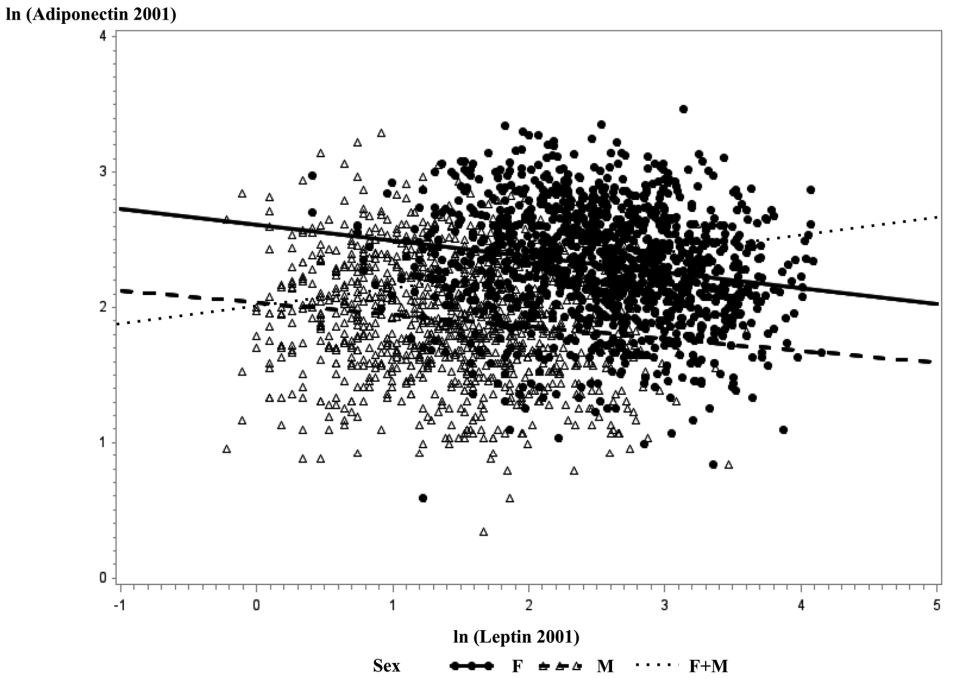

Figure 11. Correlation of serum leptin levels with adiponectin levels in adulthood in females (F) and males (M).

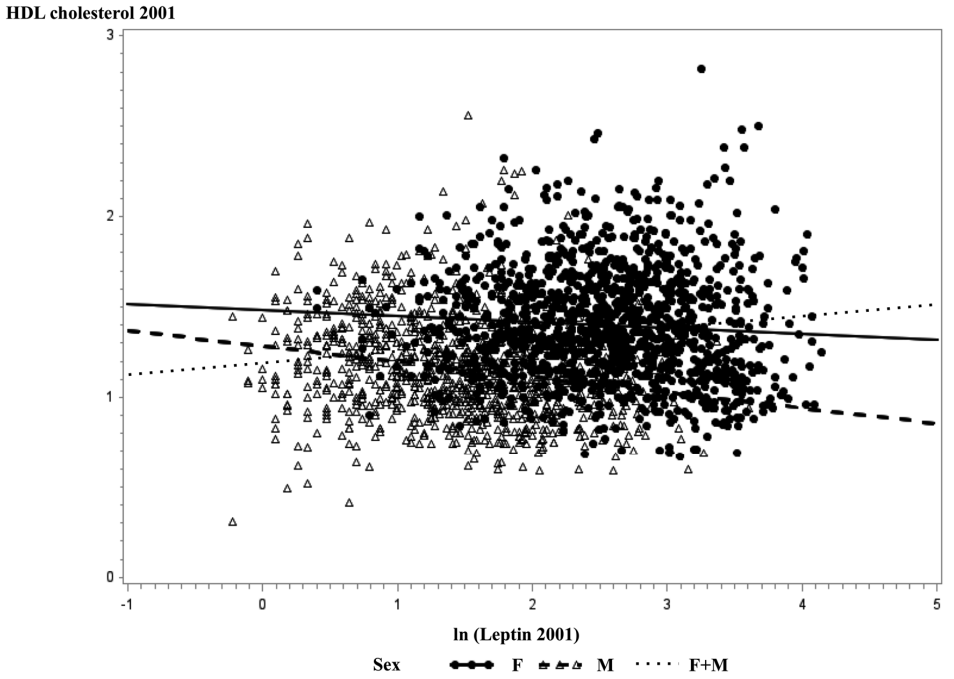

Figure 12. Correlation of serum leptin levels with HDL cholesterol levels in adulthood in females (F) and males (M).

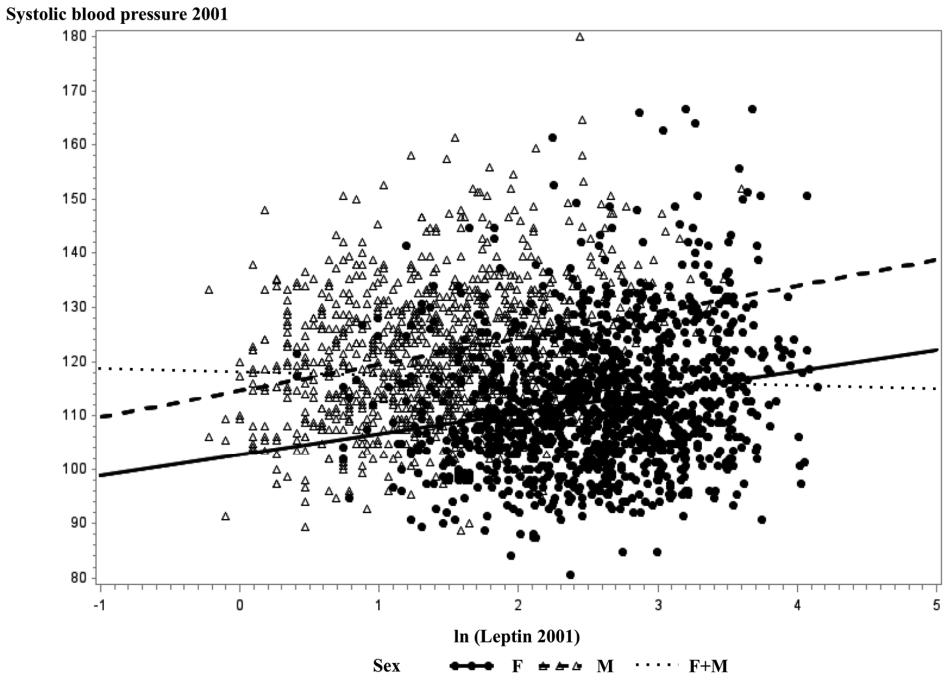

Figure 13. Correlation of serum leptin levels with systolic blood pressure levels in adulthood in females (F) and males (M).

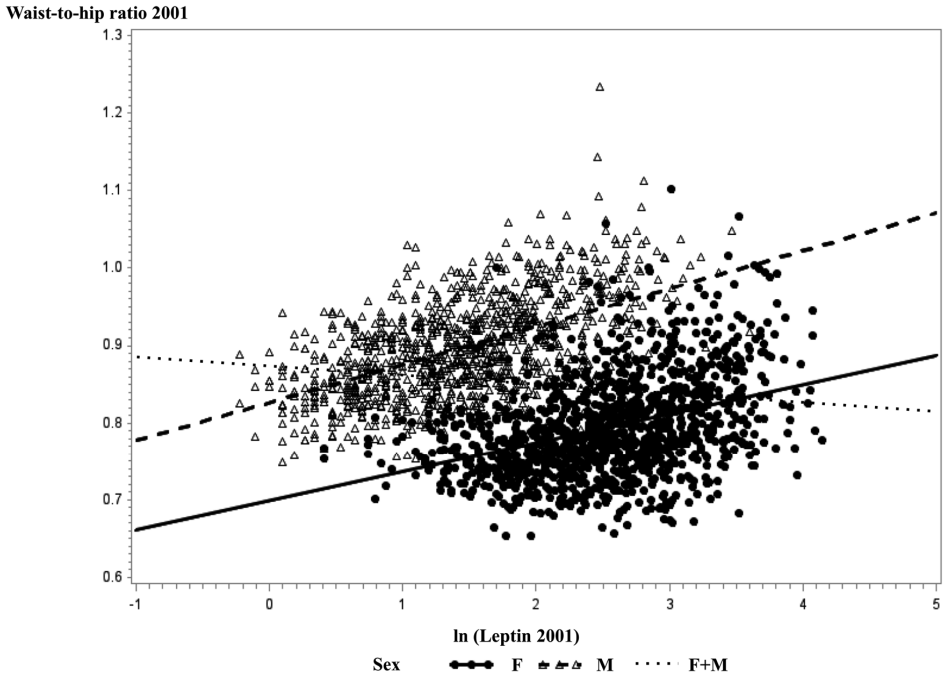

Figure 14. Correlation of serum leptin levels with waist-to-hip ratio in adulthood in females (F) and males (M).

In childhood, the age-adjusted associations of serum leptin levels with weight and BMI were positive in both males and females. In females, leptin levels were also positively associated with insulin and systolic blood pressure levels whereas in boys, there was a negative association with non-HDL cholesterol levels. Similar associations have been reported in other studies (Hassink *et al.* 1996, Garcia-Mayor *et al.* 1997, Atabek *et al.* 2004).

Overall, in adults, serum adiponectin levels tend to correlate with a favorable cardiovascular risk factor profile whereas serum leptin levels correlate with a non-favorable risk factor profile. The correlations in childhood are more complex, probably due to pubertal changes affecting risk factor levels.

Low serum adiponectin levels are correlated with each component of MetS: increased waist circumference, high triglycerides, low HDL cholesterol, high blood pressure and insulin resistance. In study IV, higher baseline adiponectin levels were predictive of a decreased 6-year incidence of MetS independent of age, sex, individual MetS components as well as inflammatory markers, leptin and insulin levels, dyslipidemia, smoking, and family history of coronary artery disease. Of the individual MetS components, adiponectin was independently associated with a decreased incidence of hyperglycemia. The results are in concordance with other studies reporting associations between low adiponectin levels and prevalent MetS (Ryo *et al.* 2004, Hara *et al.* 2006, Santaniemi *et al.* 2006) as well as a study reporting low levels of HMW adiponectin levels to predict incident MetS (Seino *et al.* 2009).

6.3.3 Adiponectin, leptin, and inflammation

CRP is a marker of inflammation and its production by the hepatocytes is regulated by cytokines in acute infections and in chronic inflammatory states such as obesity (Wang and Nakayama 2010). Serum leptin levels correlated positively with CRP levels whereas there was a weak negative correlation between adiponectin and CRP levels. In study I, the association between serum leptin and CRP levels remained independent when adjusted with obesity indices and serum lipid levels. Serum leptin levels were the main determinant of CRP levels in males and the second most important determinant of CRP levels in females. The effect of adiponectin levels was small, and remained significant only in females. These results were similar to previous reports from studies on smaller populations (Kazumi *et al.* 2003, Shamsuzzaman *et al.* 2004, Ble *et al.* 2005).

Results from experimental studies have shown that leptin stimulates the production of inflammatory cytokines from human monocytes (Santos-Alvarez *et al.* 1999) which could be one mechanism for circulating leptin to increase CRP production in the liver. Leptin may also have a direct effect on CRP production, since leptin has been shown to stimulate CRP expression in human hepatocytes *in vitro* (Chen et al. 2006).

The association between leptin and CRP could be explained by obesity, since the production of inflammatory cytokines regulating CRP production is increased in obesity (Kern *et al.* 2001). However, in this study the association between CRP and leptin remained independent after adjustment for BMI and waist circumference, and was also observed in subgroup analysis that included normal weight individuals only.

The use of oral contraceptives has been reported to increase CRP levels (Dreon *et al.* 2003, Williams *et al.* 2004, Raitakari *et al.* 2005). Oral contraceptive (OC) use was the main determinant of CRP levels in females. Although CRP levels were higher in OC users compared with non-users, no such difference was seen for leptin levels. This suggests that CRP levels would not have a direct effect on leptin levels.

The results of the haplotypic Mendelian randomization analyses did not support a causal role for CRP in the regulation of leptin levels. The inherence of haplotypes in the CRP gene is not affected by environmental factors that potentially confound the association between circulating CRP and leptin and the haplotypes are also likely to be associated with life-long differences in average CRP levels. Therefore use of CRP haplotypes is assumed to represent a good instrument for CRP levels that is largely free from confounding. In this study, CRP haplotypes were not related to leptin to the extent predicted by the magnitude of their association with average circulating CRP levels suggesting that CRP is unlikely to be a causal factor for leptin. Genetic variability at the leptin receptor locus has been reported to associate with CRP levels (Zhang *et al.* 2007).

6.3.4 Adiponectin, leptin, and atherosclerosis

Previous reports from the Cardiovascular Risk in Young Finns Study and other longitudinal cohorts have shown that cardiovascular risk factor levels in childhood are predictive of subclinical atherosclerosis in adulthood (Davis *et al.* 2001, Li *et al.* 2003, Raitakari *et al.* 2003). Elevated blood pressure levels,

overweight/obesity and dyslipidemia have been most consistently associated with subclinical atherosclerosis.

An important aim of this thesis was to analyze the associations of serum leptin and adiponectin levels with subclinical atherosclerosis. In cross-sectional analyses in adults (Study II), adiponectin levels were inversely associated with carotid IMT and directly with brachial FMD and carotid artery distensibility. Serum leptin levels were directly associated with FMD but not with IMT or carotid artery distensibility. After adjustment for sex, age, and conventional cardiovascular risk factors, the associations of adiponectin levels with IMT and FMD remained independent.

In longitudinal analyses of the association of childhood risk factor levels with subclinical atherosclerosis in adulthood (Study III), serum adiponectin levels were associated with increased IMT of the common carotid artery as well as with atherosclerotic plaque in the carotid artery bifurcation. These associations also remained significant after adjustments for sex, age and conventional childhood risk factors. To my knowledge, Study III is the first publication reporting such associations.

To assess the performance of the risk prediction model, AUC was used to describe the overall performance of the model in discriminating individuals with and without the outcome, and the integrated discrimination index was used to determine if adding a new risk factor to a prediction model increases the average predicted risks between the individuals with and without the outcome. In Study III, adding child adiponectin levels to conventional child risk factors for the prediction of adult carotid plaque/abnormal IMT increased the AUC from 0.733 to 0.748 (difference in AUC=0.015). These data suggest a medium effect size of adiponectin levels, considering that the improvement in the AUC depends strongly on the baseline model (Pencina *et al.* 2012). Consistent with the AUC results, a significant improvement in the integrated discrimination index was found. When adult adiponectin levels were taken into account, childhood adiponectin still had a residual independent effect on adult high IMT or plaque, whereas the association with the continuous common carotid IMT was diluted to non-significant. This could indicate that exposure to low levels of adiponectin in childhood may have a role in the initiation of atherosclerosis or that childhood adiponectin levels are indicative of adult adiponectin levels but that adult levels have a more important pathophysiological role.

In humans, the cross-sectional association between adiponectin and carotid IMT is well established in all age groups. In pediatric populations, an inverse correlation between adiponectin and IMT has been reported for obese adolescents (Pilz *et al.* 2005, Beauloye *et al.* 2007). An inverse correlation has

also been reported in the STRIP study in healthy adolescents (Jaakkola *et al.* 2015) as well as in a population of 100 healthy women aged 24-59 years (Lo *et al.* 2006). In middle-aged populations there are several publications reporting on the association between low adiponectin levels and increased IMT (Iglseder *et al.* 2005, Behre *et al.* 2006, Dullaart *et al.* 2007, Gardener *et al.* 2012). Other studies have not found an independent association between adiponectin levels and IMT. In a study on Swedish middle-aged adults, the association was attenuated to non-significant after adjustment for metabolic risk factors (Nilsson *et al.* 2006). Similarly, there was no independent association between adiponectin levels and IMT in a study on European males and females, however in that study, variation in the promoter region of the ADIPOQ gene encoding adiponectin was independently associated with IMT (Patel *et al.* 2008).

In study IV, baseline adiponectin levels in adults were associated with carotid IMT cross-sectionally as well as IMT measured after 6-year follow-up. The inverse correlation was stronger in subjects with MetS suggesting that subjects with MetS might be more vulnerable to low adiponectin levels. Although the baseline adiponectin levels were not associated with IMT progression in this study, there was a weak inverse correlation between 6-year change in adiponectin levels and IMT progression.

Previously, an inverse association with baseline adiponectin level and IMT and age-adjusted adiponectin in the lowest quartile have been reported to associate with progression of IMT after 12 months follow-up (Störk *et al.* 2007). Recent results from a longitudinal international multicenter study showed an independent association with adiponectin levels and baseline mean bifurcation IMT and progression of mean carotid IMT in men but not in women. Moreover, in that study a gene score of adiponectin-raising alleles was inversely associated with baseline mean bifurcation IMT in men (Persson *et al.* 2015).

In Study II, low adiponectin levels were also associated with attenuated FMD responses. Previous data on the relation between adiponectin and brachial FMD are available from relatively small studies. Adiponectin has been reported to be independently associated with FMD in subjects with type 2 diabetes and early diabetic nephropathy (Yilmaz *et al.* 2008). Conversely, no association between adiponectin and FMD was found in nearly 300 British adolescents (Singhal *et al.* 2005). This may be due to the young age of subjects in that study or a narrow range for endothelial function in healthy adolescents. In experimental studies, adiponectin has been shown to have many beneficial effects on endothelial cells, such as inhibition of monocyte adhesion to endothelial cells (Ouchi *et al.* 1999) and induction of nitric oxide production (Chen *et al.* 2003), both of which could positively affect endothelial function.

The results from this study support the hypothesis of a beneficial role for adiponectin in early atherosclerosis. However, the role of childhood adiponectin levels in the prediction of IMT in adulthood needs to be studied in other populations.

6.4 Strengths and limitations

An important strength of this study is the large, randomly selected population-based, racially homogenous cohort of males and females representing the age range from early childhood to adulthood. The study design provides a setting for not only cross-sectional but also prospective analyses to study the associations of childhood cardiovascular risk factors with subclinical atherosclerosis in adulthood and, in the future, with cardiovascular events.

The study cohort is comprised of racially homogenous young adults and therefore, the generalizability of the reported results is limited to white European like populations. The cohort is still without clinical manifestations of cardiovascular disease and therefore it is not yet possible to study associations between risk factor levels and cardiovascular events.

Adiponectin circulates in three different complexes of which the high molecular weight form of adiponectin has been suggested to have stronger association with adverse metabolic effects compared to total adiponectin levels (Wang and Scherer 2016). In this study, only total adiponectin levels were measured. If the associations of adiponectin levels with cardiovascular risk factors and subclinical atherosclerosis were mainly related with high molecular weight form, using total adiponectin levels in the analyses could attenuate the observed associations but would not be likely cause false positive associations.

Adipose tissue secretes hundreds of proteins affecting inflammation, the immune system, satiety and energy expenditure, glucose metabolism and insulin sensitivity as well as heart and vasculature (Blüher and Mantzoros 2015). Of those adipokines, only leptin and adiponectin were used as markers of adipose tissue adipokine secretion in this thesis. Leptin and adiponectin are thought to act in different directions in inflammation and the key processes in early atherosclerosis, but more comprehensive evaluation of different adipokine levels could provide a wider perspective in examining the associations between adipokines and atherosclerosis.

6.5 Clinical implications

Children and adolescents at high risk for obesity, type 2 diabetes and cardiovascular disease are not always recognized early enough, and preventive measures reach the families in need insufficiently. Finland is, however, one of the few countries that follow growth regularly through childhood, and efforts for prevention are made at population level in well-baby clinics and school health care.

As a reaction to the increasing prevalence of overweight and obesity in Finland, the National Institute for Health and Welfare launched the first National Obesity Programme 2012-2015 in order to promote healthy lifestyle via collaboration between health services, schools and child day care, food industry and sports organizations (Working Group for the National Obesity Programme, 2013). The primary tools used currently include the evaluation of height and weight against national growth charts. The importance of a physically active lifestyle and healthy diet as well as negative effects of smoking are emphasized. Family history of cardiovascular diseases and type 2 diabetes is inquired.

The secondary tools, in primary care and in pediatric clinics in hospitals, reserved for overweight or obese patients and for high risk families are measurements of plasma glucose and insulin, oral glucose tolerance test, and measurements of serum total cholesterol, HDL cholesterol and triglyceride levels. Lipoprotein(a) levels can be measured in patients with high family risk for cardiovascular diseases, especially with no evident cause (Lapinleimu *et al.* 2015). Blood pressure levels are also evaluated. Furthermore, measurements of alanine amino transferase and ultrasonic evaluation of the liver are used to find non-alcoholic fatty liver disease.

Recommendations for screening of risk factor levels in childhood have been, wordwide, rather conservative. Expert panels and associations have recommended general prepubertal and postpubertal screening in order to detect especially familial hypercholesterolemia already in childhood. However, general screening is not performed in clinical practice and diagnosis in childhood is most often obtained by screening of relatives of patients with familial hypercholesterolemia (Gidding 2016). A recent recommendation statement from the US Preventive Services Task Force concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for lipid disorders in children and adolescents (US Preventive Services Task Force *et al.* 2016).

At present, there are no guidelines for interpreting an individual's adiponectin or leptin level in respect to currently used risk factors, nor are there clinical cut-off values for abnormal levels for adiponectin or leptin. For now, there is no data to support adding measurements of either adiponectin or leptin levels to the clinical toolbox of everyday practice, but they are reserved for scientific use.

6.6 Future research needs

The association of adiponectin levels in childhood and adolescence with carotid atherosclerosis in adulthood is scientifically interesting and merits further research. This association should be confirmed in other populations.

The cross-sectional association of adiponectin and surrogate markers of atherosclerosis is well documented in adolescent and adult populations, but the associations in elderly populations especially concerning prediction of cardiovascular events are controversial and need further research.

Once sufficient numbers of clinical events accumulate in the Cardiovascular Risk in Young Finns Study, it will be of interest to determine if child and adolescent adiponectin levels predict cardiovascular events later in adulthood. A more comprehensive evaluation of adipokine levels and their associations is a vast research area of which very little is known at present.

7 CONCLUSIONS

In this study, the following conclusions can be made:

1. Serum adiponectin levels decrease from childhood to adolescence both in females and in males. In adulthood, adiponectin levels are higher in females than in males. Serum leptin levels increase from childhood to adolescence in females and tend to decrease in males. In adulthood, leptin levels are higher in females than males and are strongly correlated with obesity.

2. The tracking of serum adiponectin from childhood to adulthood is relatively strong and comparable to the tracking of serum cholesterol levels. Thus, measuring serum adiponectin levels in childhood as a part of risk evaluation might be worthwhile. The tracking of serum leptin levels from childhood to adulthood is only moderate and therefore measuring serum leptin levels in childhood in this respect is not reasonable.

3. Overall, serum adiponectin levels are associated with a favorable cardiovascular risk factor profile, whereas serum leptin levels are associated with a non-favorable cardiovascular risk factor profile in adults. Low adiponectin levels predict incident MetS, especially incident hyperglycemia. High serum leptin levels are associated with inflammation assessed with serum CRP levels.

4. Low serum adiponectin levels are associated with increased carotid IMT and attenuated brachial FMD in adulthood independently of age, sex, obesity, cigarette smoking and conventional cardiovascular risk factor levels. Individuals with a non-favorable risk factor profile assessed with prevalent MetS may be more vulnerable to low adiponectin levels. Serum leptin levels are not independently associated with subclinical atherosclerosis in this population.

5. Serum adiponectin levels in childhood and adolescence are associated with increased carotid IMT in adulthood and slightly improve the prediction of carotid plaque over conventional cardiovascular risk factors. If these results are confirmed in other populations, childhood adiponectin levels might have a role in cardiovascular risk prediction in childhood in the future.

ACKNOWLEDGEMENTS

This study is a part of the Cardiovascular Risk in Young Finns Study and was carried out at the Research Centre of Applied and Preventive Cardiovascular Medicine, the Department of Internal Medicine, and the Department of Pharmacology, Drug Development and Therapeutics, University of Turku, during the years 2003-2016.

I express my deepest gratitude to my supervisors. I thank Professor Olli Raitakari for patience and I admire his profound knowledge of cardiovascular risk factors, statistics, and medical writing. It has been a privilege to work with Professor Risto Huupponen whose expertise, encouraging attitude and valuable comments have been essential during these years. I am especially grateful to my father, Professor Jorma Viikari, for introducing the Cardiovascular Risk in Young Finns Study to me. His role in the initiation and continuance of the Young Finns Study has been enormous. I admire his efficiency, his vast knowledge of science, as well as his never-ending innovative ideas on different perspectives in the evaluation of data.

Professor Markus Juonala has been my unofficial supervisor, and I warmly thank him for collaboration and for his friendly support over the years. I sincerely wish to thank all other co-authors: Professor Terho Lehtimäki, Professor Mika Kivimäki, Professor Mika Kähönen, Professor Mikko Hurme, Professor Tomi Laitinen, Docent Eero Jokinen, Docent Jukka Marniemi, Docent Nina Hutri-Kähönen, Docent Mervi Oikonen, Docent Costan Magnussen, Leena Taittonen, Leo-Pekka Lyytikäinen, Carita Eklund, and Juha Koskinen for their valuable comments and help in the preparation of the manuscripts.

I warmly thank the members of my follow-up committee, Professor Tapani Rönnemaa, Professor Pekka Kääpä and Professor Jaakko Hartiala for their encouragement during these years.

Docent Hannu Vanhanen and Docent Päivi Keskinen are greatly acknowledged for the skilful review of the manuscript of this thesis and for their valuable comments and constructive criticism. I value our conversations during the review process. I was fortunate to have Docent Costan Magnussen to revise the language providing me also with his expertise on the subject.

I have had the pleasure to share my journey towards the dissertation with friends and colleagues. Noora Matsson, Tuomas Koskinen and Kristiina Pälve, I thank you for the memorable moments and the team spirit. I also wish to thank late Docent Mervi Oikonen for her always positive and encouraging attitude. It has been a privilege to work at the Research Centre of Applied and Preventive Cardiovascular Medicine. Nina Ruotsalainen, Anni Pakarinen, Nina Aalto, and Katja Pahkala, thank you for the support and help during these years. I am deeply grateful to Irina Lisinen for friendship and endurance with my statistical problems. I also wish to thank Ville Aalto and Noora Kartiosuo for statistical advice. I am grateful to Elina Kahra and Satu Mäkelä for performing the measurements of adiponectin and leptin.

The Department of Pediatrics and Adolescent Medicine is my professional home and I thank Professor Erika Isolauri and Professor Liisa Lehtonen for their encouraging attitude towards research. I am indebted to Professor Jussi Mertsola for luring me into the field of pediatrics and challenging me during these years. I am deeply grateful to my mentor, Docent Kirsti Näntö-Salonen, for her friendship and her warm and caring guidance in the intriguing world of pediatric endocrinology. I admire Professor Jorma Toppari for his incredible expertise in pediatric endocrinology and Professor Harri Niinikoski for his vast knowledge of metabolic diseases and enthusiasm in discovering rare disorders. I warmly thank Docent Leena Kainulainen and Anne Kinnala for collaboration and all my colleagues for inspiring conversations, social gatherings and the supportive atmosphere. Sinikka Karppinen, Lauri Ivaska and Laura Toivonen, thank you for peer support and practical tips in finalizing the thesis and Docent Helena Lapinleimu for your invaluable advice on how to use Turnitin.

I thank my dear friend Johanna Lempainen for our long, enjoyable conversations. Your advice, support, positivity, and encouragement have been extremely important in the preparation of this thesis and in life in general. Lasse, Johanna and Kari, Aino, Tuomas and Elena, Jarkko and Annica, Paulina and Jari, Katariina, Louise and Jarkko, Ilona and Riku, Tuomas and Aida, Anssi and Helena, Marikki and Henri, Heikki and Hanna, Pia and Janne, Anna and Ville, Salla and Jyrki, and all others, I warmly thank you all for the enjoyable moments spent together and to come. Friendship is one of the most important things in life.

Finally, I wish to thank my family. I owe my deepest gratitude to my mother Marjukka, who has always been selflessly there to help when needed. I am grateful to my sisters Laura, Paula, Nora, and Heidi for being there for me. We support each other in ways that only sisters can. I especially wish to thank Paula for her firm believe in me completing this thesis. I warmly thank my brothers-in law Jussi and Mika as well as my mother-in-law Helena and my brother-in-law Michael for widening my perspective in life. My nieces and nephews bring joy and sense of reality to my life. I especially thank my husband Tuukka for sharing all the ups and downs of life, work, leisure, sailing, and research with me. You and our dear sons Tommi and Sami make my day.

This study was financially supported by grants from the Turku University Foundation, the Orion-Farmos Research Foundation, the Finnish Cultural Foundation, the Finnish Medical Foundation, the Foundation of Turku University Hospital, the Turku University Hospital Medical Funds and the Doctoral Programme of Clinical Investigation, University of Turku.

Turku, January 2017

Liisa Saarikoski

REFERENCES

ACCORD Study Group, Ginsberg, HN, Elam, MB, Lovato, LC, Crouse, JR, 3rd, Leiter, LA*, et al.* Effects of combination lipid therapy in type 2 diabetes mellitus. *N Engl J Med*. 2010;362:1563-74.

Ai, M, Otokozawa, S, Asztalos, BF, White, CC, Cupples, LA, Nakajima, K*, et al.* Adiponectin: an independent risk factor for coronary heart disease in men in the Framingham offspring Study. *Atherosclerosis*. 2011;217:543-8.

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.

Alberti, KG, Eckel, RH, Grundy, SM, Zimmet, PZ, Cleeman, JI, Donato, KA*, et al.* Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120:1640-5.

Alberti, KG, Zimmet, P, Shaw, J and IDF Epidemiology Task Force Consensus Group. The metabolic syndrome--a new worldwide definition. *Lancet*. 2005;366:1059-62.

Alberti, KG and Zimmet, PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998;15:539-53.

Andersen, KK, Frystyk, J, Wolthers, OD, Heuck, C and Flyvbjerg, A. Gender differences of oligomers and total adiponectin during puberty: a cross-sectional study of 859 Danish school children. *J Clin Endocrinol Metab*. 2007;92:1857-62.

Andersen, UO and Jensen, GB. Trends and determinant factors in hypertension control in a population study with 25 years of follow-up. *J Hypertens*. 2010;28:1091-6.

Arita, Y, Kihara, S, Ouchi, N, Takahashi, M, Maeda, K, Miyagawa, J*, et al.* Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun*. 1999;257:79-83.

Arslanian, S, Suprasongsin, C, Kalhan, SC, Drash, AL, Brna, R and Janosky, JE. Plasma leptin in children: relationship to puberty, gender, body composition, insulin sensitivity, and energy expenditure. *Metabolism*. 1998;47:309-12.

Assmann, G, Cullen, P and Schulte, H. Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Münster (PROCAM) study. *Circulation*. 2002;105:310-5.

Atabek, ME, Kurtoglu, S, Demir, F and Baykara, M. Relation of serum leptin and insulin-like growth factor-1 levels to intima-media thickness and functions of common carotid artery in children and adolescents with type 1 diabetes. *Acta Paediatr*. 2004;93:1052-7.

Bäck, M and Hansson, GK. Anti-inflammatory therapies for atherosclerosis. *Nat Rev Cardiol*. 2015;12:199-211.

Balkau, B and Charles, MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med*. 1999;16:442-3.

Barton, M. Mechanisms and therapy of atherosclerosis and its clinical complications. *Curr Opin Pharmacol*. 2013;13:149-53.

Beauloye, V, Zech, F, Tran, HT, Clapuyt, P, Maes, M and Brichard, SM. Determinants of early atherosclerosis in obese children and adolescents. *J Clin Endocrinol Metab*. 2007;92:3025-32.

Behre, CJ, Brohall, G, Hulthe, J, Wikstrand, J and Fagerberg, B. Are serum adiponectin concentrations in a population sample of 64-year-old Caucasian women with varying glucose tolerance associated with ultrasound-assessed atherosclerosis? *J Intern Med*. 2006;260:238-44.

Berenson, GS, Srinivasan, SR, Bao, W, Newman, WP, 3rd, Tracy, RE and Wattigney, WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med*. 1998;338:1650-6.

Besseling, J, Sjouke, B and Kastelein, JJ. Screening and treatment of familial hypercholesterolemia - Lessons from the past and opportunities for the future (based on the Anitschkow Lecture 2014). *Atherosclerosis*. 2015;241:597-606.

Blacher, J, Pannier, B, Guerin, AP, Marchais, SJ, Safar, ME and London, GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension*. 1998;32:570-4.

Ble, A, Windham, BG, Bandinelli, S, Taub, DD, Volpato, S, Bartali, B*, et al.* Relation of plasma leptin to C-reactive protein in older adults (from the Invecchiare nel Chianti study). *Am J Cardiol*. 2005;96:991-5.

Blüher, M and Mantzoros, CS. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. *Metabolism*. 2015;64:131-45.

Bodary, PF, Gu, S, Shen, Y, Hasty, AH, Buckler, JM and Eitzman, DT. Recombinant leptin promotes atherosclerosis and thrombosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2005;25:e119-22.

Booth, A, Magnuson, A, Fouts, J and Foster, MT. Adipose tissue: an endocrine organ playing a role in metabolic regulation. *Horm Mol Biol Clin Investig*. 2016;26:25-42.

Borch-Johnsen, K and Wareham, N. The rise and fall of the metabolic syndrome. *Diabetologia*. 2010;53:597-9.

Bots, ML, Hoes, AW, Koudstaal, PJ, Hofman, A and Grobbee, DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation*. 1997;96:1432-7.

Bouloumie, A, Drexler, HC, Lafontan, M and Busse, R. Leptin, the product of Ob gene, promotes angiogenesis. *Circ Res*. 1998;83:1059-66.

Bouloumie, A, Marumo, T, Lafontan, M and Busse, R. Leptin induces oxidative stress in human endothelial cells. *FASEB J*. 1999;13:1231-8.

Böttner, A, Kratzsch, J, Müller, G, Kapellen, TM, Blüher, S, Keller, E*, et al.* Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. *J Clin Endocrinol Metab*. 2004;89:4053-61.

Cabia, B, Andrade, S, Carreira, MC, Casanueva, FF and Crujeiras, AB. A role for novel adipose tissue-secreted factors in obesity-related carcinogenesis. *Obes Rev*. 2016;17:361-76.

Cannon, CP, Blazing, MA, Giugliano, RP, McCagg, A, White, JA, Theroux, P*, et al.* Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. *N Engl J Med*. 2015;372:2387-97.

Cao, R, Brakenhielm, E, Wahlestedt, C, Thyberg, J and Cao, Y. Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. *Proc Natl Acad Sci U S A*. 2001;98:6390-5.

Capewell, S, Morrison, CE and McMurray, JJ. Contribution of modern cardiovascular treatment and risk factor changes to the decline in coronary heart disease mortality in Scotland between 1975 and 1994. *Heart*. 1999;81:380-6.

Carbone, F, Mach, F and Montecucco, F. The role of adipocytokines in atherogenesis and atheroprogression. *Curr Drug Targets*. 2015;16:295-320.

Carlson, CS, Aldred, SF, Lee, PK, Tracy, RP, Schwartz, SM, Rieder, M*, et al.* Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. *Am J Hum Genet*. 2005;77:64-77.

Celermajer, DS, Sorensen, KE, Gooch, VM, Spiegelhalter, DJ, Miller, OI, Sullivan, ID*, et al.* Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111-5.

Chai, SB, Sun, F, Nie, XL and Wang, J. Leptin and coronary heart disease: a systematic review and meta-analysis. *Atherosclerosis*. 2014;233:3-10.

Chambless, LE, Heiss, G, Folsom, AR, Rosamond, W, Szklo, M, Sharrett, AR*, et al.* Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. *Am J Epidemiol*. 1997;146:483-94.

Chan, JL and Mantzoros, CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. *Lancet (London, England)*. 2005;366:74-85.

Chan, SY, Mancini, GB, Kuramoto, L, Schulzer, M, Frohlich, J and Ignaszewski, A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. *J Am Coll Cardiol*. 2003;42:1037-43.

Chen, H, Charlat, O, Tartaglia, LA, Woolf, EA, Weng, X, Ellis, SJ*, et al.* Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell*. 1996;84:491-5.

Chen, H, Montagnani, M, Funahashi, T, Shimomura, I and Quon, MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem*. 2003;278:45021-6.

Chen, K, Li, F, Li, J, Cai, H, Strom, S, Bisello, A*, et al.* Induction of leptin resistance through direct interaction of C-reactive protein with leptin. *Nat Med*. 2006;12:425-32.

Chiba, T, Shinozaki, S, Nakazawa, T, Kawakami, A, Ai, M, Kaneko, E*, et al.* Leptin deficiency suppresses progression of atherosclerosis in apoE-deficient mice. *Atherosclerosis*. 2008;196:68-75.

Chou, K and Perry, CM. Metreleptin: first global approval. *Drugs*. 2013;73:989-97.

Clement, K, Vaisse, C, Lahlou, N, Cabrol, S, Pelloux, V, Cassuto, D*, et al.* A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*. 1998;392:398-401.

Cnop, M, Havel, PJ, Utzschneider, KM, Carr, DB, Sinha, MK, Boyko, EJ*, et al.* Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia*. 2003;46:459-69.

Cnop, M, Landchild, MJ, Vidal, J, Havel, PJ, Knowles, NG, Carr, DR*, et al.* The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations : distinct metabolic effects of two fat compartments. *Diabetes*. 2002;51:1005-15.

Cohen, JC, Boerwinkle, E, Mosley, TH, Jr. and Hobbs, HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264-72.

Colditz, GA and Hankinson, SE. The Nurses' Health Study: lifestyle and health among women. *Nat Rev Cancer*. 2005;5:388-96.

Coleman, DL. Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia*. 1973;9:294-8.

Coleman, DL and Hummel, KP. The influence of genetic background on the expression of the obese (Ob) gene in the mouse. *Diabetologia*. 1973;9:287-93.

Collins, R, Reith, C, Emberson, J, Armitage, J, Baigent, C, Blackwell, L*, et al.* Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet*. 2016;388:2532-61

Conroy, RM, Pyörälä, K, Fitzgerald, AP, Sans, S, Menotti, A, De Backer, G*, et al.* Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J*. 2003;24:987-1003.

Considine, RV, Sinha, MK, Heiman, ML, Kriauciunas, A, Stephens, TW, Nyce, MR*, et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med*. 1996;334:292-5.

Cook, KS, Min, HY, Johnson, D, Chaplinsky, RJ, Flier, JS, Hunt, CR*, et al.* Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. *Science*. 1987;237:402-5.

Critchley, J, Liu, J, Zhao, D, Wei, W and Capewell, S. Explaining the increase in coronary heart disease mortality in Beijing between 1984 and 1999. *Circulation*. 2004;110:1236-44.

D'Agostino, RB, Sr., 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.

Danesh, J, Wheeler, JG, Hirschfield, GM, Eda, S, Eiriksdottir, G, Rumley, A*, et al.* C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med*. 2004;350:1387-97.

Dastani, Z, Hivert, MF, Timpson, N, Perry, JR, Yuan, X, Scott, RA*, et al.* Novel loci for adiponectin levels and their influence on type 2 diabetes and metabolic traits: a multi-ethnic meta-analysis of 45,891 individuals. *PLoS Genet*. 2012;8: e1002607.

Davey Smith, G and Ebrahim, S. What can mendelian randomisation tell us about modifiable behavioural and environmental exposures? *BMJ (Clinical research ed)*. 2005;330:1076-9.

Davis, PH, Dawson, JD, Riley, WA and Lauer, RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: The Muscatine Study. *Circulation*. 2001;104:2815-9.

Dawber, TR, Kannel, WB, Revotskie, N, Stokes, J, 3rd, Kagan, A and Gordon, T. Some factors associated with the development of coronary heart disease: six years' follow-up experience in the Framingham study. *Am J Public Health Nations Health*. 1959;49:1349-56.

Dawber, TR, Meadors, GF and Moore, FE, Jr. Epidemiological approaches to heart disease: the Framingham Study. *Am J Public Health Nations Health*. 1951;41:279-81.

Dawber, TR, Moore, FE and Mann, GV. Coronary heart disease in the Framingham study. *Am J Public Health Nations Health*. 1957;47:4-24.

Dreon, DM, Slavin, JL and Phinney, SD. Oral contraceptive use and increased plasma concentration of C-reactive protein. *Life Sci*. 2003;73:1245-52.

Dubey, L and Hesong, Z. Role of leptin in atherogenesis. *Exp Clin Cardiol*. 2006;11:269-75.

Dullaart, RP, de Vries, R, van Tol, A and Sluiter, WJ. Lower plasma adiponectin is a marker of increased intima-media thickness associated with type 2 diabetes mellitus and with male gender. *Eur J Endocrinol*. 2007;156:387-94.

Dwyer, T, Sun, C, Magnussen, CG, Raitakari, OT, Schork, NJ, Venn, A*, et al.* Cohort Profile: the international childhood cardiovascular cohort (i3C) consortium. *Int J Epidemiol*. 2013;42:86-96.

Einhorn, D, Reaven, GM, Cobin, RH, Ford, E, Ganda, OP, Handelsman, Y*, et al.* American College of Endocrinology position statement on the insulin resistance syndrome. *Endocr Pract*. 2003;9:237-52.

Engeli, S, Feldpausch, M, Gorzelniak, K, Hartwig, F, Heintze, U, Janke, J*, et al.* Association between adiponectin and mediators of inflammation in obese women. *Diabetes*. 2003;52:942-7.

Enos, WF, Holmes, RH and Beyer, J. Coronary disease among United States soldiers killed in action in Korea; preliminary report. *JAMA*. 1953;152:1090-3.

Farooqi, IS, Jebb, SA, Langmack, G, Lawrence, E, Cheetham, CH, Prentice, AM*, et al.* Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med*. 1999;341:879-84.

Ference, BA, Robinson, JG, Brook, RD, Catapano, AL, Chapman, MJ, Neff, DR*, et al.* Variation in PCSK9 and HMGCR and Risk of Cardiovascular Disease and Diabetes. *N Engl J Med*. 2016;375:2144-53.

Ference, BA, Yoo, W, Alesh, I, Mahajan, N, Mirowska, KK, Mewada, A*, et al.* Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. *J Am Coll Cardiol*. 2012;60:2631-9.

Ford, ES. Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence. *Diabetes Care*. 2005;28:1769-78.

Ford, ES, Ajani, UA, Croft, JB, Critchley, JA, Labarthe, DR, Kottke, TE*, et al.* Explaining the decrease in U.S. deaths from coronary disease, 1980-2000. *N Engl J Med*. 2007;356:2388-98.

Forsblom, C, Thomas, MC, Moran, J, Saraheimo, M, Thorn, L, Waden, J*, et al.* Serum adiponectin concentration is a positive predictor of all-cause and cardiovascular mortality in type 1 diabetes. *J Intern Med*. 2011;270:346-55.

Freedman, DS, Shear, CL, Burke, GL, Srinivasan, SR, Webber, LS, Harsha, DW*, et al.* Persistence of juvenile-onset obesity over eight years: the Bogalusa Heart Study. *Am J Public Health*. 1987;77:588-92.

Frick, MH, Elo, O, Haapa, K, Heinonen, OP, Heinsalmi, P, Helo, P*, et al.* Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. *N Engl J Med*. 1987;317:1237-45.

Friedewald, WT, Levy, RI and 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.

Frühbeck, G. Intracellular signalling pathways activated by leptin. *Biochem J*. 2006;393:7-20.

Garcia-Mayor, RV, Andrade, MA, Rios, M, Lage, M, Dieguez, C and Casanueva, FF. Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. *J Clin Endocrinol Metab*. 1997;82:2849-55.

Gardener, H, Sjoberg, C, Crisby, M, Goldberg, R, Mendez, A, Wright, CB*, et al.* Adiponectin and carotid intima-media thickness in the northern Manhattan study. *Stroke*. 2012;43:1123-5.

Gaziano, JM. Fifth phase of the epidemiologic transition: the age of obesity and inactivity. *JAMA*. 2010;303:275-6.

Gidding, SS. Why Cholesterol Testing in Children and Adolescents Matters. *JAMA Cardiol*. 2016;1:859-61

Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration. Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. *Lancet Diabetes Endocrinol*. 2014;2:634-47.

Goldstein, JL and Brown, MS. Familial hypercholesterolemia: identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. *Proc Natl Acad Sci U S A*. 1973;70:2804-8.

Goldstein, JL and Brown, MS. The LDL receptor. *Arterioscler Thromb Vasc Biol*. 2009;29:431-8.

Golledge, J, Leicht, AS, Crowther, RG, Glanville, S, Clancy, P, Sangla, KS*, et al.* Determinants of endothelial function in a cohort of patients with peripheral artery disease. *Cardiology*. 2008;111:51-6.

Grant, DB. Fasting serum insulin levels in childhood. *Arch Dis Child*. 1967;42:375-8.

Greenland, S. An introduction to instrumental variables for epidemiologists. *Int J Epidemiol*. 2000;29:722-9.

Grundy, SM, Cleeman, JI, Daniels, SR, Donato, KA, Eckel, RH, Franklin, BA*, et al.* Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005;112:2735-52.

Haapanen, A, Koskenvuo, M, Kaprio, J, Kesäniemi, YA and Heikkilä, K. Carotid arteriosclerosis in identical twins discordant for cigarette smoking. *Circulation*. 1989;80:10-6.

Hakanen, M, Rönnemaa, T, Talvia, S, Rask-Nissila, 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.

Halaas, JL, Gajiwala, KS, Maffei, M, Cohen, SL, Chait, BT, Rabinowitz, D*, et al.* Weight-reducing effects of the plasma protein encoded by the obese gene. *Science (New York, N Y)*. 1995;269:543-6.

Hanon, O, Luong, V, Mourad, JJ, Bortolotto, LA, Jeunemaitre, X and Girerd, X. Aging, carotid artery distensibility, and the Ser422Gly elastin gene polymorphism in humans. *Hypertension*. 2001;38:1185-9.

Hansson, GK. Atherosclerosis--an immune disease: The Anitschkov Lecture 2007. *Atherosclerosis*. 2009;202:2-10.

Hao, G, Li, W, Guo, R, Yang, JG, Wang, Y, Tian, Y*, et al.* Serum total adiponectin level and the risk of cardiovascular disease in general population: a meta-analysis of 17 prospective studies. *Atherosclerosis*. 2013;228:29-35.

Hara, K, Horikoshi, M, Yamauchi, T, Yago, H, Miyazaki, O, Ebinuma, H*, et al.* Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. *Diabetes Care*. 2006;29:1357-62.

Hassink, SG, Sheslow, DV, de Lancey, E, Opentanova, I, Considine, RV and Caro, JF. Serum leptin in children with obesity: relationship to gender and development. *Pediatrics*. 1996;98:201-3.

Heinonen, MV, Laaksonen, DE, Karhu, T, Karhunen, L, Laitinen, T, Kainulainen, S*, et al.* Effect of diet-induced weight loss on plasma apelin and cytokine levels in individuals with the metabolic syndrome. *Nutr Metab Cardiovasc Dis*. 2009;19:626-33.

Herbert, V, Lau, KS, Gottlieb, CW and Bleicher, SJ. Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab*. 1965;25:1375-84.

Hjermann, I. A randomized primary preventive trial in coronary heart disease: the Oslo study. *Prev Med*. 1983;12:181-4.

Hoefer, IE, Steffens, S, Ala-Korpela, M, Back, M, Badimon, L, Bochaton-Piallat, ML*, et al.* Novel methodologies for biomarker discovery in atherosclerosis. *Eur Heart J*. 2015;36:2635-42.

Hoffmann, A, Ebert, T, Kloting, N, Dokas, J, Jeromin, F, Jessnitzer, B*, et al.* Leptin dose-dependently decreases atherosclerosis by attenuation of hypercholesterolemia and induction of adiponectin. *Biochim Biophys Acta*. 2016;1862:113-20.

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

Hort, W. Arteriosclerosis: its morphology in the past and today. *Basic Res Cardiol*. 1994;89 Suppl 1:1-15.

Hossain, MM, Mukheem, A and Kamarul, T. The prevention and treatment of hypoadiponectinemia-associated human diseases by up-regulation of plasma adiponectin. *Life Sci*. 2015;135:55-67.

Iglseder, B, Mackevics, V, Stadlmayer, A, Tasch, G, Ladurner, G and Paulweber, B. Plasma adiponectin levels and sonographic phenotypes of subclinical carotid artery atherosclerosis: data from the SAPHIR Study. *Stroke*. 2005;36:2577-82.

Isomaa, B, Almgren, P, Tuomi, T, Forsen, B, Lahti, K, Nissen, M*, et al.* Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*. 2001;24:683-9.

Jaakkola, JM, Pahkala, K, Viitala, M, Rönnemaa, T, Viikari, J, Niinikoski, H*, et al.* Association of Adiponectin with Adolescent Cardiovascular Health in a Dietary Intervention Study. *J Pediatr*. 2015;167:353-60 e1.

Jacobsson, LT, Turesson, C, Gulfe, A, Kapetanovic, MC, Petersson, IF, Saxne, T*, et al.* Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis. *J Rheumatol*. 2005;32:1213-8.

Joannides, R, Haefeli, WE, Linder, L, Richard, V, Bakkali, EH, Thuillez, C*, et al.* Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation*. 1995;91:1314-9.

Jousilahti, P, Laatikainen, T, Peltonen, M, Borodulin, K, Männistö, S, Jula, A*, et al.* Primary prevention and risk factor reduction in coronary heart disease mortality among working aged men and women in eastern Finland over 40 years: population based observational study. *Br Med J*. 2016;352:i721.

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.

Jun, JY, Ma, Z, Pyla, R and Segar, L. Leptin treatment inhibits the progression of atherosclerosis by attenuating hypercholesterolemia in type 1 diabetic Ins2(+/Akita):apoE(-/-) mice. *Atherosclerosis*. 2012;225:341-7.

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, Kähönen, M, Taittonen, L, Laitinen, T, Hutri-Kähönen, N*, et al.* Life-time risk factors and progression of carotid atherosclerosis in young adults: the Cardiovascular Risk in Young Finns study. *Eur Heart J*. 2010;31:1745-51.

Juonala, M, Viikari, JS and Raitakari, OT. Main findings from the prospective Cardiovascular Risk in Young Finns Study. *Curr Opin Lipidol*. 2013;24:57-64.

Juonala, M, Viikari, JS, Rönnemaa, T, Taittonen, L, Marniemi, J and 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*. 2006;26:1883-8.

Kadowaki, T, Yamauchi, T, Kubota, N, Hara, K, Ueki, K and Tobe, K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest*. 2006;116:1784-92.

Kanhai, DA, Kranendonk, ME, Uiterwaal, CS, van der Graaf, Y, Kappelle, LJ and Visseren, FL. Adiponectin and incident coronary heart disease and stroke. A systematic review and meta-analysis of prospective studies. *Obes Rev*. 2013;14:555-67.

Kazumi, T, Kawaguchi, A, Hirano, T and Yoshino, G. C-reactive protein in young, apparently healthy men: associations with serum leptin, QTc interval, and high-density lipoprotein-cholesterol. *Metabolism*. 2003;52:1113-6.

Keech, A, Simes, RJ, Barter, P, Best, J, Scott, R, Taskinen, MR*, et al.* Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet*. 2005;366:1849-61.

Kern, PA, Ranganathan, S, Li, C, Wood, L and Ranganathan, G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab*. 2001;280:E745-51.

Ketola, E, Laatikainen, T and Vartiainen, E. Evaluating risk for cardiovascular diseases--vain or value? How do different cardiovascular risk scores act in real life. *Eur J Public Health*. 2010;20:107-12.

Keys, A, Aravanis, C, Blackburn, HW, Van Buchem, FS, Buzina, R, Djordjevic, BD*, et al.* Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. *Acta Med Scand Suppl*. 1966;460:1-392.

Kilpeläinen, TO, Carli, JF, Skowronski, AA, Sun, Q, Kriebel, J, Feitosa, MF*, et al.* Genome-wide meta-analysis uncovers novel loci influencing circulating leptin levels. *Nat Commun*. 2016;7:10494.

Kistorp, C, Faber, J, Galatius, S, Gustafsson, F, Frystyk, J, Flyvbjerg, A*, et al.* Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation*. 2005;112:1756-62.

Kojima, S, Funahashi, T, Maruyoshi, H, Honda, O, Sugiyama, S, Kawano, H*, et al.* Levels of the adipocyte-derived plasma protein, adiponectin, have a close relationship with atheroma. *Thromb Res*. 2005;115:483-90.

Kumada, M, Kihara, S, Sumitsuji, S, Kawamoto, T, Matsumoto, S, Ouchi, N*, et al.* Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol*. 2003;23:85-9.

Lakka, HM, Laaksonen, DE, Lakka, TA, Niskanen, LK, Kumpusalo, E, Tuomilehto, J*, et al.* The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA*. 2002;288:2709-16.

Lapinleimu, J, Raitakari, OT, Lapinleimu, H, Pahkala, K, Rönnemaa, T, Simell, OG*, et al.* High lipoprotein(a) concentrations are associated with impaired endothelial function in children. *J Pediatr*. 2015;166:947-52 e1-2.

LaRosa, JC, He, J and Vupputuri, S. Effect of statins on risk of coronary disease: a meta-analysis of randomized controlled trials. *JAMA*. 1999;282:2340-6.

Lauer, RM, Connor, WE, Leaverton, PE, Reiter, MA and Clarke, WR. Coronary heart disease risk factors in school children: the Muscatine study. *J Pediatr*. 1975;86:697-706.

Lauer, RM, Lee, J and Clarke, WR. Factors affecting the relationship between childhood and adult cholesterol levels: the Muscatine Study. *Pediatrics*. 1988;82:309-18.

Lawlor, DA, Davey Smith, G, Ebrahim, S, Thompson, C and Sattar, N. Plasma adiponectin levels are associated with insulin resistance, but do not predict future risk of coronary heart disease in women. *J Clin Endocrinol Metab*. 2005;90:5677-83.

Lee, BP, Lloyd-Laney, HO, Locke, JM, McCulloch, LJ, Knight, B, Yaghootkar, H*, et al.* Functional characterisation of ADIPOQ variants using individuals recruited by genotype. *Mol Cell Endocrinol*. 2016;

Lewington, S, Clarke, R, Qizilbash, N, Peto, R, Collins, R and Prospective Studies, C. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360:1903-13.

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. *JAMA*. 2003;290:2271-6.

Li, S, Shin, HJ, Ding, EL and van Dam, RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA*. 2009;302:179-88.

Libby, P, Ridker, PM and Hansson, GK. Progress and challenges in translating the biology of atherosclerosis. *Nature*. 2011;473:317-25.

Lindberg, S, Mogelvang, R, Pedersen, SH, Bjerre, M, Frystyk, J, Flyvbjerg, A*, et al.* Relation of serum adiponectin levels to number of traditional atherosclerotic risk factors and all-cause mortality and major adverse cardiovascular events (from the Copenhagen City Heart Study). *Am J Cardiol*. 2013;111:1139-45.

Lindsay, RS, Resnick, HE, Zhu, J, Tun, ML, Howard, BV, Zhang, Y*, et al.* Adiponectin and coronary heart disease: the Strong Heart Study. *Arterioscler Thromb Vasc Biol*. 2005;25:e15-6.

Lithell, H, Aberg, H, Selinus, I and Hedstrand, H. The primary preventive study in Uppsala. Fatal and non-fatal myocardial infarction during a 10-year follow-up of a middle-aged male population with treatment of high-risk individuals. *Acta Med Scand*. 1984;215:403-9.

Lloyd, DJ, McCormick, J, Helmering, J, Kim, KW, Wang, M, Fordstrom, P*, et al.* Generation and characterization of two novel mouse models exhibiting the phenotypes of the metabolic syndrome: Apob48-/-Lepob/ob mice devoid of ApoE or Ldlr. *Am J Physiol Endocrinol Metab*. 2008;294:E496-505.

Lo, J, Dolan, SE, Kanter, JR, Hemphill, LC, Connelly, JM, Lees, RS*, et al.* Effects of obesity, body composition, and adiponectin on carotid intima-media thickness in healthy women. *J Clin Endocrinol Metab*. 2006;91:1677-82.

Lonnqvist, F, Arner, P, Nordfors, L and Schalling, M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. *Nat Med*. 1995;1:950-3.

Luukkaa, V, Pesonen, U, Huhtaniemi, I, Lehtonen, A, Tilvis, R, Tuomilehto, J*, et al.* Inverse correlation between serum testosterone and leptin in men. *J Clin Endocrinol Metab*. 1998;83:3243-6.

Ma, W, Huang, T, Zheng, Y, Wang, M, Bray, GA, Sacks, FM*, et al.* Weight-loss diets, adiponectin, and changes in cardiometabolic risk in the 2-year POUNDS Lost Trial. *J Clin Endocrinol Metab*. 2016;101:2415-22.

Maahs, DM, Ogden, LG, Kinney, GL, Wadwa, P, Snell-Bergeon, JK, Dabelea, D*, et al.* Low plasma adiponectin levels predict progression of coronary artery calcification. *Circulation*. 2005;111:747-53.

Mach, F, Schönbeck, U, Sukhova, GK, Atkinson, E and Libby, P. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. *Nature*. 1998;394:200-3.

Maeda, K, Okubo, K, Shimomura, I, Funahashi, T, Matsuzawa, Y and Matsubara, K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun*. 1996;221:286-9.

Maeda, N, Shimomura, I, Kishida, K, Nishizawa, H, Matsuda, M, Nagaretani, H*, et al.* Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med*. 2002;8:731-7.

Magnussen, CG, Koskinen, J, Chen, W, Thomson, R, Schmidt, MD, Srinivasan, SR*, et al.* Pediatric metabolic syndrome predicts adulthood metabolic syndrome, subclinical atherosclerosis, and type 2 diabetes mellitus but is no better than body mass index alone: the Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study. *Circulation*. 2010;122:1604-11.

Mahmud, A and Feely, J. Adiponectin and arterial stiffness. *Am J Hypertens*. 2005;18:1543-8.

Mahoney, LT, Burns, TL, Stanford, W, Thompson, BH, Witt, JD, Rost, CA*, et al.* Coronary risk factors measured in childhood and young adult life are associated with coronary artery calcification in young adults: the Muscatine Study. *J Am Coll Cardiol*. 1996;27:277-84.

Maingrette, F and Renier, G. Leptin increases lipoprotein lipase secretion by macrophages: involvement of oxidative stress and protein kinase C. *Diabetes*. 2003;52:2121-8.

Mark, AL, Shaffer, RA, Correia, ML, Morgan, DA, Sigmund, CD and Haynes, WG. Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice. *J Hypertens*. 1999;17:1949-53.

Marma, AK, Berry, JD, Ning, H, Persell, SD and Lloyd-Jones, DM. Distribution of 10-year and lifetime predicted risks for cardiovascular disease in US adults: findings from the National Health and Nutrition Examination Survey 2003 to 2006. *Circ Cardiovasc Qual Outcomes*. 2010;3:8-14.

Martin, SS, Blaha, MJ, Muse, ED, Qasim, AN, Reilly, MP, Blumenthal, RS*, et al.* Leptin and incident cardiovascular disease: the Multi-ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*. 2015;239:67-72.

Martin-Romero, C, Santos-Alvarez, J, Goberna, R and Sanchez-Margalet, V. Human leptin enhances activation and proliferation of human circulating T lymphocytes. *Cell Immunol*. 2000;199:15-24.

Matsuzawa, Y, Funahashi, T and Nakamura, T. The concept of metabolic syndrome: contribution of visceral fat accumulation and its molecular mechanism. *J Atheroscler Thromb*. 2011;18:629-39.

Matthews, DR, Hosker, JP, Rudenski, AS, Naylor, BA, Treacher, DF and 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.

Maury, E and Brichard, SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol*. 2010;314:1-16.

McNamara, JJ, Molot, MA, Stremple, JF and Cutting, RT. Coronary artery disease in combat casualties in Vietnam. *JAMA*. 1971;216:1185-7.

Montague, CT, Farooqi, IS, Whitehead, JP, Soos, MA, Rau, H, Wareham, NJ*, et al.* Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*. 1997;387:903-8.

Morioka, T, Emoto, M, Yamazaki, Y, Kawano, N, Imamura, S, Numaguchi, R*, et al.* Leptin is associated with vascular endothelial function in overweight patients with type 2 diabetes. *Cardiovasc Diabetol*. 2014;13:10.

Morrison, JA, deGroot, I, Edwards, BK, Kelly, KA, Rauh, JL, Mellies, M*, et al.* Plasma cholesterol and triglyceride levels in 6,775 school children, ages 6--17. *Metabolism*. 1977;26:1199-211.

Morrow, DA and de Lemos, JA. Benchmarks for the assessment of novel cardiovascular biomarkers. *Circulation*. 2007;115:949-52.

Motoshima, H, Wu, X, Mahadev, K and Goldstein, BJ. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. *Biochem Biophys Res Commun*. 2004;315:264-71.

Muoio, DM and Lynis Dohm, G. Peripheral metabolic actions of leptin. *Best Pract Res Clin Endocrinol Metab*. 2002;16:653-66.

Murphy, WA, Jr., Nedden Dz, D, Gostner, P, Knapp, R, Recheis, W and Seidler, H. The iceman: discovery and imaging. *Radiology*. 2003;226:614-29.

Napoli, C, D'Armiento, FP, Mancini, FP, Postiglione, A, Witztum, JL, Palumbo, G*, et al.* Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest*. 1997;100:2680-90.

Neunteufl, T, Katzenschlager, R, Hassan, A, Klaar, U, Schwarzacher, S, Glogar, D*, et al.* Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis*. 1997;129:111-8.

Ng, M, Fleming, T, Robinson, M, Thomson, B, Graetz, N, Margono, C*, et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384:766-81.

Nguyen, QM, Srinivasan, SR, Xu, JH, Chen, W and Berenson, GS. Racial (black-white) divergence in the association between adiponectin and arterial stiffness in asymptomatic young adults: the Bogalusa heart study. *Am J Hypertens*. 2008;21:553-7.

Nicoletti, A, Kaveri, S, Caligiuri, G, Bariety, J and Hansson, GK. Immunoglobulin treatment reduces atherosclerosis in apo E knockout mice. *J Clin Invest*. 1998;102:910-8.

Niiranen, TJ and Vasan, RS. Epidemiology of cardiovascular disease: recent novel outlooks on risk factors and clinical approaches. *Expert Rev Cardiovasc Ther*. 2016;14:855-69.

Nikam, N, Steinberg, TB and Steinberg, DH. Advances in stent technologies and their effect on clinical efficacy and safety. *Med Devices (Auckl)*. 2014;7:165-78.

Nilsson, PM, Engström, G, Hedblad, B, Frystyk, J, Persson, MM, Berglund, G*, et al.* Plasma adiponectin levels in relation to carotid intima media thickness and markers of insulin resistance. *Arterioscler Thromb Vasc Biol*. 2006;26:2758-62.

Nishimura, M, Izumiya, Y, Higuchi, A, Shibata, R, Qiu, J, Kudo, C*, et al.* Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase dependent mechanisms. *Circulation*. 2008;117:216-23.

Norata, GD, Raselli, S, Grigore, L, Garlaschelli, K, Dozio, E, Magni, P*, et al.* Leptin:adiponectin ratio is an independent predictor of intima media thickness of the common carotid artery. *Stroke*. 2007;38:2844-6.

Ntaios, G, Gatselis, NK, Makaritsis, K and Dalekos, GN. Adipokines as mediators of endothelial function and atherosclerosis. *Atherosclerosis*. 2013;227:216-21.

O'Leary, DH, Polak, JF, Kronmal, RA, Manolio, TA, Burke, GL and Wolfson, SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med*. 1999;340:14-22.

Oda, A, Taniguchi, T and Yokoyama, M. Leptin stimulates rat aortic smooth muscle cell proliferation and migration. *Kobe J Med Sci*. 2001;47:141-50.

Ohashi, K, Ouchi, N and Matsuzawa, Y. Adiponectin and hypertension. *Am J Hypertens*. 2011;24:263-9.

Okada-Iwabu, M, Yamauchi, T, Iwabu, M, Honma, T, Hamagami, K, Matsuda, K*, et al.* A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. *Nature*. 2013;503:493-9.

Okamoto, Y, Folco, EJ, Minami, M, Wara, AK, Feinberg, MW, Sukhova, GK*, et al.* Adiponectin inhibits the production of CXC receptor 3 chemokine ligands in macrophages and reduces T-lymphocyte recruitment in atherogenesis. *Circ Res*. 2008;102:218-25.

Okamoto, Y, Kihara, S, Ouchi, N, Nishida, M, Arita, Y, Kumada, M*, et al.* Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation*. 2002;106:2767-70.

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

Ouchi, N. Adipocytokines in Cardiovascular and Metabolic Diseases. *J Atheroscler Thromb*. 2016;23:645-54.

Ouchi, N, Kihara, S, Arita, Y, Maeda, K, Kuriyama, H, Okamoto, Y*, et al.* Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*. 1999;100:2473-6.

Ouchi, N, Kihara, S, Arita, Y, Nishida, M, Matsuyama, A, Okamoto, Y*, et al.* Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation*. 2001;103:1057-63.

Ouchi, N and Walsh, K. A novel role for adiponectin in the regulation of inflammation. *Arterioscler Thromb Vasc Biol*. 2008;28:1219-21.

Patel, S, Flyvbjerg, A, Kozakova, M, Frystyk, J, Ibrahim, IM, Petrie, JR*, et al.* Variation in the ADIPOQ gene promoter is associated with carotid intima media thickness independent of plasma adiponectin levels in healthy subjects. *Eur Heart J*. 2008;29:386-93.

Pedersen, TR, Olsson, AG, Faergeman, O, Kjekshus, J, Wedel, H, Berg, K*, et al.* Lipoprotein changes and reduction in the incidence of major coronary heart disease events in the Scandinavian Simvastatin Survival Study (4S). *Circulation*. 1998;97:1453-60.

Pencina, MJ, D'Agostino, RB, Pencina, KM, Janssens, AC and Greenland, P. Interpreting incremental value of markers added to risk prediction models. *Am J Epidemiol*. 2012;176:473-81.

Pencina, MJ, D'Agostino, RB, Sr., D'Agostino, RB, Jr. and Vasan, RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med*. 2008;27:157-72; discussion 207-12.

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

Persson, J, Strawbridge, RJ, McLeod, O, Gertow, K, Silveira, A, Baldassarre, D*, et al.* Sex-Specific Effects of Adiponectin on Carotid Intima-Media Thickness and Incident Cardiovascular Disease. *J Am Heart Assoc*. 2015;4:e001853.

Pesonen, E, Norio, R and Sarna, S. Thickenings in the coronary arteries in infancy as an indication of genetic factors in coronary heart disease. *Circulation*. 1975;51:218-25.

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

Pilz, S, Horejsi, R, Moller, R, Almer, G, Scharnagl, H, Stojakovic, T*, et al.* Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. *J Clin Endocrinol Metab*. 2005;90:4792-6.

Pischon, T, Girman, CJ, Hotamisligil, GS, Rifai, N, Hu, FB and Rimm, EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA*. 2004;291:1730-7.

Poli, A, Tremoli, E, Colombo, A, Sirtori, M, Pignoli, P and Paoletti, R. Ultrasonographic measurement of the common carotid artery wall thickness in hypercholesterolemic patients. A new model for the quantitation and follow-up of preclinical atherosclerosis in living human subjects. *Atherosclerosis*. 1988;70:253-61.

Porkka, KV, Viikari, JS, Taimela, S, Dahl, M and Åkerblom, HK. Tracking and predictiveness of serum lipid and lipoprotein measurements in childhood: a 12-year follow-up. The Cardiovascular Risk in Young Finns study. *Am J Epidemiol*. 1994;140:1096-110.

Prineas, RJ, Gillum, RF, Horibe, H and Hannan, PJ. The Minneapolis children's blood pressure study. Part 1: standards of measurement for children's blood pressure. *Hypertension*. 1980;2:I18-24.

Puska, P, Tuomilehto, J, Salonen, J, Neittaanmäki, L, Mäki, J, Virtamo, J*, et al.* Changes in coronary risk factors during comprehensive five-year community programme to control cardiovascular diseases (North Karelia project). *Br Med J*. 1979;2:1173-8.

Puska, P, Vartiainen, E, Nissinen, A, Laatikainen, T and Jousilahti, P. Background, Principles, Implementation, and General Experiences of the North Karelia Project. *Glob Heart*. 2016;11:173-8.

Raitakari, M, Mansikkaniemi, K, Marniemi, J, Viikari, JSA and 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, 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. *JAMA*. 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.

Reaven, GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*. 1988;37:1595-607.

Reaven, GM. The metabolic syndrome: time to get off the merry-go-round? *J Intern Med*. 2011;269:127-36.

Reilly, MP, Iqbal, N, Schutta, M, Wolfe, ML, Scally, M, Localio, AR*, et al.* Plasma leptin levels are associated with coronary atherosclerosis in type 2 diabetes. *J Clin Endocrinol Metab.* 2004;89:3872-8.

Reis, JP, Macera, CA, Wingard, DL, Araneta, MR, Lindsay, SP and Marshall, SJ. The relation of leptin and insulin with obesity-related cardiovascular risk factors in US adults. *Atherosclerosis*. 2008;200:150-60.

Rhee, CM, Nguyen, DV, Moradi, H, Brunelli, SM, Dukkipati, R, Jing, J*, et al.* Association of Adiponectin With Body Composition and Mortality in Hemodialysis Patients. *Am J Kidney Dis*. 2015;66:313-21.

Ridker, PM. From C-Reactive Protein to Interleukin-6 to Interleukin-1: Moving Upstream To Identify Novel Targets for Atheroprotection. *Circ Res*. 2016;118:145-56.

Ridker, PM, Buring, JE, Cook, NR and Rifai, N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation*. 2003;107:391-7.

Rifkind, BM. Lipid Research Clinics Coronary Primary Prevention Trial: results and implications. *Am J Cardiol*. 1984;54:30C-4C.

Rimm, EB, Giovannucci, EL, Willett, WC, Colditz, GA, Ascherio, A, Rosner, B*, et al.* Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet*. 1991;338:464-8.

Robinson, JG, Farnier, M, Krempf, M, Bergeron, J, Luc, G, Averna, M*, et al.* Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372:1489-99.

Rönnemaa, T, Knip, M, Lautala, P, Viikari, J, Uhari, M, Leino, A*, et al.* Serum insulin and other cardiovascular risk indicators in children, adolescents and young adults. *Ann Med*. 1991;23:67-72.

Rosendorff, C, Lackland, DT, Allison, M, Aronow, WS, Black, HR, Blumenthal, RS*, et al.* Treatment of hypertension in patients with coronary artery disease: a scientific statement from the American Heart Association, American College of Cardiology, and American Society of Hypertension. *Circulation*. 2015;131: e435-70.

Ross, R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*. 1993;362:801-9.

Ross, R. Atherosclerosis--an inflammatory disease. *New Engl J Med*. 1999;340:115-26.

Ross, R, Glomset, J and Harker, L. Response to injury and atherogenesis. *Am J Pathol*. 1977;86:675-84.

Rubin, DA, McMurray, RG, Hackney, AC and Harrell, JS. Relationship between cardiovascular risk factors and adipokines in adolescents. *Horm Res Paediatr*. 2011;76:123-9.

Ryo, M, Nakamura, T, Kihara, S, Kumada, M, Shibazaki, S, Takahashi, M*, et al.* Adiponectin as a biomarker of the metabolic syndrome. *Circ J*. 2004;68:975-81.

Saad, MF, Damani, S, Gingerich, RL, Riad-Gabriel, MG, Khan, A, Boyadjian, R*, et al.* Sexual dimorphism in plasma leptin concentration. *J Clin Endocrinol Metab*. 1997;82:579-84.

Sabatine, MS, Giugliano, RP, Wiviott, SD, Raal, FJ, Blom, DJ, Robinson, J*, et al.* Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372:1500-9.

Salonen, JT, Puska, P and Mustaniemi, H. Changes in morbidity and mortality during comprehensive community programme to control cardiovascular diseases during 1972-7 in North Karelia. *Br Med J*. 1979;2:1178-83.

Santaniemi, M, Kesäniemi, YA and Ukkola, O. Low plasma adiponectin concentration is an indicator of the metabolic syndrome. *Eur J Endocrinol*. 2006;155:745-50.

Santaniemi, M, Ukkola, O, Malo, E, Bloigu, R and Kesäniemi, YA. Metabolic syndrome in the prediction of cardiovascular events: the potential additive role of hsCRP and adiponectin. *Eur J Prev Cardiol*. 2014;21:1242-8.

Santos-Alvarez, J, Goberna, R and Sanchez-Margalet, V. Human leptin stimulates proliferation and activation of human circulating monocytes. *Cell Immunol*. 1999;194:6-11.

Sattar, N, McConnachie, A, Shaper, AG, Blauw, GJ, Buckley, BM, de Craen, AJ*, et al.* Can metabolic syndrome usefully predict cardiovascular disease and diabetes? Outcome data from two prospective studies. *Lancet*. 2008;371: 1927-35.

Savage, DB and O'Rahilly, S. Leptin: a novel therapeutic role in lipodystrophy. *J Clin Invest*. 2002;109:1285-6.

Savill, J, Dransfield, I, Gregory, C and Haslett, C. A blast from the past: clearance of apoptotic cells regulates immune responses. *Nat Rev Immunol*. 2002;2:965-75.

Scherer, PE, Williams, S, Fogliano, M, Baldini, G and Lodish, HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem*. 1995;270:26746-9.

Schäfer, K, Halle, M, Goeschen, C, Dellas, C, Pynn, M, Loskutoff, DJ*, et al.* Leptin promotes vascular remodeling and neointimal growth in mice. *Arterioscler Thromb Vasc Biol*. 2004;24:112-7.

Schönbeck, U and Libby, P. Inflammation, immunity, and HMG-CoA reductase inhibitors: statins as antiinflammatory agents? *Circulation*. 2004;109:II18-26.

Seino, Y, Hirose, H, Saito, I and Itoh, H. High-molecular-weight adiponectin is a predictor of progression to metabolic syndrome: a population-based 6-year follow-up study in Japanese men. *Metabolism*. 2009;58:355-60.

Shamsuzzaman, ASM, Winnicki, M, Wolk, R, Svatikova, A, Phillips, BG, Davison, DE*, et al.* Independent association between plasma leptin and C-reactive protein in healthy humans. *Circulation*. 2004;109:2181-5.

Shek, EW, Brands, MW and Hall, JE. Chronic leptin infusion increases arterial pressure. *Hypertension*. 1998;31:409-14.

Shibata, R, Sato, K, Pimentel, DR, Takemura, Y, Kihara, S, Ohashi, K*, et al.* Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat Med*. 2005;11:1096-103.

Shimabukuro, M, Higa, N, Asahi, T, Oshiro, Y, Takasu, N, Tagawa, T*, et al.* Hypoadiponectinemia is closely linked to endothelial dysfunction in man. *J Clin Endocrinol Metab*. 2003;88:3236-40.

Simell, O, Niinikoski, H, Rönnemaa, T, Raitakari, OT, Lagström, H, Laurinen, M*, et al.* Cohort Profile: the STRIP Study (Special Turku Coronary Risk Factor Intervention Project), an Infancy-onset Dietary and Life-style Intervention Trial. *Int J Epidemiol*. 2009;38:650-5.

Simmons, RK, Alberti, KG, Gale, EA, Colagiuri, S, Tuomilehto, J, Qiao, Q*, et al.* The metabolic syndrome: useful concept or clinical tool? Report of a WHO Expert Consultation. *Diabetologia*. 2010;53:600-5.

Sinaiko, AR, Donahue, RP, Jacobs, DR, Jr. and Prineas, RJ. Relation of weight and rate of increase in weight during childhood and adolescence to body size, blood pressure, fasting insulin, and lipids in young adults. The Minneapolis Children's Blood Pressure Study. *Circulation*. 1999;99:1471-6.

Singhal, A, Farooqi, IS, Cole, TJ, O'Rahilly, S, Fewtrell, M, Kattenhorn, M*, et al.* Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? *Circulation*. 2002;106:1919-24.

Singhal, A, Jamieson, N, Fewtrell, M, Deanfield, J, Lucas, A and Sattar, N. Adiponectin predicts insulin resistance but not endothelial function in young, healthy adolescents. *J Clin Endocrinol Metab*. 2005;90:4615-21.

Stary, HC, Chandler, AB, Dinsmore, RE, Fuster, V, Glagov, S, Insull, W, Jr*., et al.* A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*. 1995;92:1355-74.

Stary, HC, Chandler, AB, Glagov, S, Guyton, JR, Insull, W, Jr., Rosenfeld, ME*, et al.* A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*. 1994;89:2462-78.

Steinberg, D. In celebration of the 100th anniversary of the lipid hypothesis of atherosclerosis. *J Lipid Res*. 2013;54:2946-9.

Störk, S, Bots, ML, Angerer, P, von Schacky, C, Grobbee, DE, Angermann, CE*, et al.* Low levels of adiponectin predict worsening of arterial morphology and function. *Atherosclerosis*. 2007;194:e147-53.

Strandberg, TE, Salomaa, V, Strandberg, AY, Vanhanen, H, Sarna, S, Pitkälä, K*, et al.* Cohort Profile: The Helsinki Businessmen Study (HBS). *Int J Epidemiol*. 2016;45:1074-4h.

Takemura, Y, Ouchi, N, Shibata, R, Aprahamian, T, Kirber, MT, Summer, RS*, et al.* Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. *J Clin Invest*. 2007;117:375-86.

Tartaglia, LA, Dembski, M, Weng, X, Deng, N, Culpepper, J, Devos, R*, et al.* Identification and expression cloning of a leptin receptor, OB-R. *Cell*. 1995;83:1263-71.

The National Institutes of Health Consensus Development Panel. Lowering blood cholesterol to prevent heart disease. NIH Consensus Development Conference statement. *Arteriosclerosis*. 1985;5:404-12.

Thomas, GS, Wann, LS, Allam, AH, Thompson, RC, Michalik, DE, Sutherland, ML*, et al.* Why did ancient people have atherosclerosis?: from autopsies to computed tomography to potential causes. *Glob Heart*. 2014;9:229-37.

Thompson, DR, Obarzanek, E, Franko, DL, Barton, BA, Morrison, J, Biro, FM*, et al.* Childhood overweight and cardiovascular disease risk factors: the National Heart, Lung, and Blood Institute Growth and Health Study. *J Pediatr*. 2007;150:18-25.

Thompson, RC, Allam, AH, Lombardi, GP, Wann, LS, Sutherland, ML, Sutherland, JD*, et al.* Atherosclerosis across 4000 years of human history: the Horus study of four ancient populations. *Lancet*. 2013;381:1211-22.

Thoonen, R, Hindle, AG and Scherrer-Crosbie, M. Brown adipose tissue: the heat is on the heart. *Am J Physiol Heart Circ Physiol*. 2016;310:H1592-605.

Tounian, P, Aggoun, Y, Dubern, B, Varille, V, Guy-Grand, B, Sidi, D*, et al.* Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. *Lancet*. 2001;358:1400-4.

Trayhurn, P and Wood, IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr*. 2004;92:347-55.

Tsao, CW and Vasan, RS. Cohort Profile: The Framingham Heart Study (FHS): overview of milestones in cardiovascular epidemiology. *Int J Epidemiol*. 2015;44:1800-13.

Tsioufis, C, Dimitriadis, K, Selima, M, Thomopoulos, C, Mihas, C, Skiadas, I*, et al.* Low-grade inflammation and hypoadiponectinaemia have an additive detrimental effect on aortic stiffness in essential hypertensive patients. *Eur Heart J*. 2007;28:1162-9.

Turer, AT and Scherer, PE. Adiponectin: mechanistic insights and clinical implications. *Diabetologia*. 2012;55:2319-26.

US Preventive Services Task Force, Bibbins-Domingo, K, Grossman, DC, Curry, SJ, Davidson, KW, Epling, JW, Jr., *et al.* Screening for Lipid Disorders in Children and Adolescents: US Preventive Services Task Force Recommendation Statement. *JAMA*. 2016;316:625-33.

van den Oord, SC, Sijbrands, EJ, ten Kate, GL, van Klaveren, D, van Domburg, RT, van der Steen, AF*, et al.* Carotid intima-media thickness for cardiovascular risk assessment: systematic review and meta-analysis. *Atherosclerosis*. 2013;228:1-11.

Van Gaal, LF, Mertens, IL and De Block, CE. Mechanisms linking obesity with cardiovascular disease. *Nature*. 2006;444:875-80.

Vartiainen, E, Laatikainen, T, Salomaa, V, Jousilahti, P, Peltonen, M and Puska, P. Sydäninfarkti- ja aivohalvausriskin arviointi FINRISKI -tutkimuksessa (in Finnish). *Suom Lääkäril*. 2007;62:4507-13.

Velarde, GP, Sherazi, S, Kraemer, DF, Bravo-Jaimes, K, Butterfield, R, Amico, T*, et al.* Clinical and Biochemical Markers of Cardiovascular Structure and Function in Women With the Metabolic Syndrome. *Am J Cardiol*. 2015;116:1705-10.

Venn, AJ, Thomson, RJ, Schmidt, MD, Cleland, VJ, Curry, BA, Gennat, HC*, et al.* Overweight and obesity from childhood to adulthood: a follow-up of participants in the 1985 Australian Schools Health and Fitness Survey. *Med J Aust*. 2007;186:458-60.

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.

Virtanen, KA, Lidell, ME, Orava, J, Heglind, M, Westergren, R, Niemi, T*, et al.* Functional brown adipose tissue in healthy adults. *N Engl J Med*. 2009;360:1518-25.

Voors, AW, Webber, LS, Frerichs, RR and Berenson, GS. Body height and body mass as determinants of basal blood pressure in children--The Bogalusa Heart Study. *Am J Epidemiol*. 1977;106:101-8.

Wallace, AM, McMahon, AD, Packard, CJ, Kelly, A, Shepherd, J, Gaw, A*, et al.* Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation*. 2001;104:3052-6.

Wang, Z and Nakayama, T. Inflammation, a link between obesity and cardiovascular disease. *Mediators Inflamm*. 2010;2010:535918.

Wang, ZV and Scherer, PE. Adiponectin, the past two decades. *J Mol Cell Biol*. 2016;8:93-100.

Webber, BJ, Seguin, PG, Burnett, DG, Clark, LL and Otto, JL. Prevalence of and risk factors for autopsy-determined atherosclerosis among US service members, 2001-2011. *JAMA*. 2012;308:2577-83.

Webber, LS, Srinivasan, SR, Wattigney, WA and Berenson, GS. Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. *Am J Epidemiol*. 1991;133:884-99.

Wensley, F, Gao, P, Burgess, S, Kaptoge, S, Di Angelantonio, E, Shah, T*, et al.* Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. *Br Med J*. 2011;342:d548.

Wiegman, A, Gidding, SS, Watts, GF, Chapman, MJ, Ginsberg, HN, Cuchel, M*, et al.* Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. *Eur Heart J*. 2015;36:2425-37.

Wilkins, JT, Ning, H, Berry, J, Zhao, L, Dyer, AR and Lloyd-Jones, DM. Lifetime risk and years lived free of total cardiovascular disease. *JAMA*. 2012;308:1795-801.

Williams, MJA, Williams, SM, Milne, BJ, Hancox, RJ and Poulton, R. Association between C-reactive protein, metabolic cardiovascular risk factors, obesity and oral contraceptive use in young adults. *Int J Obes Relat Metab Disord*. 2004;28:998-1003.

Winer, JC, Zern, TL, Taksali, SE, Dziura, J, Cali, AM, Wollschlager, M*, et al.* Adiponectin in childhood and adolescent obesity and its association with inflammatory markers and components of the metabolic syndrome. *J Clin Endocrinol Metab*. 2006;91:4415-23.

Working Group for the National Obesity Programme. Lihavuus laskuun - Hyvinvointia ravinnosta ja liikunnasta (in Finnish). National Obesity Programme 2012-2015. National Institute for Health and Welfare (THL). Directions 13/2013. Helsinki, Finland 2013.

Wright, JT, Jr., Williamson, JD, Whelton, PK, Snyder, JK, Sink, KM, Rocco, MV*, et al.* A Randomized Trial of Intensive versus Standard Blood-Pressure Control. *N Engl J Med*. 2015;373:2103-16.

Xu, SQ, Mahadev, K, Wu, X, Fuchsel, L, Donnelly, S, Scalia, RG*, et al.* Adiponectin protects against angiotensin II or tumor necrosis factor alpha-induced endothelial cell monolayer hyperpermeability: role of cAMP/PKA signaling. *Arterioscler Thromb Vasc Biol*. 2008;28:899-905.

Yaghootkar, H, Lamina, C, Scott, RA, Dastani, Z, Hivert, MF, Warren, LL*, et al.* Mendelian randomization studies do not support a causal role for reduced circulating adiponectin levels in insulin resistance and type 2 diabetes. *Diabetes*. 2013;62:3589-98.

Yahya, RS, Atwa, MA, El-Sayed, IH, El-Ghanaam, DM, Hussein, DT and El-Taweel, FA. Adipocytokines in Patients with Chronic Kidney Disease Stage 5. *Clin Lab*. 2016;62:21-30.

Yamauchi, T, Kamon, J, Ito, Y, Tsuchida, A, Yokomizo, T, Kita, S*, et al.* Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*. 2003;423:762-9.

Yamauchi, T, Nio, Y, Maki, T, Kobayashi, M, Takazawa, T, Iwabu, M*, et al.* Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med*. 2007;13:332-9.

Yilmaz, MI, Saglam, M, Qureshi, AR, Carrero, JJ, Caglar, K, Eyileten, T*, et al.* Endothelial dysfunction in type-2 diabetics with early diabetic nephropathy is associated with low circulating adiponectin. *Nephrol Dial Transplant*. 2008;23:1621-7.

Yusuf, S, Hawken, S, Ounpuu, S, Dans, T, Avezum, A, Lanas, F*, et al.* Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*. 2004;364:937-52.

Zhang, H, Mo, X, Hao, Y, Huang, J, Lu, X, Cao, J*, et al.* Adiponectin levels and risk of coronary heart disease: a meta-analysis of prospective studies. *Am J Med Sci*. 2013;345:455-61.

Zhang, Y, Proenca, R, Maffei, M, Barone, M, Leopold, L and Friedman, JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994;372:425-32.

Zhang, Y-Y, Gottardo, L, Mlynarski, W, Frazier, W, Nolan, D, Duffy, J*, et al.* Genetic variability at the leptin receptor (LEPR) locus is a determinant of plasma fibrinogen and C-reactive protein levels. *Atherosclerosis*. 2007;191:121-7.

Åkerblom, HK, Viikari, J and Kouvalainen, K. Cardiovascular risk factors in Finnish children and adolescents. *Acta Paediatr Scand Suppl*. 1985a;318:5-6.

Åkerblom, HK, Viikari, J, Uhari, M, Räsänen, L, Byckling, T, Louhivuori, K*, et al.* Atherosclerosis precursors in Finnish children and adolescents. I. General description of the cross-sectional study of 1980, and an account of the children's and families' state of health. *Acta Paediatr Scand Suppl*. 1985b;318:49-63.