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GENETIC AND EPIDEMIOLOGICAL STUDIES ON THE ROLE OF ADIPONECTIN AND PTP1B IN THE METABOLIC SYNDROME

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GENETIC AND EPIDEMIOLOGICAL STUDIES ON THE ROLE OF ADIPONECTIN AND PTP1B IN THE METABOLIC SYNDROME

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Abstract

The metabolic syndrome is a cluster of components predisposing to type 2 diabetes and cardiovascular disease. Abdominal obesity and insulin resistance seem to be central in the metabolic syndrome, although no unifying pathophysiological mechanism is available. The aim of this thesis was to determine out how the variation in PTP1B and adiponectin gene as well as variations in the plasma adiponectin concentration contribute to the risk of obesity related diseases.

PTP1B is a negative regulator of insulin signalling and therefore considered a candidate gene for type 2 diabetes. In the first study, it was found that three PTP1B polymorphisms studied have not strong impact on type 2 diabetes. However, one SNP may be slightly protective against type 2 diabetes, since it was more frequent in the healthy group compared to group of patients with type 2 diabetes. Another SNP was associated with body mass index (BMI). The combination of certain alleles of PTP1B and LEPR (leptin receptor) genes was also associated to BMI.

Adiponectin is an adipocytokine expressed in adipose tissue. It has insulin sensitizing effects in liver and muscle and it has also beneficial effects on cardiovascular health. In the second study, the contribution of adiponectin genotypes with obesity-related phenotypes was studied. In Caucasians, the carriers of rare allele of Tyr111His polymorphism were more insulin resistant and at a higher risk of developing type 2 diabetes. In African-Americans, other polymorphisms were associated with BMI and lipids. Thus, the effects of polymorphisms on obesity related phenotypes seemed to be different between ethnic groups.

Plasma adiponectin levels were measured from different study groups. In the third study, it was found out that low plasma adiponectin levels associated with different components of the metabolic syndrome and there was a trend towards reductions in adiponectin with an increasing number of components. Fourth study indicated that baseline low adiponectin level associated with a more than 2-fold risk for developing impaired glucose tolerance or type 2 diabetes in the follow-up study of normoglycemic middle-aged Finnish subjects. In the fifth study, plasma adiponectin levels were measured from postmenopausal women receiving estrogen replacement therapy. We observed a reduction in adiponectin levels in women having peroral estradiol which could be part of the "early harm" profile on cardiovascular risk factors of the peroral estrogen replacement therapy detected in clinical trials.

These studies further strengthen the role of plasma adiponectin in the obesity related diseases and bring new information of polymorphisms in the adiponectin and PTP1B genes in different populations.

Keywords: adiponectin, estrogen replacement therapy, genetic association studies, metabolic syndrome, obesity, protein tyrosine phosphatase non-receptor type 1, type 2 diabetes

Santaniemi, Merja, Geneettisiä ja epidemiologisia tutkimuksia adiponektiinin ja PTP1B:n yhteydestä metaboliseen oireyhtymään
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Tiivistelmä

Metabolinen oireyhtymä on kertymä tekijöitä, jotka altistavat tyypin 2 diabetekselle ja sydän- ja verisuonitaudeille. Keskivartalolihavuus ja insuliiniresistenssi, eli insuliinin heikentynyt teho, vaikuttavat olevan keskeisiä metabolisessa oireyhtymässä. Kuitenkaan taustalla olevaa syntymekanismia ei täysin tunneta. Väitöskirjatyön tavoitteena oli tutkia PTP1B- ja adiponektiinigeenin muuntelun sekä plasman adiponektiinitason yhteyttä metaboliseen oireyhtymään, sen osatekijöihin ja seurauksiin.

PTP1B on insuliinin toimintaa soluissa estävä molekyyli. Ensimmäisessä tutkimuksessa havaittiin että kolme tutkittua PTP1B-geenin nukleotidimuutosta eivät ole vahvasti yhteydessä tyypin 2 diabetekseen. Eräs nukleotidimuutos saattaisi olla lievästi suojaava tyypin 2 diabetesta vastaan, sillä se oli yleisempi terveillä kuin tyypin 2 diabetesta sairastavilla. PTP1B:n ja leptiinireseptorigeenin eräiden alleelien yhdistelmä oli yhteydessä painoindeksiin.

Adiponektiini on rasvakudoksen erittämä hormoni, jolla on suotuisia, insuliinin vaikutusta edesauttavia vaikutuksia elimistössä sekä edullisia vaikutuksia verenkiertoelimistössä. Toisessa työssä havaittiin että Amerikan valkoihoisilla, joilla oli eräs harvinainen adiponektiinigeenin alleeli (Tyr111His), oli heikompi insuliinin teho kuin henkilöillä joilla ei ollut kyseistä muutosta. Tämä alleeli oli yleisempi suomalaisilla tyypin 2 diabetesta sairastavilla kuin terveillä, mikä saattaa tarkoittaa että se liittyy suurentuneeseen riskiin tyypin 2 diabetekselle. Afroamerikkalaisilla taas toiset nukleotidimuutokset olivat yhteydessä lihavuuteen ja plasman rasva-arvoihin.

Adiponektiinin pitoisuutta plasmassa mitattiin erilaisissa aineistoissa. Kolmannessa tutkimuksessa havaittiin, että matala pitoisuus oli yhteydessä metabolisen oireyhtymän eri osatekijöihin ja pitoisuus oli sitä matalampi, mitä enemmän osatekijöitä henkilöllä on. Neljännessä tutkimuksessa havaittiin että matala plasman adiponektiinipitoisuus oli yhteydessä suurentuneeseen riskiin saada huonontunut glukoosin sietokyky tai tyypin 2 diabetes tulevaisuudessa. Viidennessä tutkimuksessa adiponektiinitaso määritettiin naisilta jotka olivat ohittaneet vaihdevuodet ja saivat estrogeenikorvaushoitoa. Havaittiin että plasman adiponektiinitaso laski niillä naisilla, jotka saivat korvaushoitoa suun kautta. Tämä saattaisi osittain selittää suun kautta annettavan estrogeenikorvaushoidon epäedullista vaikutusta sydän ja -verisuonitautien riskitekijöihin.

Tutkimus vahvistaa edelleen adiponektiinin merkitystä lihavuuteen liittyvissä sairauksissa ja tuo uutta tietoa adiponektiini- ja PTP1B-geenien muuntelun merkityksestä eri väestöissä.

Asiasanat: adiponektiini, estrogeenikorvaushoito, geneettinen assosiaatiotutkimus, lihavuus, metabolinen oireyhtymä, proteiini tyrosiini fosfataasi 1B, tyypin 2 diabetes

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Oulu, April 10th Merja Santaniemi

Abbreviations

AdipoR1, AdipoR2	adiponectin receptors 1 and 2
ADIPOQ	adiponectin gene
AHA/ NHLBI	American Heart Association/ National Heart, Lung and Blood Institute
AIRglucose	acute insulin response to glucose
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AMPK	5' adenosine monophosphate-activated protein kinase
BMI	body mass index
CETP	cholesterol ester transfer protein
CI	confidence intervel
CVD	Cardiovascular disease
CHD	Coronary heart disease
CREB	c-AMP response element binding
CRP	C-reactive protein
DM2	type 2 diabetes mellitus
DNA	deoxyribonucleic acid
ERT	estrogen replacement therapy
FBG	fasting blood glucose
FDR	false discovery rate
FFA	free fatty acid
FPG	fasting plasma glucose
GHbA1	glycosylated haemoglobin A1
GWA	genome wide association study
HDL	high density lipoprotein
HOMA-IR	homeostasis model assessment–insulin resistance
ICAM-1	intercellular adhesion molecule 1
IDF	international diabetes federation
IGR	impaired glucose regulation
IGF-1	insulin-like growth factor 1
IL-6	interleukin 6
IR	insulin receptor
IRS-1,2,3	insulin receptor substrates 1, 2 and 3
IVGTT	intravenous glucose tolerance test
JAK2	janus kinase 2

kDa	kilodalton
LD	linkage disequilibrium
LDL	low density lipoprotein
LepR	leptin receptor
LEPR	leptin receptor gene
LPL	lipoprotein lipase
mRNA	messenger ribonucleic acid
NCEP ATP III	National Cholesterol Education Program Adult Treatment Panel III
NFAT	nuclear factor of activated T-cells
NO	nitric oxide
OPERA	Oulu Project Elucidating Risk of Atherosclerosis
PAI-1	plasminogen activator inhibitor-1
BP	blood pressure
PCR	polymerase chain reaction
PE	peroral estrogen
PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
PTP1B	protein tyrosine phosphatase 1B
PPARγ	peroxisome proliferator-activated receptor γ
RCT	reverse cholesterol transport
SD	standard deviation
SE	standard error
SI	insulin sensitivity index
SNP	single nucleotide polymorphism
STAT	signal transducers and activators of transcription
TG	triglycerides
TE	transdermal estrogen
TNF-α	tumour necrosis factor-alpha
TZDs	thiazolidinediones
VCAM-1	vascular cell adhesion molecule 1
VLDL	very low density lipoprotein
WH-ratio	waist to hip -ratio
WHO	world health organization

List of original articles

The thesis is based on the following original articles, which are referred to in the text by their Roman numerals:

I Santaniemi M, Ukkola O & Kesäniemi YA (2004) Tyrosine phosphatase 1B and leptin receptor genes and their interaction in type 2 diabetes. J Intern Med 256(1): 48–55.

II Ukkola O, Santaniemi M, Rankinen T, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bergman R, Kesäniemi YA & Bouchard C (2005) Adiponectin polymorphisms, adiposity and insulin metabolism: HERITAGE family study and Oulu diabetic study. Ann Med 37(2): 141–50.

III Santaniemi M, Kesäniemi YA & Ukkola O (2006) Low plasma adiponectin concentration is an indicator of the metabolic syndrome. Eur J Endocrinol 155(5): 745–50.

IV Jalovaara K, Santaniemi M, Timonen M, Jokelainen J, Kesäniemi YA, Ukkola O, Keinänen-Kiukaanniemi S & Rajala U (2008) Low serum adiponectin level as a predictor of impaired glucose regulation and type 2 diabetes mellitus in a middle-aged Finnish population. Metabolism 57(8): 1130–4.

V Kunnari A, Santaniemi M, Jokela M, Karjalainen AH, Heikkinen J, Ukkola O & Kesäniemi YA (2008) Estrogen replacement therapy decreases plasma adiponectin but not resistin in postmenopausal women. Metabolism 57(11): 1509–15.

Contents

1 Introduction

Our present environment with its excess nutritional energy and modern lifestyle with minimum physical activity can be said to have created an epidemic of obesity related diseases. Obesity and the associated insulin resistance are clearly linked to the metabolic syndrome, a constellation of risk factors of cardiovascular disease and type 2 diabetes. In recent decades, cardiovascular disease and diabetes have become the major cause of mortality in the Western developed world (Reviewed by Oda 2008.) In the year 2000 in Finland, 20.1% of men and 24.1% of women aged over 30 years were obese (body mass index (BMI) ≥ 30 kg/m^2). Over the past decades, overall obesity has become more common but also a trend towards more severe grades of obesity has taken place (Lahti-Koski *et al.* 2009.) Worldwide, more than 300 million people now have BMI values of 30 kg/m^2. This increasing level of obesity has a major impact on physical, mental, psychosocial and economic health. The mortality risk increases progressively with BMI especially within the BMI range above 30. The economic burden of obesity to society is considerable and anticipated to increase (Reviewed by Dixon 2009.)

There are several findings indicating that genetics is an important contributing factor in both obesity and type 2 diabetes. For instance, 10% of type 2 diabetic patients display normal weight, and many obese subjects never develop type 2 diabetes, indicating that type 2 diabetes is not exclusively caused by environmental factors. Type 2 diabetes is considered as a polygenic and a multifactorial disease. Single nucleotide polymorphisms (SNPs), exchanges of single base pairs in deoxyribonucleic acid (DNA), are responsible for approximately 90% of the sequence variation within the human genome (Reviewed in Staiger *et al.* 2009.) Therefore SNPs in the genes modulating for example adiposity, insulin sensitivity or insulin signaling could be determinants of an individual`s predisposition to complex diseases.

Based on the hypothesis above it is no wonder, that considerable effort is being expended on understanding the risk factors, biology and genetics behind the obesity related diseases. Since obesity associates with insulin resistance, the molecules involved in insulin signaling are of great interest. One of these molecules, protein tyrosine phosphatase 1B (PTP1B) has been found as negative regulator of insulin signaling (Reviewed by Koren & Fantus 2007). Also the way in which the excessive obesity acts to drive an inflammatory cascade is another exciting research area. Adipose tissue is an important regulatory, endocrine organ

playing a role in obesity associated conditions. Therefore, new molecules have been sought and extracted from adipose tissue. One such molecule is adiponectin, which is an insulin sensitizing and anti-inflammatory peptide hormone released from adipose tissue to circulation and its plasma levels are reduced in obesity (Reviewed by Andersson *et al.* 2008.)

In the present study, PTP1B and ADIPOQ genes have been evaluated as candidate genes for obesity and type 2 diabetes. In recent years, the plasma adiponectin level has been found as a marker of metabolic syndrome and as a possible link between obesity and type 2 diabetes and cardiovascular diseases (Reviewed in Brooks *et al.* 2007). This was the hypothesis tested in the original studies of this thesis. The following literature review presents the concept of the metabolic syndrome and the obesity as its core and the involvement of PTP1B and adiponectin in these processes.

2 Review of literature

2.1 Definition and components of metabolic syndrome

The concurrance of features of the metabolic syndrome was already described in the 1920s (Reviewed in Laaksonen *et al.* 2004). However, the finding that risk factors for heart diseases tend to cluster and seem clearly relate to risk of type 2 diabetes, only attracted public attention after the report of Reaven published in 1988. He postulated that insulin resistance led to clustering of risk factors and was the underlying cause of many cardiovascular diseases (CVD). He named the condition "syndrome X" and even though he did not actually include obesity into the cluster of risk factors, he did recommend weight loss as "treatment" for "syndrome X" (Reaven 1988). Since then, obesity has been added to the syndrome and the syndrome has been also called the "deadly quartet", "insulin resistance syndrome", "visceral fat syndrome" and "atherogenic metabolic triad" (Reviewed by Oda 2008).

A definition for metabolic syndrome was introduced by the World Health Organization (WHO) in 1998 (Alberti & Zimmet 1998). The definition was intended to achieve a better classification of individuals in research, rather than serve as a strict clinical definition. WHO mandated that insulin resistance must be present for the patient to have the metabolic syndrome. It was originally proposed that insulin resistance should be measured by the euglycemic hyperinsulinemic clamp. Other organizations have developed similar, but not identical definitions. The NCEP ATP III (National Cholesterol Education Program Adult Treatment Panel III) definition did not require direct demonstration of insulin resistance and thus definition is less stringent, requiring an individual to have three or more of the following: raised fasting plasma glucose, elevated blood pressure, elevated triglycerides, reduced HDL (high density lipoprotein), and abdominal obesity, defined by waist circumference (Reviewed by Huang 2009). The recent definition proposed by International Diabetes Federation (IDF) in 2005 for metabolic syndrome requires the individual to have central obesity, defined by waist circumference, plus any two of the following factors: increased triglycerides or treatment thereof; reduced HDL cholesterol or treatment thereof; increased blood pressure or treatment thereof; or raised fasting plasma glucose or previously diagnosed diabetes (Alberti *et al.* 2005). AHA/NHLBI (American Heart Association/National Heart, Lung and Blood Institute) presented in 2005 that in

contrast to IDF, they would maintain the ATP III criteria except for minor modifications. The threshold for IFG was reduced from 110 to 100 mg/dl which corresponds to the recently modified American Diabetes Association (ADA) criteria for impaired fasting glucose (IGR) (Grundy *et al.* 2005).

The most commonly used definitions (WHO, NCEP-ATPIII, AHA/NHLBI and IDF) for metabolic syndrome are presented in Table 1.

Table 1. Three definitions of the metabolic syndrome.

Factors	WHO (1999)	NCEP ATP III (2001) AHA/NHLBI (2005)	IDF (2005)
Required factor	High insulin levels, an elevated fasting blood glucose or an elevated post meal glucose alone:		Central obesity (as waist circumference ≥ 94cm for Europid men and ≥ 80cm for women, with ethnicity specific values for other groups)
Additional factors	Plus two or more of the following:	Any three or more of the following:	Plus two of the following:
	Abdominal obesity (waist to hip ratio of > 0.9 (men), > 0.85 (women), BMI of at least 30 kg/m^2	Abdominal obesity (waist girth > 102 cm (men), > 88 cm (women))	Raised TG level: ≥ 150 mg/dl (1.7 mmol/l), or specific treatment
	A TG level of at least 150 mg/dl or an HDL cholesterol lower than 35 mg/dl.	Serum TG > 150 mg/dl (1.7 mmol/l)	HDL cholesterol: < 40 mg/dl (1.03 mmol/l*) (males), < 50 mg/dl (1.29 mmol/l*) (females), or treatment
	BP of 140/90 mmHg or above (or on treatment)	HDL cholesterol 40mg/dl or lower (men) and 50mg/dl or lower (women)	Raised blood pressure: systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg, or treatment
		Blood pressure of 130/85 mm Hg or more or treatment	Raised fasting plasma glucose ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes
		> 110 mg/dL (6.1 mmol/l) (NCEP ATP III) / > 100mg/dl (5.6 mmol/l) (AHA/NHLBI) or treatment	

AHA/NHLBI = American Heart Association/ National Heart, Lung and Blood Institute, BMI = body mass index, BP = blood pressure, HDL = high density lipoprotein, IDF = International Diabetes Federation, NCEP ATP III = National Cholesterol Education Program Adult Treatment Panel III, TG = triglycerides, WHO = World Health Organization

Recently, the several major organizations met in an attempt to unify and harmonize the existing different criteria for metabolic syndrome. It was recommended that three abnormal findings out of five according to previous IDF criteria would qualify a person as suffering from the metabolic syndrome. It was decided that the obligatory waist criteria is not necessary. There is not enough data for ethnic specific waist measurements and therefore national or regional cut points for waist circumference can be used. In Europe, the IDF or NCEP ATP III criteria for waist can be used (Alberti *et al.* 2009).

The prevalence of metabolic syndrome is increasing throughout the world but the estimates of prevalence differ depending on the definition. There is an increasing prevalence of obesity in almost all countries. Grundy (2008) has reviewed the evidence and claimed that in most countries between 20% and 30% of the adult population can be characterized as having the metabolic syndrome. In Finland, one study of 45–64 year old subjects reported that the prevalence would be 38.8% in men and 22% in women according to the definitions of WHO (Ilanne-Parikka *et al.* 2004). Another study of 24–39 year old subjects, estimated a prevalence of 14.3% in men and women together according to the definitions of IDF (Mattsson *et al.* 2007). It must be noted that the metabolic syndrome becomes more prevalent with each decade of life, increasing in parallel with obesity and central adiposity (Reviewed in Cornier *et al.* 2008).

2.1.1 Abdominal obesity as a core of metabolic syndrome and inducer of insulin resistance and dyslipidemia

The metabolic syndrome is clearly linked to the presence of obesity and in particular to the degree of abdominal obesity (Reviewed in Gustafson *et al.* 2007). In mammals, there are two types of adipose tissue; white adipose tissue and brown adipose tissue. Brown adipose tissue is more an energy dissipating organ whereas white adipose tissue is more of an energy storing organ. Brown adipose tissue is known to maintain normal body temperature in newborn infants. It differs morphologically from white adipose tissue having more mitochondria (Reviewed by Lean 1989). Interestingly, a recent study indicated that brown adipose tissue is present in healthy adults and when exposed to cold, activated brown adipose tissue may have a potential to contribute substantially to energy expenditure (Virtanen *et al.* 2009). The white adipose tissue consists of subcutaneous and visceral fat. In relation to the metabolic syndrome, visceral fat has received more attention, because it is considered more metabolically active and it releases

biologically active factors directly to the portal venous system and therefore, it can have a direct impact on the liver. However, the role of subcutaneous fat cannot be fully disregarded, because in general in men, there is much more subcutaneous fat than visceral fat (Reviewed in Gustafson *et al.* 2007.)

As individuals become obese, the size of their fat cells increase since they have to store more lipids. In addition to adipose cells, adipose tissue is composed of preadipocytes, inflammatory cells, endothelial cells and mesenchymal stem cells. Under normal condition, the mesenchymal cells in adipose tissue should recruit new, small adipocytes but a failure in this process due to impaired adipocyte differentiation, possibly of genetic origin, leads to a reduction in the capacity of the adipose tissue to accumulate lipids. Failure to recruit new adipose cells can lead to enlargement of the existing fat cells and furthermore to ectopic accumulation of lipids in other tissues, e.g. muscle and liver (Figure 1). Larger adipocytes are more metabolically active and have a higher rate of lipolysis, and therefore obesity is associated with increased levels of free fatty acids (FFAs) in circulation (Reviewed by Danforth 2000, Gustafson *et al.* 2007.)

Elevations of FFAs and ectopic fat accumulation can evoke peripheral (muscle) and liver insulin resistance (Krssak *et al.* 1999, Seppälä-Lindroos *et al.* 2002). FFAs can directly inhibit insulin signalling, partly via tyrosine phosphorylation of insulin receptor substrates (Griffin *et al.* 1999). Insulin signalling is also impaired because lipids accumulate inside the muscle and hepatic cells in the form of triglycerides and long-chain fatty acyl-CoA esters. Lipids can also accumulate within pancreatic cells and thus hamper insulin secretion (Reviewed by Boden 2004, in Moller & Kaufman 2005).

In addition to FFAs, obesity triggers also other mechanisms that affect insulin sensitivity (Figure 1). Adipose cell enlargement evokes inflammation in the adipose tissue in a way that is not fully understood. Obesity seems to be associated with an increased number of macrophages in adipose tissue. For example, proinflammatory molecules such as TNF-α (tumour necrosis factor alpha) and IL-6 (interleukin 6) are secreted from inflamed adipose tissue. In fact, it is mainly the visceral fat, which seems to determine the plasma IL-6 concentration. In addition, the infiltration rate of monocytes into visceral fat is higher than the corresponding rate into subcutaneous fat (Reviewed in Hajer *et al.* 2008.) It has been noted that increased TNF-α in obesity is mainly attributable to infiltrating macrophages surrounding the adipocytes. Proinflammatory molecules impair insulin signalling through different mechanisms including decreased tyrosine phosphorylation of key signalling molecules and downexpression of

several proteins involved in insulin signalling. These effects lead to insulin resistance i.e. the decreased sensitivity of target cells to the actions of insulin, a further increase in lipolysis and reduced glucose uptake in adipose tissue (Reviewed in Gustafson *et al.* 2007.)

Obesity also contributes to insulin resistance by altering the levels of adipocyte-derived hormones. The tremendous scientific interest in adipose tissue over the past decades has revealed that this organ is not solely an energy store but an active endocrine organ releasing a number of bioactive molecules in response to changes in metabolic status. Many hormones are secreted from adipose tissue, e.g. leptin, adiponectin, adipsin and resistin; these share some structural properties of cytokines that are often secreted by immune cells activating and recruiting further immune cells to increase the system's response to the pathogen. Therefore, hormones secreted by adipose tissue, "adipocytokines" are collectively referred as adipokines (Reviewed by Saltiel 2001.) These molecules have a variety of actions e.g. in glucose homeostasis and energy expenditure. The role of the two most important adipokines, adiponectin and leptin will be discussed in 2.3.2 and 2.4.

The increased availability of FFAs in circulation can lead to dyslipidemia, i.e. abnormalities in plasma lipoproteins (Reviewed by Berneis & Krauss 2002). There are five lipoprotein classes: high density (HDL), low density (LDL), intermediate density (IDL), and very low density (VLDL) lipoproteins and chylomicrons. They are formed together with the plasma lipids, phospholipids, cholesterol, triglycerides, and cholesteryl esters and apolipoproteins. Plasma apolipoproteins are proteins of varying composition and size, classified as the apoAs (apoA-I, apoA-II, and apoA-IV), apoBs (apoB-100 and apoB-48), apoCs (apoC-I, apoC-Il, apoC-Ill) and apoE. Apolipoproteins can belong to different lipoproteins, with the exception of apoB-100, which is the sole constituent of LDL. Apolipoproteins, in addition to lipid binding properties, can act as cofactors for lipolytic enzymes or interact with cellular receptors (Rossenau & Labeur 1995.) In brief, VLDL and chylomicrons transport lipids to the peripheral tissues from the liver and intestine. When lipid is removed from VLDL, the particle is transformed to LDL. HDL instead, is primarly involved in returning lipid, mostly cholesterol, to the liver in a process called reverse cholesterol transport (Reviewed by Biggerstaff & Wooten 2003.)

Dyslipidemia means abnormalities in plasma lipid levels, such as elevated LDL-cholesterol and triglyceride levels and/or decreased HDL cholesterol levels. The insulin resistance in liver plays a major role in the lipid abnormities in patients with the metabolic syndrome. In that condition, the excess FFAs result in

increased production of glucose and triglycerides. A high level of plasma triglyceride is associated with larger VLDL particles that are lipolyzed less efficiently by lipoprotein lipase (LPL), giving rise to remnant particles. These remnants further delay lipolysis and also lead to reduced receptor-mediated plasma clearance. The remnants are further lipolyzed by the combined action of LPL and hepatic lipase (HL), and also undergo exchange of triglyceride for cholesterol derived from LDL and HDL, a process mediated by cholesterol ester transfer protein (CETP). The resulting triglyceride can be also delipidated and remodeled to form smaller, lipid-depleted LDL. These particles have a lower affinity for the LDL receptor. Triglyceride-rich LDLs and HDLs are degraded further by HL, leading to yet smaller LDLs and to smaller and less stable HDLs that are more rapidly catabolized, resulting in reduced levels of HDL cholesterol. Thus, the insulin resistance may lead to an increased risk for cardiovascular disease that has been designated as atherogenic dyslipidemia (Reviewed by Berneis & Krauss 2002.)

The metabolic changes induced by obesity and described in this chapter are shown in Figure 1.

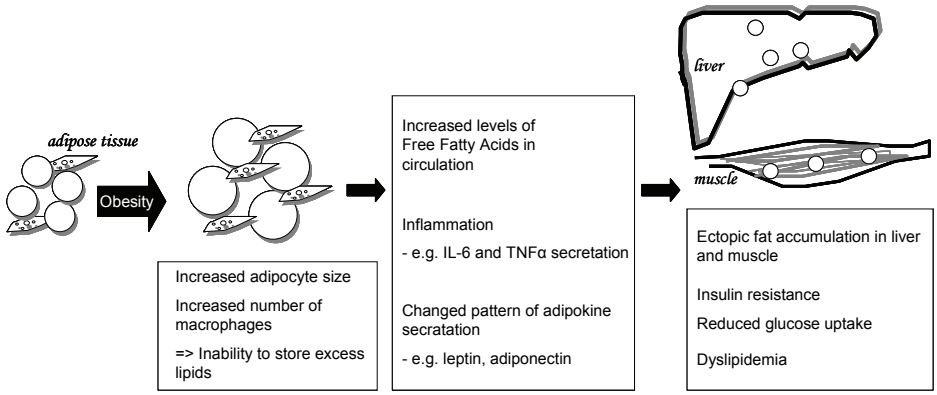

Fig. 1. Schematic representation of how obesity leads to insulin resistance. IL-6 = interleukin 6, TNF-α = tumour necrosis factor α.

2.2 Health implications of the metabolic syndrome

The objective of definitions of metabolic syndrome is to provide a simple, diagnostic and clinical tool to identify those persons at a great risk of developing type 2 diabetes and CVD (cardiovascular disease). Individuals with the metabolic

syndrome have a greater risk of developing type 2 diabetes (Reviewed in Cornier *et al.* 2008) and they are more likely to die from and to experience a heart attack or stroke compared to people not suffering from the syndrome (Reviewed by Kahn *et al.* 2005). For example, in a study of 4483 Finns and Swedes aged 35–70 years, the risk for CHD (coronary heart disease) and stroke was increased threefold in subjects with the metabolic syndrome as defined by WHO (Isomaa *et al.* 2001). For comparison, in the study of Americans over 50 years, the metabolic syndrome (NCEP(ATPIII) -defined) was a significant univariate predictor of prevalent CHD (OR 2.07, 95% CI 1.66–2.59) (Alexander *et al.* 2003). However, in that study, blood pressure, HDL cholesterol, and diabetes, but not the presence of metabolic syndrome, were significant multivariate predictors of prevalent CHD. The increased risk for CHD in subjects with the metabolic syndrome in the studies shows a wide variation from 30% up to 400%, probably due to which population is being studied, the precise definition of the syndrome and the length of the follow-up (Reviewed by Kahn *et al.* 2005).

However, it is unclear, whether the criteria of metabolic syndrome are helpful in clinical practice. The criteria do not necessarily enhance CHD prediction over the simpler Framingham-based risk scores. The dichotomous nature of metabolic syndrome criteria combined with their failure to include age, low-density lipoprotein cholesterol levels and smoking habits account for their inferior predictive value (Reviewed by Sattar 2008). It has also been claimed, that glucose tolerance or diabetes appears to account for most of the CVD prediction of metabolic syndrome (Hunt *et al.* 2004). Kahn and associates (2005) also raised the question whether the whole is greater than the sum of its parts, criticizing the concept that the presence of syndrome itself adds anything to CVD prediction.

2.2.1 Cardiovascular diseases

Coronary heart disease (CHD) is a disease where an atherosclerotic plaque located within the coronary arteries causes angina or potentially ruptures resulting in an acute CHD event such as a myocardial infarction. Major risk factors for CHD include a history of atherosclerosis (e.g. peripheral artery disease) or metabolic disease (type 2 diabetes, high blood pressure, dyslipidemia). Important modifiable risk factors are cigarette smoking and obesity (pathogenic adipose tissue). Family history of CHD (CHD in a male first-degree relative < 55 years old or CHD in a female first degree relative < 65 years old) is a not modifiable risk factor. The risk of CHD increases after the age of 45 years in men and 55

years in women (Reviewed by Bays 2009.) There is indeed some difference between women and men in CHD. CHD is the number one cause of death in women, but they tend to develop the disease 10–15 years later than men. Estrogens are believed to protect women from cardiovascular diseases before the age of the menopause. After the menopause, the declining estrogen level is associated with ongoing atherosclerosis (Reviewed in Baker *et al.* 2003.)

Many of the above risk factors of CHD are components of the metabolic syndrome and therefore metabolic syndrome does not necessarily predict risk of CHD any better than assessment of individual risk factors (Reviewed by Kahn *et al.* 2005, Bays 2009). Metabolic syndrome and obesity related insulin resistance might, however, involve some supplemental mechanisms that contribute to atherosclerosis. The proinflammatory state in adipose tissue leads to monocyte recruitment and the accumulation of monocytes in vascular lesions as well as their differentiation into macrophages which are subsequently activated. The prothrombotic state in metabolic syndrome means that patients have elevated levels of coagulation factors such as fibrinogens and PAI-1 in their circulation. In the metabolic syndrome, there might also be reduced vasodilation or endothelial dysfunction, as a result of impaired nitric oxide synthesis from endothelium. Hyperinsulinemia also affects directly the risk of atherosclerosis by activating lipid synthesis in the arterial wall and promoting vascular smooth muscle cell proliferation (Reviewed by Moller & Kaufman 2005.)

Based on prospective long-term study of cardiovascular outcomes, it appears that the metabolic syndrome is associated with a risk that is not entirely accounted for by traditional risk scoring paradigms (Girman *et al.* 2004). In that study, patients with the metabolic syndrome still showed increased risk of major coronary events irrespective of their Framingham-calculated 10-year risk score category. Therefore, it can be postulated that some indexes related to both inflammation, insulin sensitivity and CHD risk (e.g. C-reactive protein (CRP) or adiponectin) may also be useful predictive tools or additions to the definition of the metabolic syndrome (Reviewed in Kahn *et al.* 2005).

2.2.2 Type 2 diabetes

Type 2 diabetes is a complex disease caused by both environmental and genetic factors. It is characterised by chronically elevated blood glucose concentrations, resulting from both defects in insulin production and insulin action. Insulin resistance is considered one of the hallmarks of prediabetes, but also defects in

insulin secretion are regarded as a key pathophysiological characteristic of type 2 diabetes. Although type 2 diabetes is a heterogeneous disease, most patients have insulin resistance and the metabolic syndrome before they suffer the onset of type 2 diabetes. It has been noted, that insulin resistance, hyperinsulinemia, dyslipidemia, and obesity precede the progression to type 2 diabetes in 75% to 85% of patients (Reviewed in Cornier *et al.* 2008.) When tissues become insulin resistant, glucose levels do not initially rise in the circulation. Instead, the body develops a compensatory hyperinsulinemia, a state where the pancreas secretes additional insulin. However, when pancreas is no longer able to stabilize the glucose levels, the onset of impaired glucose intolerance or type 2 diabetes is present (Fortson *et al.* 2008.) However, in subjects with impaired glucose tolerance, the onset of type 2 diabetes can still be prevented or delayed effectively with appropriate life style changes (Tuomilehto *et al.* 2001).

2.3 The role of protein tyrosine phosphatase 1B (PTP1B) in insulin resistance and obesity

In both insulin and leptin signalling, tyrosine phosphorylation is the key mechanism in regulating receptor mediated actions in cells. The regulation of tyrosine phosphorylation represents a balance between two enzyme activities: protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). It can be hypothesized that a disequilibrium in enzyme activity between the insulin and/or leptin receptor and PTPs could contribute to the risk of insulin resistance or type 2 diabetes. This hypothesis is supported by the finding that PTP1B (protein tyrosine phosphatase 1B) -knockout mice have enhanced insulin sensitivity and these animals were unexpectedly protected from obesity (Elchebly *et al.* 1999, Klaman *et al.* 2000). PTP1B is expressed ubiquitously throughout the body including tissues that are key regulators of insulin metabolism such as liver, muscle, fat and brain (Reviewed in Goldstein *et al.* 1998). One study showed that PTP1B activity and protein content are higher in adipose tissue of obese human compared to lean individuals (Cheung *et al.* 1999). In another study, obese human had increased PTPIB activity in skeletal muscle compared to lean control group (Ahmad *et al.* 1997a). Enhanced insulin sensitivity following weight loss correlated with the reduction in the abundance of PTP1B (Ahmad *et al.* 1997b). In a recent report it was shown that PTP1B overexpression in multiple tissues in obesity is regulated by inflammation. In that study, treatment with the inflammatory marker TNF-α itself was sufficient to increase PTP1B mRNA and

protein levels in cultured cells and in insulin- and leptin-target tissues of mice (Zabolotny *et al.* 2008). These studies are evidence that obesity induced inflammation could increase the activity of PTP1B and inhibit the actions on insulin leading to insulin resistance. However, it must be borne in mind that there are some studies that do not agree with that conclusion; it has also been reported that the PTP1B content was reduced in insulin resistant states in humans (Kusari *et al.* 1994).

2.3.1 Insulin signalling

Insulin is the major anabolic hormone whose actions are essential for growth, development and homeostasis of glucose, fat and protein metabolism. It is produced by pancreatic β-cells. Insulin modifies the expression and activity of many molecules involved in glucose influx to cells. Insulin signalling is mediated by a complex network beginning when insulin binds to its cell-surface receptor, insulin receptor (IR) (White & Kahn 1994). The IR belongs to a family of PTKs. It is a transmembrane protein consisting of two extracellular α-subunits and two transmembrane β-subunits. After the binding of insulin, IR undergoes autophosphorylation on several tyrosine residues in the β-subunits which activates the tyrosine kinase activation of IR. The kinase activity of IR mediates the triggering of downstream signalling events, further signalling events, tyrosine phosphorylation of IRS proteins (insulin receptor substrates) and some other adaptor proteins. These proteins, via the activation of P13K-AKT/protein kinase B (PKB) pathway, are responsible for the metabolic effects of insulin in the cells (Reviewed by Koren & Fantus 2007.)

Since the phosphorylation is a very crucial part of insulin signalling, the opposite process i.e. dephosphorylation is essential for diminishing or blocking insulin signalling. There are several PTPs in the signalling route of insulin. The most important of those is PTP1B, which blocks tyrosine phosphorylation of IR and dephosphorylates IRS1 and IRS2 thus inhibiting the actions of insulin (Reviewed by Koren & Fantus 2007.) The actions of PTP1B in insulin signalling are presented in the Figure 2.

2.3.2 Leptin signalling

The endocrine role of white adipose tissue became evident, when the cytokine leptin was discovered in 1994. White adipose tissue could no longer simply be

considered as an energy store. The leptin or *ob*-gene was identified as a gene explaining the fatness of mutant *ob/ob* -mouse. It was observed that the mutation of *ob* and thus a deficiency in the leptin hormone resulted in profound obesity and the appearance of type 2 diabetes (Zhang *et al.* 1994). Since the discovery of leptin it has become clear that leptin, in the same way as insulin, has crucial effects on energy homeostasis. Leptin is produced approximately in proportion to fat stores. The primary site of leptin action is the hypothalamus, where it controls the energy expenditure by reducing food take and promoting weight loss. Leptin also limits the accumulation of triglycerides in liver and skeletal muscle thereby improving insulin sensitivity (Reviewed by Havel 2004). In the majority of cases with obesity, where leptin levels are high, leptin fails to induce weight loss. This diminished response to leptin`s anorexigenic and insulin sensitizing effects is called leptin resistance (Reviewed in Myers *et al.* 2008).

Leptin signalling is mediated via the leptin receptor (LepR). Alternative splicing can generate several isoforms of LepR with identical ligand binding domains, but different perimembrane and intracellular domains. Membrane-bound LepRs consist of long (LepRb) and short (LepRa, among others) isoforms. Long LepRb has an approximately 300 amino acid intracellular tail that contains several docking sites for proteins critical for signal transduction (Robertson *et al.* 2008). The leptin receptor is a class-I cytokine receptor which itself has no enzymatic activity, but instead needs a tyrosine kinase Jak2 (Janus kinase 2) in its intracellular domain to mediate the signalling cascade. Leptin binding to its receptor activates JaK2, which undergoes autophosphorylation and thus phosphorylates three tyrosine residues in the intracellular part of receptor. These residues stimulate the nuclear translocation of transcriptional activation at least of multiple STAT-isoforms (signal transducers and activators of transcription), but possibly also other signalling molecules. Studies have shown that PTP1B negatively regulates the signalling of leptin in part by dephosphorylating JAK2 and STAT3 molecules (Reviewed by Koren & Fantus 2007.) The role of PTP1B in insulin and leptin signalling and the metabolic functions of these molecules is presented in Figure 2.

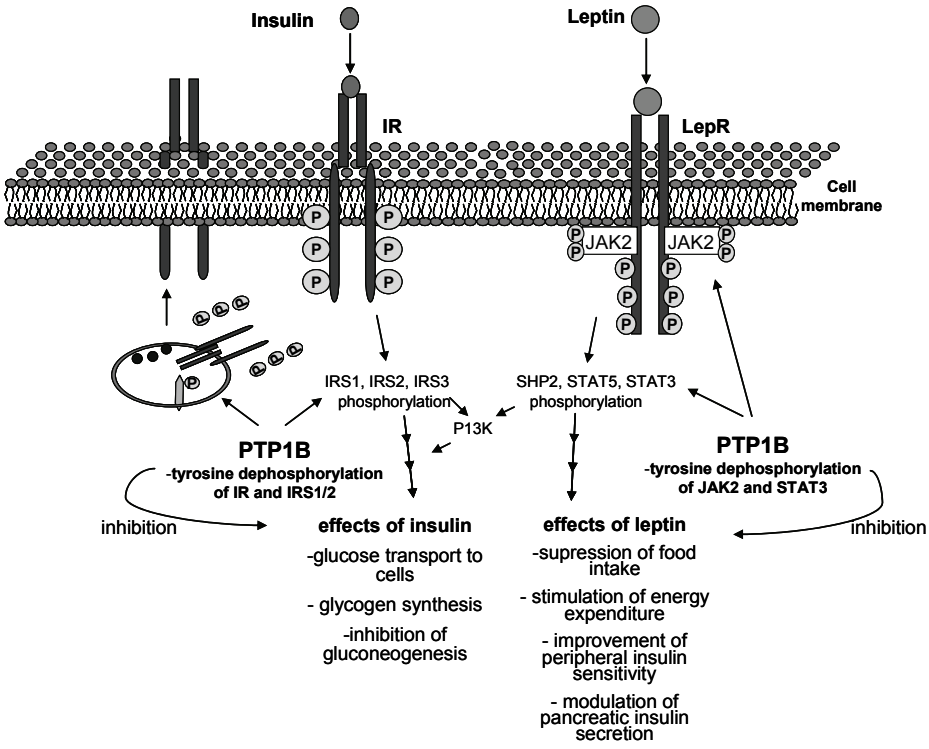

Fig. 2. The role of PTP1B in the insulin and leptin signalling and the major effects of insulin and leptin. P = phosphate, IR = Insulin receptor, LepR = Leptin receptor, PTP1B = protein tyrosine phosphatase 1B, IRS1, IRS2, IRS3 = insulin receptor substrates 1,2 and 3, STAT5, STAT3 = signal transducers and activators of transcription 5 and 3, JAK2 = janus kinase 2. (Modified from Koren & Fantus 2007 and Ukkola & Santaniemi 2002).

2.4 Adiponectin and components of metabolic syndrome

2.4.1 What is adiponectin

In conjunction with leptin, adiponectin is one of the most widely studied adipokines. Adiponectin was originally identified by four independent groups, and it is therefore also called Acrp30 (Adipose complement-related protein of 30 kDa), GBP28 (Gelatine Binding Protein of 28 kDa), apM1 (adipose Most abundant gene transcript 1) and AdipoQ (adiponectin, C1Q and collagen domain containing) (Scherer *et al.* 1995, Hu *et al.* 1996, Maeda *et al.* 1996, Nakano *et al.*

1996). Its sequence bears a strong similarity with C1q, a complement factor. The adiponectin molecule has a signal sequence at the N-terminus followed by a species-specific variable region, collagenous domain and the globular head domain. The basic building block for adiponectin is a homotrimer, where monomers are formed through globular domain. Through interactions between collagenous domains, the trimer adiponectins can form complexes (Reviewed in Berg *et al.* 2002.) Adiponectin is therefore found in several different isoforms, including trimer, low-molecular weight (-hexamers), and high-molecular weight (HMW) (18mers) forms. Different adiponectin oligomers hold distinct biological functions (Pajvani *et al.* 2003, Banga *et al.* 2008). In plasma, there also exists a smaller globular fragment of adiponectin. It is generated by proteolytic cleavage from the whole adiponectin protein (Waki *et al.* 2005). Adiponectin binds to two receptors, AdipoR1 and AdipoR2, which are expressed in liver, fat and muscle (Yamauchi *et al.* 2003). It has also been proposed that T-cadherin, which is abundantly expressed in injured vascular endothelial and smooth muscle cells in atherosclerotic regions, is a third receptor especially binding to HMW adiponectin (Hug *et al.* 2004).

Arita and colleagues (Arita *et al.* 1999) first measured adiponectin from human plasma where it is present in high concentrations (2–10 ug/ml). The adiponectin concentration is significantly higher in women than in men. They also noted, that in contrast to the leptin level which is in general proportional to overall adipose mass, adiponectin levels are significantly lower in obese than non-obese individuals. It is thought that adiponectin is primarily produced by visceral fat. In a cross-sectional study including obese and lean men and women, the negative relationship between plasma adiponectin and visceral fat was significantly stronger than that with subcutaneous fat. It has also been reported that in vitro studies, omental adipocytes secrete more adiponectin than adipocytes isolated from subcutaneous fat (Reviewed by Havel 2004.)

In adipose tissue, adiponectin acts in an autocrine and paracrine manner by promoting adipocyte differentiation. There is a regulatory feedback loop by which adiponectin downregulates its own production and also the expression of its receptor, AdipoR2 (Bauche *et al.* 2006). Regulation of adiponectin expression does not seem to be well understood. In obesity, increased fat mass results in a hypoxic microenvironment, which has been shown to suppress adiponectin expression via the hypoxia-induced factor-1. A low-grade chronic inflammatory state with increased production of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-18 reduce adiponectin gene expression. Several transcription factors,

such as CREB (c-AMP response element binding) and NFAT (nuclear factor of activated T-cells) act as repressors that contribute to obesity-induced down-regulation of adiponectin gene transcription (Reviewed by Liu & Liu 2010).

As expected, the adiponectin concentration decreases with weight gain and its expression increases as result of weight loss. However, plasma adiponectin seems to be even more closely associated with insulin resistance, since adiponectin levels are low in insulin resistant subjects irrespective of whether they are obese. The plasma adiponectin levels in different stages are well documented (Reviewed by Trujillo & Scherer 2005.) The most important factors and disease stages affecting up- or down-regulation of plasma adiponectin levels are shown in Figure 3. In addition, also lifestyle habits may modulate adiponectin levels, since in Japanese men, both the smoking status and dietary factors influenced adiponectin levels (Tsukinoki *et al.* 2005). A mediterrenean diet and exercise have also been documented to increase adiponectin levels (Reviewed in Antoniades *et al.* 2009).

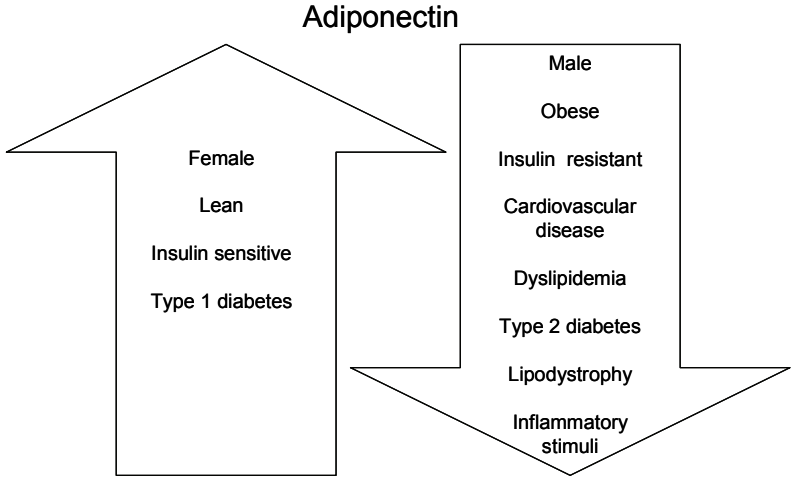

Fig. 3. Most important factors and disease states that lead to low or high plasma adiponectin level (Modified from Trujillo & Scherer 2005).

2.4.2 Adiponectin, obesity and glucose homeostasis

The role of adiponectin in controlling glucose homeostasis has been studied by many research groups and it has been found to have actions in muscle, liver, pancreas and central nervous system (Table 2). In mouse studies, administration

of recombinant adiponectin evoked increased glucose uptake and an increase in fatty acid oxidation by muscle (Fruebis *et al.* 2001) at least in part by altering the expression of molecules involved in both fatty-acid combustion and energy dissipation (Yamauchi *et al.* 2001). In isolated hepatocytes, adiponectin increases the ability of insulin to suppress glucose production (Berg *et al.* 2001). The metabolic effects of adiponectin can be at least partially explained by the direct activation of 5'-adenosine monophosphate (AMP)-activated protein kinase. AMP-activated protein kinase is an energy sensor within the cells, as it activates the cellular processes that produce energy, e.g. fatty acid oxidation and glucose uptake. Adiponectin modulates AMPK via receptor AdipoR1 while another receptor AdipoR2, is more involved in PPARγ mediated actions. Thiazolidinediones (TZDs), which are PPARγ -agonists and used as insulin sensitizing drugs, at least partially, act through upregulating adiponectin (Combs *et al.* 2002).

In addition to insulin sensitizing effects, adiponectin can affect glucose metabolism in other ways. Adiponectin induced the secretion of insulin from pancreas *in vitro* in pancreatic cell studies and *in vivo* after intravenous injection of adiponectin into mice (Okamoto *et al.* 2008). It was also shown that adiponectin stimulated food intake and decreased energy expenditure during fasting through its effects in the central nervous system e.g. in the arcuate hypothalamus (Kubota *et al.* 2007 abstract).

Table 2. The actions of adiponectin in controlling the glucose homeostasis.

Target of action	Function
Muscle	Increased glucose uptake
	Increased fat oxidation
Liver	Reduced glucose production
	Increased fat oxidation
Pancreas	Stimulation of insulin secretation
Central Nervous System	Modulation of food intake and energy expenditure

2.4.3 Adiponectin and plasma lipids

In large cross-sectional studies, the plasma adiponectin concentration has been positively correlated with HDL-cholesterol and negatively with triglycerides (Cnop *et al.* 2003, Tschritter *et al.* 2003). It is not clear whether there is a causal link between low adiponectin and dyslipidemia. Adiponectin levels may modulate

the production of triglyceride-rich lipoproteins and hepatic lipid metabolism by modulating the fat content of the liver. The increased fat content is associated with overproduction of triglyceride-rich large VLDL particles in humans (Adiels *et al.* 2006).

As reviewed by Lara-Castro *et al.* (2007), adiponectin might affect on plasma lipoproteins by altering the levels and activity of three enzymes; hepatic lipase, lipoprotein lipase and CETP (Cholesteryl Ester Transfer Protein). CETP is a molecule in plasma that transports cholesteryl esters and triglycerides between the lipoproteins. CETP removes cholesteryl esters from the LDL particle core and replaces it with triglycerides from VLDL and chylomicrons. Subsequently, the LDL particles become good substrates for hepatic lipase. Hepatic lipase functions as a lipolytic enzyme that hydrolyzes the triglycerides and phospholipids present in circulating plasma lipoproteins and also serves as a ligand that facilitates lipoprotein uptake by cell surface receptors and proteoglycans, thereby directly affecting cellular lipid delivery. High hepatic lipase activity has been demonstrated as being a strong contributor to small dense LDL-levels. Low adiponectin levels are associated with increased hepatic lipase activity in vivo (Schneider *et al.* 2005). A low level of adiponectin is also associated with decreased lipoprotein lipase, which is in turn is linked with large buoyant LDL-particles (Saiki *et al.* 2007). Through these two molecules, low adiponectin could enhance the formation small dense LDL particles, which are most detrimental in vessels and typical in insulin resistance.

It is possible that adiponectin may have a direct role on HDL catabolism. It has been reported that adiponectin deficiency might impair the HDL synthesis in the liver. In adiponectin knockout mice, the synthesis of apoA-I in the liver was reduced compared with wild-type mice. It has been demonstrated that adiponectin might have some ability to accelerate reverse cholesterol transport and protect against atherosclerosis by increasing apoA-I-mediated cholesterol efflux by enhancing the ABCA1 (ATP-binding cassette transporter) pathway in macrophages, as well as promoting the HDL assembly in the liver (Tsubakio-Yamamoto *et al.* 2008).

2.4.4 Adiponectin as a link between obesity and CVD

There are many studies investigating the role of adiponectin in the vasculature. One of the most interesting is work with the adiponectin knockout mouse – an animal that displays striking vascular alterations including severe neointimal

thickening and increased proliferation of vascular smooth muscle cells in injured arteries (Kubota *et al.* 2002). This already implies the possible role for adiponectin linking the adipocytes to vascular function.

The atherosclerosis is known to be initiated by endothelial dysfunction occurring in response to a variety of stimuli, such as diabetes. The site of the atherosclerotic plaque is inflamed and this promotes the adhesion of monocytes to the vessel wall. Once monocytes have migrated from the arterial wall to the intima, they develop into macrophages and as they take up oxidized LDL through scavenger receptors, they differentiate into foam cells. This is the early, first lesion of atherosclerosis called the fatty-streak. The synthesis of vasodilating NO (nitric oxide) by endothelial NO synthase is also impaired (Kaperonis *et al.* 2006).

Adiponectin seems to be an anti-inflammatory vasoprotective adipokine in these early stages of atherosclerosis. At first, high adiponectin levels seem to inhibit the transformation of macrophages into foam cells because in in vitro studies adiponectin has suppressed the expression of scavenger receptors in macrophages thus inhibiting the binding of modified LDL (Ouchi *et al.* 2001). In cell culture studies, adiponectin inhibited TNF-α -induced monocyte adhesion to endothelial cells and the expression of adhesion molecules VCAM-1, E-selectin, and ICAM-1, which have been detected also in human atherosclerotic lesions (Ouchi *et al.* 1999).

Adiponectin regulates eNOS enzymatic activity and stimulates NO production and in that way, controls vascular tone (Reviewed by Wang & Scherer 2008). The association between adiponectin and hypertension has been demonstrated in epidemiological studies which have revealed that hypoadiponectinemia is a risk factor for hypertension independent of insulin resistance and diabetes (Iwashima *et al.* 2004, Chow *et al.* 2007). In addition to its effects on endothelium, adiponectin is also linked to vascular tone through angiotensin II and the central nervous system. Angiotensin II, a peptide causing vessels to constrict has an inhibitory role in adiponectin production. The central nervous system overdrive increases blood pressure and also inhibits the production of adiponectin (Reviewed by Wang & Scherer 2008). Recently, it was found out that a high adiponectin concentration was associated with low ambulatory blood pressure and daytime systolic values (Vasunta *et al.* 2009). In addition, a low level of adiponectin associated with left ventricular hypertrophy, a common complication in patients with high blood pressure, and this association persisted even after adjustment for the commonly recognised risk factors (Pääkkö *et al.* 2010).

2.5 The role of genetics in the metabolic syndrome and associated diseases

According to the "thrifty genotype hypothesis", factors in early humans that favoured fat deposition during periods when food was abundant were crucial for survival during periods of nutritional hardship. This might have led to the positive selection of genes that promote fat deposition and cause insulin resistance. This hypothesis was originally proposed by Neel in 1962 (Neel 1962). Later, this characteristic became detrimental in populations which experienced rapid socioeconomic modernisation and had constant access to an abundance of food and who were less physically active than their parents and grandparents. It is not expected that evolution would have created one single "thrifty genotype". The most likely situation is that several genes have been selected in different combinations in different populations (Reviewed by Dowse & Zimmet 1993.) The hypothesis of Neel has however been criticized, for instance because the impact of famines on fertility might consequently be insufficient to cause selection. Therefore, alternative scenarios favoring genetic drift, not positive selection, have been proposed (Speakman 2008). No consistent footprint of selection has been found across the loci that would support the concept of a universal mechanism to explain the high prevalence of type 2 diabetes and obesity (Southam *et al.* 2009). On the other hand, as discussed later, the truly causal genetic loci for type 2 diabetes and obesity, have not yet been identified.

Not all people are affected equally by an unhealthy lifestyle. There is convincing evidence that genetic determinants contribute to the development of type 2 diabetes (Reviewed by van Tilburg *et al.* 2001). There are higher concordance rates in monozygotic twins than in dizygotic twins, for example in the Finnish twin study 34% and 16% (Kaprio *et al.* 1992), but 83% and 40% in a Japanese twin study (Committee on Diabetic Twins, Japan Diabetes Society), respectively. There are also studies showing a lifetime risk of 40% for the development of the disease in non-diabetic offspring, siblings and dizygotic twins and increasing to 70% if both parents have type 2 diabetes mellitus. There are large variations in the values reported in these kinds of studies between populations, which may reflect the different environmental factors as well as differences in genetic susceptibilities. Type 2 diabetes seems to be a very multifactorial disease in which genes not only interact with each other but also with environmental factors (Reviewed by Hansen & Pedersen 2005.) According to heritability studies, around 70% of the individual variation in BMI and

comparable other measures of adiposity (skinfold thickness, waist circumference and total and regional fat distribution) can be traced to genetic factors (Reviewed by Blakemore & Froguel 2008).

When searching the genes influencing the common type 2 diabetes, it is possible to seek either functional candidate genes or positional candidate genes. Functional genes are those whose products are known to play a role in glucose homeostasis, for example. Positional candidate genes are genes located in chromosomal regions that have been identified in linkage studies (Doria *et al.* 2008.)

2.5.1 ADIPOQ and PTP1B as candidate genes

ADIPOQ and PTP1B genes can be seen as both functional and positional candidate genes for the metabolic syndrome and associated diseases. Since they have profound effects on metabolism, genetic variability in these molecules could be determinants of insulin resistance and adiposity. ADIPOQ is located on chromosome 3q27 where various quantitative trait loci for the metabolic syndrome and a locus for type 2 diabetes have been identified (Kissebah *et al.* 2000, Vionnet *et al.* 2000, Mori *et al.* 2002). There are also a number of publications examining various populations showing that the region 13q of the chromosome 20 containing the PTP1B gene displays linkage signals with type 2 diabetes, BMI (body mass index), fat mass and energetic intake (Bowden *et al.* 1997, Lembertas *et al.* 1997, Hunt *et al.* 2001, Dong *et al.* 2003, Collaku *et al.* 2004).

The role of genetic factors to explain the variance of circulating adiponectin is estimated to be quite strong. The levels of adiponectin seem to be under multigenic control but mostly the variability at the adiponectin locus itself contributes to regulation (Comuzzie *et al.* 2001, Menzaghi *et al.* 2004). Recently, a whole genome-wide linkage and association analyses have been performed to evaluate the genetic influences on plasma adiponectin levels. In one genome wide association analysis of 2000 subjects, plasma adiponectin levels also associated with the variation in CDH13, a cadherin superfamily gene recently found to be expressed in vascular endothelial cells and smooth muscle cells interacting with hexameric and high-molecular weight species of adiponectin (Ling *et al.* 2009). In another large recent study, an intronic SNP located in the ARL15 (ADP-ribosylation factor-like 15) gene with unknown function, was robustly associated

with decreased adiponectin levels (Richards *et al.* 2009). In these studies, the SNPs in ADIPOQ were also strongly associated with plasma adiponectin levels.

2.5.2 Different types of genetic studies on type 2 diabetes

The most common polymorphism in the human genome and which has been most extensively used in genetic studies, is the single-nucleotide polymorphism (SNP). Polymorphisms are terminologically distinguished from mutations by a frequency criterion meaning that different forms of the polymorphism termed alleles are observed more often in the general population than mutations, with a population frequency of < 1% often used as a cutoff value. Currently, more than 9 million SNP are described in databases. SNPs occur on average once every 200 base pairs in the human genome, many of those however being very rare (Reviewed by Crawford & Nickerson 2005.)

Currently, the modest effect sizes of the known type 2 diabetes susceptibility variants limit their use, individually or in combination, in the prediction of disease risk (Lango *et al.* 2008). It is not yet known how much the epigenetic effects, such as the maternal uterine environment impact on the risk of diabetes in the offsprings and thus inflate the estimates of heritability (Reviewed by Dabalea & Pettitt 2001). In addition, not only the SNPs used in most studies but also the copy number variants in genome may associate with risk of type 2 diabetes. Gene-gene interactions and gene-environmental interactions could be contributory factors in the difficulties in finding type 2 diabetes genes (McCarthy & Zeggini 2009). Three aspects on the study of type 2 diabetes genes are presented in the following paragraphs.

Linkage scans

In typical genetic linkage studies, correlations between inheritance of a trait and chromosomal regions within family units, such as sibling pairs or multigenerational pedigrees, are investigated. Linkage studies have had some success in identifying the molecular basis of monogenic diseases, but less successes with common, more complex phenotypes, such as type 2 diabetes or atherosclerosis (Hegele 2002). In the late 1980 and 1990s, genome mapping provided genomewide collections of markers and technologies for typing linkages in hundreds of individuals. Multiple genome wide linkage scans for type 2 diabetes have been done, but no single region in the genome has been widely

replicated in these studies. Recently, a large study combining 83 linkage reports was published and it provided some evidence that there might be major genes for type 2 diabetes in chromosomal locations 6q, 1q,1p, 2q, 20q, 17pq, 8p, 19q and 9q (Lillioja & Wilton 2009). However, there has been no clear path for progressing from linkage to gene identification (Reviewed in Florez *et al.* 2003). Studies are variable between populations. If only a subset of type 2 susceptibility genes are required for the disease, and the frequencies of these genes differ between populations, the results are variable. When the risk allele is present at a high frequency and has only a modest impact, linkage suffers a loss of power. This is because allele sharing of used markers is only observed if the risk allelele is inherited from only one side of the pedigree (Reviewed in Florez *et al.* 2003.)

Candidate gene studies

Candidate genes, either positional or functional, can be studied by linkage based methods or association studies. The typical association study is a case-control study, where the difference in allele frequency of candidate gene polymorphisms is examined between affected individuals and unrelated controls, for example between type 2 diabetes patients and healthy individuals in this thesis. There are three possible explanations for a positive association between disease and allele. First, there might be a true association so that the gene variant actually has a causative role in the development of the disease. Second, the positive association can arise if the marker studied is in linkage disequilibrium with another truly causal locus. Linkage disequilibrium takes place, when two genetic markers occur together more frequently than would be expected from random association. This means that marker allele is so close to the other allele that these alleles are inherited together over many generations. This can happen because of a variety of reasons, including recent admixture of populations with different allele frequencies, selection in favour of a specific allele, genetic drift or population bottlenecks or new mutations (Weeks & Lathrop 1995.)

The linkage disequilibrium between alleles can be measured with some parameters. D is the most common measure, and can be calculated as $P_{AB} - P_A \times P_B$, where P_A is a frequency of allele A in the first loci and P_B the frequency of allele B in the second loci. The value of D is dependent on allele frequencies, but another measure r^2 is the correlation of alleles at the two sites, and is formed by dividing D^2 by the product of the four allele frequencies at the two loci. The case of $r^2 = 1$ is known as perfect LD. In perfect LD, observations at

one marker provide complete information about the other marker (Ardlie *et al.* 2002.)

Selection bias or another bias can also lead to false positive association. For instance, some population substrata may be more susceptible to develop type 2 diabetes because of an unmeasured factor, such as ethnicity or genetic background, for which the genotype is merely an indirect marker (Hegele 2002). Therefore, genetic isolates, such as Finns with a history of a small founder population, long-lasting isolation and population bottlenecks represent exceptional resources in the identification of disease genes. It can also be assumed that the vast majority of cases are caused by the same mutation, and disease alleles reveal linkage disequilibrium (LD) with markers over significant genetic intervals compared to older populations (Peltonen 2000.)

Candidate gene studies have had disappointing outcomes in the field of type 2 diabetes. Many studies have been limited in power, the small sample sizes are inadequate to detect the kinds of effects that are known to be realistic in complex traits. It might also be that the candidate genes are selected on the basis of limited knowledge of the etiology of the disease. In addition, there might have been a poor understanding of the architecture of genetic variation. The genotyping methods have been time-consuming and also the irreproducibility of results may have been further reinforced by liberal thresholds for defining significance and the tendency to over-interpret the results (McCarthy and Zeggini 2009.)

Genome wide association studies

With the commercially available chips, a very large number of SNPs (10^5 -10^6) in the genome can nowadays be detected from an individual (Reviewed in Grant & Hakonarson 2008). At least 30 genome wide association (GWA) scans for type 2 diabetes have been performed (McCarthy & Zeggini 2009). For example, 386 731 SNPs of 1464 patients with type 2 diabetes and 1467 matched controls were studied and three loci were associated with type 2 diabetes, including for example the loci IGF2BP2 (Insulin-like growth factor 2 mRNA-binding protein 2) and FTO (fat mass and obesity associated) (Saxena *et al.* 2007). GWAs have proved to be successful in identifying susceptibility variants, but the power of individual studies to detect small or modest effects at common SNPs has been limited (McCarthy & Zeggini 2009). It is very disconcerting that the GWA studies and linkage studies detect different genes; GWA studies may detect genes that linkage studies have been insufficiently powered to detect but linkage studies may

identify genes that have multiple variants within the same gene and variants rare enough individually will be undetected by current GWA studies (Lillioja & Wilton 2009). Continuous efforts through GWA meta-analysis and fine mapping may represent the way to explain the "missing heritability" of type 2 diabetes, since it is claimed that about 20 known variants explain only 5–10% of the inherited predisposition of type 2 diabetes (McCarthy & Zeggini 2009).

3 Purpose of the study

The purpose of this study was to determine how the variation in both protein tyrosine phosphatase 1B (PTP1B) and adiponectin (ADIPOQ) genes and plasma adiponectin concentration contribute to the metabolic syndrome and its components as well as on its health consequences. The specific aims of the study are the following:

1. To examine how the variation in PTP1B and LEPR (gene coding leptin receptor) genes or possible gene–gene interactions between the polymorphisms in these genes is associated with type 2 diabetes in a Finnish population
2. To study how polymorphisms and haplotypes in ADIPOQ are involved in the adiposity and insulin metabolism in a large study group of Caucasian and African American families and in type 2 diabetes in Finnish subjects
3. To test how good marker of the metabolic syndrome is the plasma adiponectin level in a Finnish population in a cross-sectional study with using two criteria for the metabolic syndrome and to test how plasma adiponectin levels relate to different components of the metabolic syndrome
4. To study how plasma adiponectin levels predict the development of type 2 diabetes or impaired glucose tolerance in healthy people in a progressive study
5. To evaluate the influence of two types of estrogen replacement therapy on plasma adiponectin levels in postmenopausal women and to establish the possible role of adiponectin in the risk of atherosclerosis

4 Subjects and methods

4.1 Study subjects

All the study groups used in the original papers I–V are previously defined. The names of the study groups, the original reasons for recruiting and original publications are defined in Table 3.

Table 3. Study groups used in the studies.

Study	Name of the study	Original reason for collecting the data	N
I and II	DIABETIC healthy, age and sex - matched control population	association of SNPs in lipid regulatory proteins with micro- and macroangiopathy (Ukkola *et al.* 1993)	257 285
II	HERITAGE (HEalth, RIsk factors, exercise Training And GEnetics)	the role of the genotype in response to aerobic exercise training (Bouchard *et al.* 1995)	779
III	OPERA (Oulu Project Elucidating the Risk of Atherosclerosis)	risk factors and end points of atheroclerosis (Kauma *et al.* 1996, Rantala *et al.* 1999)	1041
IV	OULU55	the prevalence of diabetes and IGT (Rajala *et al.* 2001, Rajala *et al.* 2002)	201
v	ERT (Estrogen replacement therapy)	the effects of peroral and transdermal estrogen replacement therapy (Karjalainen *et al.* 2000, Karjalainen *et al.* 2001)	79

In short, subjects in the first study were middle aged (mean age 58 years), obese or overweight (mean BMI 28 kg/m^2) type 2 diabetic patients having poor metabolic control. The DNA was collected in 1988–1990 when the patients came to Oulu University Hospital for the evaluation of their diabetes treatment. The control group in the first study was the subgroup of the OPERA -study used in the third study. The control subjects were chosen by matching them with type 2 diabetes patients as closely as possible for age and sex.

The study cohort in the second study, the HERITAGE, is a multicenter clinical trial conducted in five institutions in United States and Canada. It is a family study, and in the original paper II, the sample consisted of 779 subjects, including 503 Caucasians and 276 African Americans. The people enrolled in the

study were required to be in good health (no diabetes, cardiovascular diseases or other chronic diseases) and to be sedentary at baseline (no regular strenuous physical activity over the previous six months).

The OPERA study group used in the study III was collected in the 1990 in the Department of Internal Medicine, University of Oulu. It consists of hypertensive subjects that were chosen from the Social Insurance Institute register of individuals receiving reimbursement of antihypertensive medication. All subjects were in the highest reimbursement class. For each hypertensive subject, an age and sex matched control was randomly selected from the national health register. People receiving reimbursement for antihypertensive medication were not selected to control group. Both hypertensive and control subjects were living in Oulu and were aged 40–59 at the time of selection of subjects.

The study cohort used in the progressive study IV, is a part of a population based survey, that consisted of persons born in 1935 and living in Oulu. Shortly, subjects attended to the first phase in 1990–1992 and were classified as normoglygemic at that time. The second phase was in 1996–1998. The study cohort used in the study IV consisted of 201 subjects of those that had participated in the second phase. There were no statistically significant differences in HDL cholesterol, triglycerides, systolic and diastolic blood pressures, or BMI in the subjects with or without measured adiponectin levels.

Subjects in study V were postmenopausal women seeking hormone substitution therapy for relief of climacteric symptoms. The women were 45–65 years old with their postmenopausal status confirmed and they were previously hysterectomized with at least one remaining ovary. The study took place in Oulu in 1993–1994 and a total of 79 women were randomly chosen to receive either estradiol valerate orally with placebo gel or estradiol gel with placebo tablets. Plasma samples were obtained in the morning after an overnight fast at baseline and after 6 months of therapy.

4.2 Genotyping of genes PTP1B, LEPR and ADIPOQ

From PTP1B gene, three polymorphisms (G82A (rs6096013), Pro303Pro (rs2230604) and Pro387Leu (rs16995309)) were genotyped. From ADIPOQ (adiponectin gene), three polymorphisms were also genotyped (T45G (rs2241766), G276T (rs1501299), Tyr111His (rs17366743)). One LEPR polymorphism (Gln223Arg (rs1137101)) was also genotyped. The studied SNPs and their locations in genes are illustrated in Figure 4.

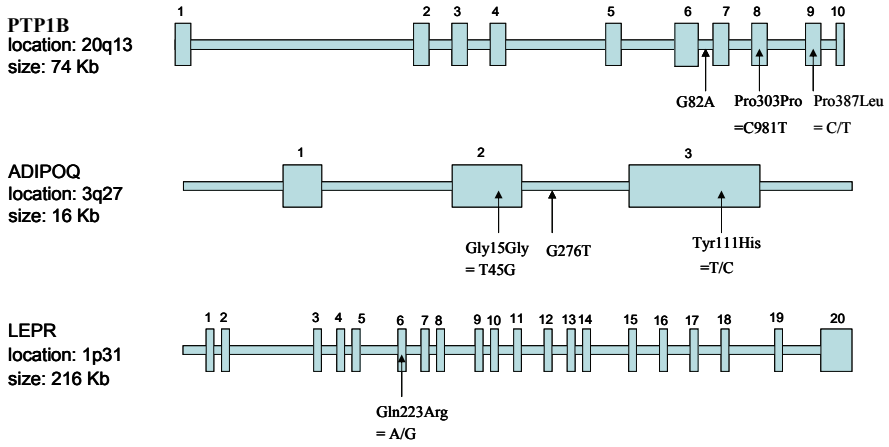

Fig. 4. The single nucleotide polymorphisms determined in the study. PTP1B = tyrosine phosphatase 1B gene, ADIPOQ = adiponectin gene, LEPR = Leptin receptor gene.

Since polymorphism changes the DNA sequence, the altered nucleotide can be identified with a restriction enzyme that cuts the DNA from a particular sequence. The method is called restriction fragment length polymorphism (RFLP). First the DNA- samples from studied subjects are multiplied by polymerase chain reaction (PCR) and then digested with restriction enzymes and visualized based on the length of the formed fragments. The RFLP – methods are described in detail in studies I and II.

4.3 Linkage disequilibrium –calculations and haplotype reconstruction

Haplotypes frequencies and their differences between type 2 diabetic patients and control population in the study II were obtained using the program PHASE version 2.1 available in the internet. It is a Bayesian software for haplotype reconstruction from an unrelated population. Since the second study population HERITAGE in the study II, is a family cohort, the genotypes could be obtained using family structures and parental genotypes. Rare haplotypes ($n < 9$) were not included in the analysis. Only parents were examined in the study.

4.4 Measurements of plasma adiponectin concentrations

Three methods for the measurement of plasma adiponectin were used. In studies III and IV, an ELISA- (enzyme-linked immunoassay) method devised in our laboratory was used. The method was based on two adiponectin antibodies (R&D Systems, catalog MAB10651 and BAM1065) as described in study III and study IV. Measurements were conducted in a random order. The intra-assay variation of the measurements was 13.9% and 9.8%, and interassay variation was 15.9% and 17.0% (6.5% after correction) in studies III and IV, respectively. In study II, adiponectin was measured with a commercial radioimmunoassay. In study V, commercially available ELISA-kit was used as described in the paper. The samples obtained from subject at baseline and after therapy were measured in the same assay. The interassay variation was 12.5%.

4.5 Other clinical measurements

All the blood pressure measurements were recorded with an automatic oscillometric blood pressure recorder.

The lipids in studies I, III, IV and V were measured as follows: VLDLs were measured with an enzymatic colorimetric method from the fraction formed during ultracentrifugation and HDL was measured in the same way from the supernatant after precipitation of lower density lipoproteins. The low-density lipoprotein (LDL) cholesterol concentration was then calculated by subtracting the cholesterol concentration in HDL from that in the VLDL-free fraction. The concentrations of total cholesterol and triglycerides in the plasma and lipoprotein fractions were determined by enzymatic colourimetric methods.

Body mass index was calculated with the formula BMI = weight in kilograms/ height in meters^2 (studies I–V). Body composition studies in study II (HERITAGE cohort) included hydrostatic weighing determination of body density from which the fat mass was estimated. The sum of eight skinfolds were used to determine the subcutaneous fat level. Abdominal fat was quantified by computerized tomography.

The glucose metabolism – indexes examined in study II are described in detail in the study of Hong (Hong *et al.* 2001). In short, the IVGTT (the intravenous glucose tolerance test) was performed in the morning after an overnight (12 h) fast. The insulin sensitivity index (SI) represents the increase in net fractional glucose clearance rate per unit change in plasma insulin

concentration after the intravenous glucose load. The acute insulin response to glucose ($AIR_{Glucose}$) was computed as the incremental integrated area under the insulin curve for the first 10 min of the IVGTT and used as an index of insulin secretion. The disposition index (DI), derived as the product of SI and AIRGlucose, is a measure of the activity of the beta cells in relation to insulin resistance.

The glucose concentrations in all studies were measured with the routinely used enzymatic methods. Insulin was measured with a radioimmunoassay method (study II and III) and with two-site immunoenzymometric assay (study V). Urinary albumin concentrations (study I) were measured by an immunoturbidimetric assay and glycohaemoglobin A1 levels (study I) were determined using a commercial agar gel electroendo-osmotic method. Serum IGF-1, estradiol, estrone and free testosterone (study V) were measured with radioimmunoassay method.

In study IV, type 2 diabetes, IGR and normoglycemia were defined based on glucose tolerance test and criteria of the WHO (Alberti & Zimmet 1998). In study I (type 2 diabetic patients) type 2 diabetes was defined in the individual if he/she was over 35 years at the onset of disease, if the patient had no tendency towards ketonuria, or had stimulated serum C-peptide concentration of 1.8 ug/l or higher after glucagon injection.

4.6 Statistical methods

Statistical analyses were performed by SAS (studies I, II and IV) and SPSS version 11.5 (study III), version 14.0 (study IV and V). Continuous variables were examined for skewness and curtosis and if needed, the values were logarithmically transformed to achieve a normal distribution. A chi-square test was used for comparing frequencies or proportions of qualitative variables in groups, for example to determine whether the genotype frequencies were in Hardy-Weinberg equilibrium and to compare the allele and genotype frequencies between groups. Correlations were studied either with Pearson correlation coefficient in the case of normally distributed variables or otherwise Spearman rank correlation. A Students *t* test was used to compare two groups if the variables were normally distributed, otherwise Mann-Whitney *U*-test or Wilcoxon signed rank test were used. In the comparisons of more than two groups, ANOVA (analysis of variance) or ANCOVA (analysis of covariance) were used.

In studies III and IV, the study populations were divided into quartiles based on adiponectin levels; in study III by keeping mean, +1 and −1 standard deviations as limits in both sexes separately and in study IV, by sub-dividing the whole study group to four groups with equal percentiles. The logistic regression analysis was used to define to odds for metabolic syndrome (III) and type 2 diabetes or IGR (study IV) in adiponectin quartiles. A *P*-value of < 0.05 was considered as statistically significant.

In study V, linear regression analysis was performed to study the determinants of the adiponectin change in the peroral estrogen group.

5 Results

5.1 Interaction of two polymorphisms in PTP1B and LEPR genes associated to higher BMI in type 2 diabetic patients

The distribution of alleles to genotypes followed the Hardy-Weinberg (HW) equilibrium in the diabetics. In control subjects, the genotype Pro303Pro did not follow HW ($P = 0.003$).

The genotype and allele frequencies of PTP1B SNPs were compared between diabetics and healthy subjects. The rare homozygous genotype T/T of Pro303Pro polymorphism was observed only in healthy control population and not in the diabetic population and the genotype frequencies were statistically different (P = 0.018). However, the allele-frequencies of three studied SNPs in PTP1B did not differ between type 2 diabetic patients and control population.

Variation of risk factors and complications of type 2 diabetes did not differ in genotypes based on Pro303Pro and Pro387Leu in type 2 diabetes. G82A polymorphism was associated with BMI ($P = 0.026$), glycohaemoglobin A1 (GhbA1) ($P = 0.033$), and albuminuria ($P = 0.031$) (P-values adjusted for age and gender). BMI was highest in A/A-genotypes and GHbA1 and albuminurea differed between the genotypes A/G and G/G with heterozygous having more albuminurea and lower glycohaemoglobin A1. Heterozygotes showed the lowest prevalence of hypertension ($P = 0.028$). In addition, the lipid values (VLDL cholesterol, VLDL triglycerides, plasma triglycerides) were higher in A/A-genotypes than in the other genotypes ($P < 0.05$), but when adjusted for BMI, there was no statistical difference.

There was a statistically significant interaction effect between PTP1B and LEPR genes impacting on BMI in type 2 diabetic patients. The subjects having genotype combination A82A of PTP1B and Arg223Arg of the LEPR had higher BMI than the other genotype combinations ($P = 0.004$ for trend). The difference was statistically significant compared with all the other genotypes (P-values < 0.006). The BMIs of the genotype combinations are shown in Figure 5. The interaction effect of these two polymorphisms explained 3% of the variation in BMI, when age, sex and major effects of these polymorphisms were included on the model.

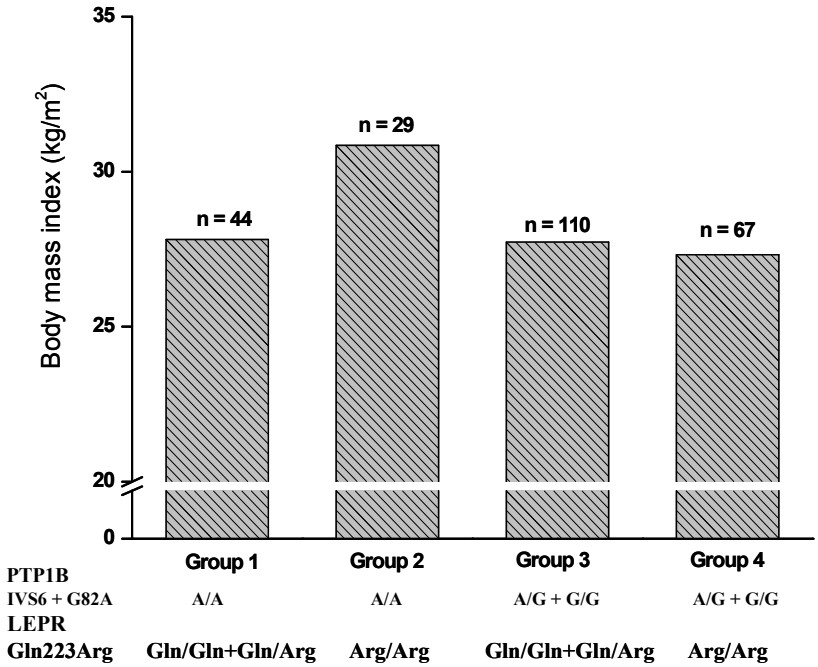

Fig. 5. The interaction effect between PTP1B (protein tyrosine phosphatase 1B) gene and LEPR (leptin receptor) gene polymorphisms on body mass index.

5.1.1 Tyr111His polymorphism of ADIPOQ associated with insulin sensitivity in Caucasians but other SNP were related to body composition in African-Americans

The three studied SNPs in the ADIPOQ were in Hardy-Weinbergs equilibrium. In the Finnish population, the genotype and allele frequencies between Type 2 diabetic patients and healthy control population was different when analyzing Tyr111His polymorphism (Table 4.) but not in other adiponectin SNPs. The calculated r^2 values were small among all the SNPs of this study which means that the SNPs are in linkage equilibrium.Therefore, haplotypes were constructed of three SNPs studied. However, the haplotype frequencies did not differ between Finnish type 2 diabetic and controls.

Table 4. Tyr111His polymorphism of ADIPOQ in Finnish study subjects.

	Genotype frequency *			Allele frequency **	
	Tyr111Tyr	Tyr111His	His111His	Tyr	His
Type 2 diabetes patients (n = 254)	229 (0.902)	24 (0.094)	1 (0.004)	0.949	0.051
Controls (n = 270)	256 (0.948)	14 (0.052)	0 (0.000)	0.974	0.026

*P = 0.034 for the genotype frequencies between type 2 diabetes patients and controls. **P = 0.033 for the alleles frequencies between type 2 diabetes patients and controls

In American study population, there were differences between African Americans and Caucasians in body composition and lipids according to ADIPOQ genotypes. In Caucasians, the carriers of His111 allele tended to have lower insulin sensitivity index and higher AIRglucose and Disposition index than the noncarriers (Figure 6). The allele frequency of His-allele was low, 0.04% (39 Tyr111His and His111His genotypes) in Caucasians and 0.01% (four Tyr111His-genotypes) in African-Americans.

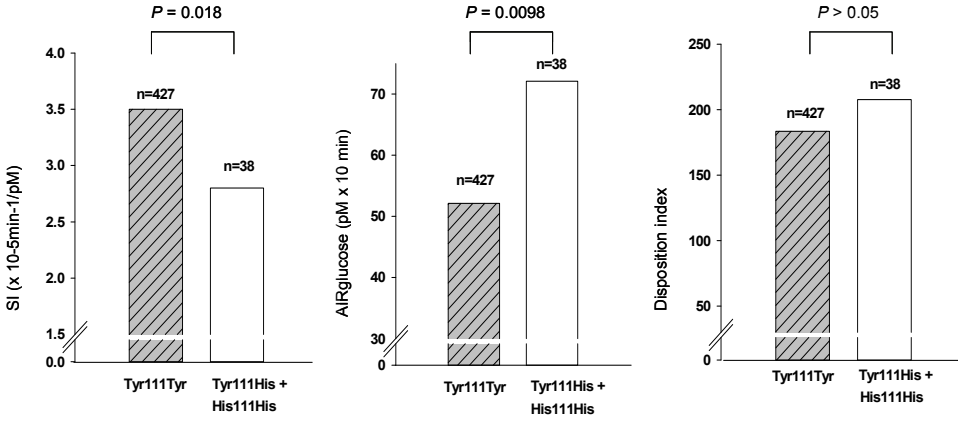

Fig. 6. Glucose metabolism phenotypes in relation to Tyr111His genotypes in Caucasians in the HERITAGE cohort. All the family members were included in the analysis. SI = insulin sensitivity index, AIRglucose = acute insulin response to glucose.

In African Americans, however, the Gly15Gly and G276T polymorphisms associated with BMI and lipids. The associations between T276G SNP with body fat and total plasma cholesterol are presented in Table 5. T276T genotypes of African Americans had significantly lower percentage body fat ($P = 0.002$) than the G276G genotypes and also a lower percentage of subcutaneous fat than

carriers of the G allele ($P = 0.014$ for trend). These genotypes also differed according to their cholesterol levels with genotype G276G having the higher total cholesterol than the other genotypes ($P = 0.005$ for trend). In African Americans, those carrying the rarer allele G in the Gly15Gly polymorphism had significantly higher total cholesterol (4.26 mmol/l (SE 0.10)) than subjects with the T allele (4.78 mmol/l (SE 0.16)) ($P = 0.009$). Haplotypes conducted of these three polymorphisms reflected the associations of independent polymorphisms

Table 5. T276G polymorphism of ADIPOQ in African American subjects.

	T276T	T276G	G276G	*P*
Percentage body fat	19.9 (2.4)	22.8 (1.8)	27.4 (1.9)	0.002*
Subcutaneous fat (sum of eight skinfolds)	123.5 (12.4)	132.0 (7.9)	153.6 (8.0)	0.014**
Total cholesterol mmol/l	4.25 (0.18)	4.15 (0.09)	4.50 (0.11)	0.005***

* $P = 0.001$ between G62G and T62T; $P = 0.008$ between G62G and G62T

** $P = 0.012$ between G62G and T62T; $P = 0.018$ between G62G and G62T

*** $P = 0.001$ between G276G and G276T

5.2 Low plasma adiponectin indicates metabolic syndrome

In both sexes and with both criteria for metabolic syndrome, plasma levels of adiponectin were lower in subjects with the metabolic syndrome compared to those without the syndrome ($P < 0.001$). There was no statistical difference between the groups with differentially diagnosed metabolic syndrome (IDF and NCEP (ATPIII)).

According to *t*-test performed between those groups positive for the individual components of metabolic syndrome and those not having any components, adiponectin levels were lower in both sexes according to waist-, triglyceride-, and HDL criteria ($P < 0.001$) (Table 6).

Table 6. Adiponectin levels as means (S.D) in subjects positive to individual component of metabolic syndrome according to International Diabetes Federation (IDF) criteria and in subjects not having any components.

	Females			Males		
	No IDF criteria present	IDF criteria present	Student`s *t*-test	No IDF criteria present	IDF criteria present	Student`s *t*-test
Metabolic	n = 315	n = 165		n = 293	n = 233	
syndrome	18,81 (6.3)	14.74 (5.6)	$P < 0.001$	14.47 (5.5)	12.28 (5.4)	$P < 0.001$
Waist	n = 233	n = 282		n = 201	n = 315	
	18.94 (6.4)	16.27 (6.0)	$P < 0.001$	15.01 (5.6)	12.86 (5.5)	$P < 0.001$
Triglycerides	n = 398	n = 118		n = 311	n = 205	
	18.32 (6.4)	14.77 (5.33)	$P < 0.001$	14.5 (5.6)	12.5 (5.4)	$P < 0.001$
HDL-	n = 352	n = 164		n = 364	n = 152	
cholesterol	18.60 (5.4)	15.17 (5.13)	$P < 0.001$	16.09 (5.4)	13.46 (5.6)	$P < 0.001$
Systolic blood	n = 96	n = 420		n = 45	n = 471	
pressure	18.25 (6.2)	17.22 (6.4)	$P = 0.203$	16.09 (5.4)	13.46 (5.6)	$P = 0.003$
Plasma glucose	n = 420	n = 96		n = 387	n = 129	
	18.18 (6.3)	14.57 (5.8)	$P < 0.001$[a]	13.74 (9.37)	13.56 (6.25)	$P = 0.455$*

*Mann-Whitney *U*-test, HDL = high density cholesterol, S.D = standard deviation

To evaluate the association between the multiplicity of components of metabolic syndrome and hypoadiponectimia, adiponectin levels were compared between groups based on how many components of metabolic syndrome could be identified on the basis of IDF criteria. As seen in Figure 7, it was observed that adiponectin levels were lowest in subjects having most features of metabolic syndrome.

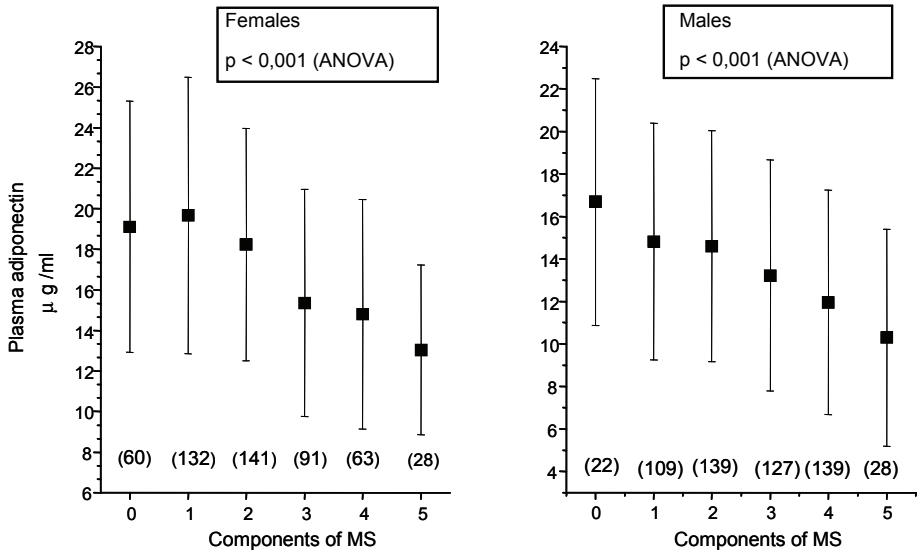

Fig. 7. Plasma adiponectin levels according to number of positive features of metabolic syndrome (IDF) in females and males. The boxes represent means, the bars represent standard deviation and the number of subjects is in parentheses. MS = metabolic syndrome.

Both sexes were divided into four groups based on adiponectin levels. The probability of metabolic syndrome defined by changes in the odds of the event compared with the group with highest adiponectin level. In both sexes, the odds of developing the metabolic syndrome was increased in every lower group of adiponectin levels. Thus the highest probability of having metabolic syndrome was in the group with the lowest plasma adiponectin level (probability 4.4 -fold compared to highest quartile (95% CI 2.3–8.5, $P < 0.001$) in males, probability 7.5 -fold (95% CI 3.6–15.6, $P < 0.001$) in females). The same model was exained also adjusting by age, smoking years, grams of alcohol consumption per day, blood pressure – and lipid medication. Then, the results were 3.1 fold risk in men (95% CI 1.5–6.3 $P = 0.002$ and 9.4 fold in women (95% CI 4.1–21.4 $P < 0.001$).

5.3 Low plasma adiponectin predicts impaired glucose regulation and type 2 diabetes

The prospective data was analyzed to clarify how well low serum adiponectin could predict impaired glucose tolerance or type 2 diabetes. The mean serum adiponectin level tended to be higher in the subjects who remained

normoglycemic compared to those who progressed to impaired glucose tolerance or type 2 diabetes ($P = 0.073$). The subjects who remained normally glucose tolerant had significantly lower levels of triglycerides and 2-hour glucose, and tended to have lower BMI, lower systolic and diastolic blood pressures, and higher HDL cholesterol than the other group at baseline.

In 15 of 41 (37%) subjects with low plasma adiponectin levels at baseline developed impaired glucose regulation or type 2 diabetes, whereas the corresponding proportion was 32 of 160 (20%) subjects with higher adiponectin levels ($P = 0.025$). Adiponectin was considered low when it was in the lowest adiponectin quartile, with the cut-off point being calculated separately for men and women.

When the subjects with lower adiponectin levels (subjects in lowest adiponectin quartile) at baseline were compared to subjects with the higher level (subjects in the highest adiponectin quartile), the odds ratio for the development of impaired glucose tolerance or type 2 diabetes was 2.3 (95% CI, 1.1–4.9). In the logistic regression analysis, the adjusted odds ratio for impaired glucose tolerance or type 2 diabetes was 2.1 (95% CI, 1.0–4.5) when adjustment was made for sex and BMI.

5.4 Peroral estrogen replacement therapy decreases plasma adiponectin in postmenopausal women

In this study, the effects of both the PE and the TE estrogen replacement therapy on plasma adiponectin levels were studied. The two treatment groups i.e. those who received either estradiol valerate orally with placebo gel (PE group) and those who received estradiol gel transdermally with placebo tablet were similar in their baseline adiponectin concentrations as well as in their BMI values. In addition, the number of smokers and previous HRT users did not differ between the treatment groups.

The correlations of adiponectin levels with various factors are presented in Table 7.

In the PE group, the plasma adiponectin concentration decreased significantly after treatment from a mean value of 13.6 ± 7.5 to 11.6 ± 5.8 mg/L ($P < 0.01$). In the TE group, the change from 12.7 ± 5.8 to 12.2 ± 6.4 mg/L did not reach statistical significance. Due to the significant change in plasma adiponectin levels in the PE group, correlations between changes in adiponectin and several other parameters were studied. There was a significant correlation between the change

in adiponectin levels and the change in waist-hip (WH) ratio ($r = -0.386$, $P = 0.029$), VLDL triglycerides ($r = -0.454$, $P = 0.009$) and IGF-1 ($r = -0.39$, $P = 0.025$). There was also a trend of correlation between adiponectin and triglycerides and VLDL cholesterol.

The linear regression model was assessed to explain the variation in the change of adiponectin concentration in the PE group. The variables that correlated with the change in adiponectin (WH-ratio, VLDL-triglycerides and IGF-1) and also the change in estradiol were included in the model. The model was able to explain 43.1% (adjusted $R^2 = 0.431$, $P = 0.001$) of the variation in the adiponectin change. Only the change in VLDL triglycerides (standardized $\beta = -0.407$, $P = 0.011$) explained significantly the variation in the change of adiponectin level.

Table 7. Correlation coefficients for associations with the plasma adiponectin concentration at the baseline.

Variable	*r*
BMI	−0.259 *
Waist hip ratio	−0.185
Estradiol	0.025
luteinizing hormone	0.243 *
free testosterone	−0.022
HDL cholesterol	0.283 *
LDL cholesterol	−0.120
VLDL cholesterol	−0.351 **
mean triglycerides	−0.385 ***
VLDL triglycerides	−0.404 ***
fasting glucose	−0.136
fasting insulin	−0.210
insulin sensitivity index	0.286 *
IGF-1	−0.043

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$,

BMI = body mass index, HDL = high density lipoprotein, LDL = low density lipoprotein, VLDL = very low density lipoprotein, IGF-1 = insulin growth factor 1

6 Discussion

6.1 Methodological considerations

6.1.1 Study populations

The strength of the study is the use of five, quite large and clinically well defined populations. However, from todays`perspective, the study groups we used are relatively small for genetic association studies. In addition, when performing genetic studies, it can always be speculated whether the controls and cases are truly appropriate. According to multifactorial model, a predisposition to the disease can be determined by many different combinations of genetic variants and environmental factors, thus the genetically disposed individuals will not necessarily develop diabetes if they avoid the relevant environmental factors. As Gerich (1998) stated, there are both diabetogenic genes and diabetes-related genes. A diabetogenic gene could be for example a gene directly affecting insulin secretion and a diabetes related gene is one affecting a risk factor of diabetes, such as obesity. From these points of view, the association study should be designed so that groups are very strictly defined, for example obese and non-obese or subjects with impaired and normal glucose tolerance. When studying type 2 diabetic patients and the healthy control subjects, there might be obese subjects or subjects having impaired glucose tolerance in the healthy control subjects. For these reasons, the control group is always difficult to match to the case population.

6.1.2 Concerns relating to association studies

It is both frustrating but understandable that the results of the published association studies are inconsistent. There might be several reasons for these inconsistencies. Firstly, the different allele frequencies and linkage disequilibrium in different populations may lead to inconsistency. Second, the genetic etiology of type 2 diabetes might be different in populations with different genetic origins. Nonetheless gene-environment interactions, which have not yet been widely studied, can also affect the results. For example, there has already been one study performed by Kang *et al.* (2003) regarding ADIPOQ SNP G45T, where carriers of the GG genotype exhibited a smaller reduction in the fasting plasma glucose

(FPG) level after rosiglitazone treatment than carriers of the other genotypes. There was also a smaller increase in the serum adiponectin concentration for the GG genotype than for the other genotypes (Kang *et al.* 2005). This can mean that already the intervention can modify the genotype, if there are users of medication in the study group. As discussed earlier, also lifestyle factors such as dietary habits can modify the amount of adiponectin in circulation and modulate the effect of SNPs between two populations. In addition, the variation in other areas of the genome may influence the results since it has been found out that at least five identified loci are associated with variations in the serum adiponectin level (Gable *et al.* 2006). When the impact of the genotype on the phenotypes of interest is small, as is expected in the case of a multifactorial disease like type 2 diabetes, these kinds of issues make it difficult to compare the results from different populations. However, it is important that the association studies are repeated. The use of several, independent populations in order to replicate the results of one study, minimizes the risk of getting spurious associations.

6.1.3 Statistical tests

When performing multiple statistical tests, there is a chance of obtaining false positive association. In the significance level $P < 0.05$, there is a 5% probability of obtaining false result. When 20 independent tests are performed, the chance of at least one test being significant is no longer 0.05, but 0.64. That should be borne in mind when analyzing the results. However, in the works of this thesis, it was also appreciated that strict adjustments like Bonferroni adjustment for multiple comparisons might be overadjustment when the hypothesis of the study is biologically relevant. The results of the study can also be interpreted without adjustments since too strict adjustments could lead to rejection of true associations in small, underpowered studies (Perneger 1998). However, in the 2000s at the time of the original publications of this thesis, the false discovery rate (FDR) (Benjamini & Hochenberg 1995) had started to be a cause for concern. FDR takes also into account the possibility of erroneous rejections.

6.1.4 Genotyping of polymorphisms

We used a PCR based RFLP method to genotyping of SNPs in the candidate genes. Within a few short years, the candidate gene approach that was used in this thesis, seems to have been replaced by genome wide association studies (GWAs).

Nowadays, array-based technologies allow very large number of SNPs to (10^5–10^6) be genotyped in large cohorts from the whole genome (Reviewed by Grant & Hakonarson 2008). When looking at the recently published genetic studies on adiponectin and PTP1B, the new array methods seem to readily permit the genotyping of tens of SNPs in genes. In addition, the International HapMap Project has yielded information from human genetic diversity and has revealed the discrete linkage disequilibrium blocks in human genome which can be readily accessed through the internet. This has allowed scientists to choose the SNPs that detect the major haplotypes with a minimal set of SNPs. However, despite being somewhat old fashioned, the method used in this thesis is quite reliable because the restriction reaction was always confirmed by evaluating samples from every genotype in every restriction reaction. This minimizes the effect of varying efficiency of enzymes from one experiment to the next.

The haplotypes for type 2 diabetic patients and controls (study II) were statistically constructed with the Bayesian method, PHASE. Thus there might be a proportion of incorrect haplotypes. The haplotypes could also be determined molecularly, for example with allele specific primers (Crawford & Nickerson 2005).

6.1.5 Measurement of plasma adiponectin levels

The measurement of adiponectin levels seems to have been appropriate based on the following studies. In the study of Shand and colleagues, only minor diurnal and post-prandial changes were observed in plasma adiponectin levels. Therefore, the collected plasma in our study are believed to provide a realistic view of the subjects` adiponectin concentrations. They also observed that the storage at −30 C ° for 33 months or three cycles of freezing and thawing had no discernible effect on adiponectin levels (Shand *et al.* 2006). However, we cannot rule out the possibility that the longer storage time and more frequent handling of OPERA plasma samples have affected the protein structure in samples and interfered with the ELISA method.

There are many different methods available with which to measure adiponectin levels. No standardized method for the measurement exists and therefore it is not yet easy to determine, for example, which level of adiponectin in an individual can be considered as being too low. This can also be seen in these studies, where different methods have been used for the adiponectin measurement. In addition, a knowledge of different adiponectin forms may bring new

information to future studies. Based on recent studies, one possible limitation to this study is that the total adiponectin concentration including all the forms of adiponectin in the circulation were assayed. However, the recent studies suggest that the multimeric, HMW-form adiponectin may be better at explaining insulin sensitivity rather than the whole circulating level (Pajvani *et al.* 2004). The stability and secretion of adiponectin are also regulated at the post-translational modification level via hydroxylation, glycosylation and disulfide bond formation. Therefore, it is also possible that defects in adiponectin may be due to complex changes in posttranslational processing (Liu & Liu 2010).

6.2 Consideration of major results

6.2.1 PTP1B polymorphisms and the interaction with LEPR polymorphism

It was decided to test how the variation in the PTP1B gene would be associated with type 2 diabetes and how the variation would be related to risk factors of type 2 diabetes and cardiovascular complications. According to this study, none of the three studied SNPs in PTP1B gene clearly associate to type 2 diabetes. The earlier studies and the studies performed after our study examining the Pro303Pro and Pro387Leu polymorphisms are somewhat contradictory. The rare T allele of Pro303Pro polymorphism was reported to protect from diabetes and IGT in Oji-Crees (Mok *et al.* 2002). We obtained similar evidence because the rare genotype was not seen in diabetics while 2.9% of controls (n = 285) were genotype T/T. However, this difference was not detected in allele frequencies. It is possible that this association is a false positive, because the number of subjects is so low. In addition the concept, that Pro303Pro did not follow HW equilibrium in the control population might make one suspicious of the results. It could also be speculated that the rare allele T is in linkage disequilibrium with another recessive allele in same or another gene and that the allele becomes protective only in homozygotes. Since Pro303Pro is a silent mutation, it does not change the amino-acids in the protein but could affect translation efficiency or have an influence on splice-enhancer regions within the exon.

The Pro387Leu polymorphism was originally identified by Echwald (Echwald *et al.* 2002) who reported that the rare variant was associated with a 3.7 genotype relative risk for suffering type 2 diabetes. That study evaluated 527 type

2 diabetes subjects and 542 glucose tolerant control subjects. The functional consequence *in vitro* of this variant was that the variant peptide reduced serine phosphorylation. Subsequently, however also negative findings have been published. In a German population, the Pro387Leu polymorphism did not associate with type 2 diabetes but the carriers of the rare allele had significantly higher triglycerides (Gouni-Berthold *et al.* 2005). The results of this present thesis indicate that Pro387Leu SNP is not associated with type 2 diabetes. However, frequencies of rare alleles of both Pro303Pro and Pro387Leu SNPs are low and our study group was too small to confirm the results as Echwald *et al.* (2002) who originally reported a much larger study group.

The polymorphism G82A had not been studied before our study. This locus is located in the noncoding region and does not change the amino acid sequence of PTP1B. In this study, the A/A genotypes had higher BMI than the other genotypes. However, the association was only moderate and the association of this genotype with lipids and lipoprotein values vanished after adjustment for BMI. Subsequently, Ukkola performed another study in American subjects where A82A genotypes showed lower BMI, percentage body fat and subcutaneous fat than other genotypes of this PTP1B G82A polymorphism (Ukkola *et al.* 2005). This is contradictory to our report which might result from different linkage disequilibrium between populations or simply indicate that this association was a false positive.

It should be pointed out that the SNPs studied in the PTP1B gene locate very closely to each other in the chromosome. According to studies conducted in French subjects (Cheyssac *et al.* 2006), Hispanic Americans (Palmer *et al.* 2004) and Pima Indians (Traurig *et al.* 2007), the polymorphisms in the PTP1B gene occur in two discrete LD blocks. One region is upstream to PTP1B and another spans the whole coding region of the gene. Also in Scandinavians, (Florez *et al.* 2005) the most common SNPs in the coding region fell within the same LD block. Therefore, it is likely that the SNPs are in linkage disequilibrium and they could also be seen as one haplotype. Recently, many studies have been performed with tagSNPs or haplotypes. According to the Cheyssacs (2006) study in French families, any of tagSNPs selected including Pro303Pro, did not associate with type 2 diabetes, however there was some association with obesity. In Pima Indians and Scandinavians, the SNPs did not associate with type 2 diabetes (Traurig *et al.* 2007, Florez *et al.* 2005). However in the haplotype analysis (Bento *et al.* 2004) several SNPs in the distal part of the gene (from exon 4 to intron 8) associated strongly with type 2 diabetes. One can speculate that since

Pro303Pro and Pro387Leu have functional consequences, it is reasonable to study both of them regardless of their close location to each other in the sequence. Spencer-Jones *et al.* (2005) reported that Pro303Pro polymorphism associated to Avignon's insulin sensitivity index. Palmer studied the SNPs and glucose homeostasis and found that many of the SNPs in the LD block that contains the SNPs studied here, associated with quantitative measures of glucose homeostasis. There is also an insertion polymorphism (1484insG) in the 3´untranslated region that has displayed higher mRNA stability and which associates with several features of insulin resistance (Di Paola *et al.* 2002). In addition, promoter variants have recently been identified and some have been reported to possess functional relevance (Meshkani *et al.* 2007).

It was found that individuals with the genotypic combination of PTP1B A82A and LEPR Arg223Arg had significantly higher BMI values than the other genotypes. In complex traits such as type 2 diabetes, there are probably many predisposing alleles and interactions between many genes. This might be one example of such an interaction. For example, these two polymorphisms might have synergestic inhibiting effects on leptin signalling.

The interaction of PTP1B and LEPR -genes also associated differentially in the study of Ukkola (Ukkola *et al.* 2005). In that study, the genotype G82G+Gln223Gln of PTP1B+LepR combination had the lowest insulin sensitivity index, which is opposite to what would have been expected based on our study, where A82A+Arg223Arg genotypes had the highest BMI values.

6.2.2 Association of adiponectin polymorphisms with adiposity and insulin sensitivity

Three SNPs (Tyr111His, T45G, T276G) in ADIPOQ and the haplotypes derived from these SNPs were analysed in Finnish Type 2 diabetic patients and healthy controls. The genotype and allele frequencies of Tyr111His SNP differed between the groups. The His allele was more frequent in diabetics than in healthy subjects. Interestingly, in the Caucasian Americans examined in the HERITAGE study, subjects having the His -allele, were more insulin resistant according to SI (insulin sensitivity index). Presumably as a compensatory mechanism, His111 carriers displayed a higher acute insulin response to glucose. Adiponectin levels tended to be lower in carriers of His allele, which is in accordance with the finding that they also were more insulin resistant. However, it is not possible to conclude that the adiponectin levels were influenced by the Tyr111His

polymorphism. In agreement with the finding in this thesis, Tyr111His was associated with diabetes incidence in a recent large Framingham Offspring Study (Hivert *et al.* 2008). In that study, Tyr111His was not associated with the plasma adiponectin level. The authors of the Framingham study hypothesized that Ty111His polymorphism might disrupt the exonic splicing enhancer, which could influence alternative splicing of the message. It is known that Tyr111His does not affect multimerization of protein (Waki *et al.* 2003). Tyr111His associated with the incidence of diabetes also in French Caucasians (Vasseur *et al.* 2002) but not in a Swedish population (Gu *et al.* 2004).

The frequency of the His -allele is very low, and therefore further studies in larger populations are needed. In African Americans, the evaluation of Tyr111His could not be completed because the allele frequency was too low. There were also other differences in the associations between Caucasian and African American samples. Based on a recent study, African Americans have different linkage equilibrium blocks in the adiponectin gene, as compared to European population (Bostrom *et al.* 2009). This could explain the differences in associations between Caucasian and African Americans. The Caucasian and African American alleles in this study are in LD with different alleles, in ADIPOQ or another gene. It is known that African Americans have higher rates of obesity and associated morbidities than Caucasians, and it might be that a sequence variation behind the results of this study is one contributing factor. These results also emphasize the difficulty of comparing the results of association studies in different populations.

The T45G polymorphism is located in exon 2 of the ADIPOQ and does not cause an amino acid change (Gly15Gly). However, in one study, G-allele was reported to have higher transcriptional activity than the T-allele since it may alter mRNA splicing or stability (Yang *et al.* 2003). The G276T is located in intron 2 and could also influence adiponectin expression. Based on this thesis study, T45G and G276T SNPs do not influence the risk of type 2 diabetes at least in Finns. Also in another Finnish study, no significant differences between offspring of type 2 diabetes patients and control subjects in SNPs T45G and Gly15Gly were found and these SNPs did not associate with the adiponectin level or with any other metabolic variables (Salmenniemi *et al.* 2005). However, in one study of young Finnish men, the T276T genotype was found to be associated with elevated serum adiponectin levels and elevated diastolic blood pressure and the T45T was associated with high HDL cholesterol levels (Mousavinasab *et al.* 2006). In our American study population, G276G was associated with a poorer lipid profile in African Americans.

After we performed our study, a large meta-analysis was conducted of the effect of ADIPOQ variability on BMI, type 2 diabetes and insulin resistance (Menzaghi *et al.* 2007). The published data concerned the SNPs G45T and G276T, which were also included in our study. After reviewing many studies, Menzaghi and the colleagues concluded that the carriers of T allele of G276T polymorphism have higher insulin sensitivity as defined by $HOMA_{IR}$ but that there was no global effect of the SNPs on type 2 diabetes or BMI.

6.2.3 Plasma adiponectin level and components of the metabolic syndrome

It was decided to measure plasma adiponectin levels in a large population to determine how the levels associate with the metabolic syndrome as diagnosed by the IDF criteria. At that time, there were only few studies of adiponectin and metabolic syndrome in large populations and none had used the criteria for metabolic syndrome of IDF.

As expected based on earlier studies, the adiponectin levels were higher in women than in men. In both sexes, low adiponectin levels associated clearly with multiplicity of the metabolic syndrome. In our study, a significant difference was noted in the adiponectin level in both sexes between subjects with the metabolic syndrome and those subjects without the metabolic syndrome. Despite the differences in definitions, both NCEP – and IDF –defined subjects had low plasma adiponectin levels and both criteria seem to work similarly in the sense that they identify subjects with low adiponectin levels. As seen from Figure 7, the more the individual has components of metabolic syndrome, the lower the adiponectin levels are likely to be.

When studying adiponectin and metabolic syndrome, it should be noted that adiponectin levels correlate significantly with BMI and measures of adiposity. Therefore, it could be speculated that the strong association of adiponectin with different components and multiplicity of metabolic syndrome is simply a consequence of fat mass. This causes problems with statistical tests. Adjusting for BMI might represent overadjusting. However, there are individuals who may have low adiponectin levels and the metabolic syndrome, perhaps more due to genetic factors, despite the fact that they are lean. There are also individuals who are obese but do not suffer from comorbidities. Therefore, it is important to identify the factors that lie beyond the obesity. Interestingly, it was stated that according to the statistical modelling performed in one study (Martin *et al.* 2005),

the relationship between adiponectin and insulin levels and blood lipids (HDL and TG) was strengthened with increasing adiposity. This could be attributable to the proinflammatory milieu in adipose tissue in overweight people but its absence in lean individuals.

Our results suggested that in females, low adiponectin levels may be even more related to the probability to suffer the metabolic syndrome than is the case in males. It has been found that females have higher proportions of high molecular weight and hexameric forms of adiponectin and lower proportions of the trimers in theis bloodstream and that the higher molecular form adiponectin serves as a precursor pool for activation by metabolic stimuli, such as an increased insulin level (Peake *et al.* 2005). Therefore, the presence of these adiponectin forms could partly explain the better insulin sensitivity of females and could also be one reason why lower adiponectin levels may have a more deleterious effect in women.

Our results are very similar to those reported for other populations despite differences in lifestyle and nutritional habits. In the large study group of Chinese aged 50–70, adiponectin levels similarly associated to multiplicity in the metabolic syndrome and the subjects in the lowest plasma adiponectin quartile were five times more likely to have the metabolic syndrome than those in highest quartile (Wang *et al.* 2008). Since low adiponectin levels associate strongly with the multiplicity of the metabolic syndrome, it is possible that the development of the metabolic syndrome is triggered by a single underlying mechanism. Our study is cross-sectional, which does not allow us to infer causality from our results. For example, it is impossible to determine whether the decreased adiponectin levels are a cause or a consequence of the metabolic state of high glycemia, dyslipidemia or inflammation. However, it is tempting to speculate that inflammation in fat tissue, genetic factors and lifestyle habits leading to reduced adiponectin level in the circulation could evoke many metabolic changes and give rise to components of the metabolic syndrome. Low adiponectin level has now been linked to metabolic syndrome in many populations (Bacha *et al.* 2004, Ryo *et al.* 2004, Salmenniemi *et al.* 2004, Xydakis *et al.* 2004, Bugianesi *et al.* 2005, Mohan *et al.* 2005) and the high molecular weight adiponectin might show an even stronger association with this complex disease (Tabara *et al.* 2008).

6.2.4 Plasma adiponectin and future impaired glucose tolerance or type 2 diabetes

One objective was to determine if a baseline low adiponectin level in normoglycemic subjects would be predictive of IGR or type 2 diabetes during a mean follow-up period of 5.1 years in middle-aged subjects. It was noted that subjects in the lowest quartile of plasma adiponectin levels at baseline, had a 2-fold risk to develop diabetes or IGR compared to the subjects in the highest adiponectin quartile.

The adiponectin-diabetes association in this study is possibly mediated to some extent by insulin resistance. This cannot however be evaluated in the study because there were no assays of insulin levels determined at the baseline. Interestingly, one study has proposed that during the initial stages of hyperinsulinemia, frequently a sign of insulin resistance, high insulin levels lead to a down-regulation of HMW adiponectin levels, which in turn decrease insulin sensitivity further, requiring an even higher level of circulating insulin to maintain glucose homeostasis (Basu *et al.* 2007). Therefore, the fact that insulin levels at baseline were not measured, is one limitation of this study.

The number of study subjects was fairly small, which prevented stratified analyses according to sex. According to other studies and also this present work with respect to the metabolic syndrome, the association of adiponectin to IGR and diabetes could be stronger in women than in men. There was also extensive variation in the BMI of study subjects and a larger study group would have permitted a stratified analyses according to BMI, since it has been proposed that the adiponectin – type 2 diabetes association may well be stronger in obese individuals than it is in their lean counterparts. Finally, many other factors may influence the risk of diabetes and adiponectin may only be a marker of these other factors. Thus, there are many variables, such as plasma lipids, plasma glucose levels at the baseline and inflammatory markers, which could have been added as adjustments in the statistical tests.

Recently, a meta-analysis including 13 prospective studies of plasma adiponectin concentrations and type 2 diabetes was performed. The findings of that study were in accordance with our study and showed that risk of type 2 diabetes appeared to decrease linearly with increasing adiponectin levels. The association was evident inmany races, e.g. whites, East Asians, Asian Indians, African Americans, and native Americans (Li *et al.* 2009).

It could be that long term exposure to low plasma adiponectin levels, due to genetic and/or environmental factors may progressively evoke a state of lipotoxicity in the tissues i.e. accumulation of ectopic fat in liver and muscle altering insulin signaling and functions, thus contributing to whole body insulin resistance and a deterioration of glucose tolerance (Reviewed by Rasouli & Kern 2008 and Dyck 2009). Several studies on metabolic flexibility i.e. capacity of tissues to acutely shift their metabolic fuel from lipids to glucose, and type 2 diabetes have been conducted recently (Reviewed in Galgani *et al.* 2008). Interestingly, in one of these reports, adiponectin-overexpressing mice displayed enhanced clearance of circulating fatty acids and increased expansion of subcutaneous adipose tissue with chronic feeding of high fat diet. These adaptive changes to the high fat diet were associated with mitochondrial density in adipocytes, smaller adipocyte size, and a general transcriptional up-regulation of factors involved in lipid storage through efficient esterification of free fatty acids (Asterholm & Scherer 2010). Hypothetically it could be that the people with low adiponectin are more prone to disruptive effects if they consume fatty food and therefore develop more type 2 diabetes.

6.2.5 Decreased plasma adiponectin in peroral estrogen replacement therapy

Since women undergo extensive physiological changes at menopause in body composition, insulin responses and risk of CHD, estrogen hormone replacement therapy has been suggested as being protective. However, whether estradiol is administered transdermally (gels or patches) or orally has a significant effect on the immediate markers of cardiovascular risk factors. Orally administered hormone therapy has been observed to increase triglyceride levels, this being linked to a decrease in the size of LDL particles and higher levels of C-reactive protein because of accumulation of estradiol to liver (Modena *et al.* 2005).

The study population of postmenopausal women receiving hormone therapy made it possible to explore how adiponectin levels are involved in this change with two different hormone therapies. This is interesting because women have higher adiponectin levels than men and sex hormones have therefore been proposed to be able to influence adiponectin levels. It was noted that peroral estradiol valerate treatment therapy decreased adiponectin levels significantly, but this did not occur in with the transdermal treatment. No correlation was found between estradiol and adiponectin at the baseline, which is in contrast to most of

the studies investigating the relationship of estrogen and adiponectin studies which have pointed to an inverse association between these hormones (Sumino *et al.* 2004, Im *et al.* 2006, Laughlin *et al.* 2006, Leung *et al.* 2009). In addition in the regression analysis conducted here, the change in estradiol after PE treatment, was not the strongest variable explaining the variation in the change in adiponectin. Therefore, it cannot be stated definitely that it was the estradiol treatment that decreased adiponectin levels. The change in adiponectin evoked by PE correlated also with the changes in WH ratio, which is unexpected because BMI or WH ratio did not change significantly after PE. It is possible that even a minor, nonsignificant change in WH ratio is sufficient to affect the plasma adiponectin concentration. This may be particulatly significant because during menopause there is an increased deposition of abdominal fat as compared to peripheral fat (Kanaley *et al.* 2001).

In the regression analysis, the change in the adiponectin level after PE treatment was explained most strongly by the change in VLDL triglycerides. Adiponectin regulates the activity of hepatic lipase and lipoprotein lipase, which are responsible for the catabolism of triglyceride rich lipoproteins. Therefore it could be that reduction in plasma adiponectin levels after PE estradiol therapy partly mediated the unfavourable effects of the treatment on the lipid profile (Modena *et al.* 2005). Therefore, the reduction in the adiponectin level could also contribute to increased risk of CHD reported in the first years of peroral treatment noted in the Woman`s Health Initiative randomized controlled trial (Anderson *et al.* 2004).

The mechanism how adiponectin decreases after PE is not easy to explain and needs to be clarified with larger cohorts and targeted experimental studies. There are very few studies investigating adiponectin and hormone replacement therapy. Chu *et al.* (2006) reported no change in plasma adiponectin after PE but a significant increase after TE, which is at odds with this study. In other studies, progesterone has been used together with PE, which complicates comparison of studies. In those reports, no change (Sumino *et al.* 2004, Cooper *et al.* 2007) or a decrease (Tanko & Christiansen 2005) in plasma adiponectin levels after hormone replacement therapy has been observed. The complex effects of sex hormones on adiponectin remains to be resolved and the hints of a reduction in the plasma adiponectin level after PE treatment must be studied in larger cohorts. It is also possible that sex hormones influence the cardiovascular risk of women by altering the relationship between HMW and total adiponectin levels.

6.3 Clinical relevance of the studies and some future aspects

While the incidences of type 2 diabetes and metabolic syndrome are growing around the world, there is an urgent need to discover new therapeutic targets. There are indeed many studies focusing on PTP1B as a medication target. The development of a selective PTP1B inhibitor would improve insulin sensitivity of patients more than for example vanadate, an agent that non-selectivically inhibits all PTP:s. Before finding and testing of such medicine, many studies will need to be performed to define possible role of PTP1B in cancers, for example. One of the most fascinating ideas behind the genetic studies is the pharmacogenetics, the variability of drug response due to inherited characteristics in individuals. For example, it could be possible that individuals with certain polymorphisms in PTP1B or ADIPOQ need certain types of medication for the metabolic syndrome. Alternatively, as recently postulated (Ferguson *et al.* 2009), people with a certain adiponectin genotype might need personalized dietary advice because they are susceptible to gene-nutrient interactions.

PTP1B, adiponectin and their signalling pathways seem to be attractive therapeutic targets for obesity related conditions. In addition to trying to avoid obesity, at the same time it could be possible to avoid the obesity induced symptoms, like type 2 diabetes or the metabolic syndrome. Therefore, the molecules in the study could be secondary targets for the treatment of diseases beyond obesity. However, the associations between low adiponectin levels and type 2 diabetes and metabolic syndrome need to be confirmed. In these kinds of population studies, it is important to repeat the studies in different study groups to rule out any possible false statistical errors or for example, population specific associations. If the subjects at a higher risk of developing obesity related diseases could be identified, it could be possible to more efectively target treatment and lifestyle instructions. Much remains to be learned about the role of different adiponectin multimers, the factors influencing the relationships between the different multimers and also the regulation of adiponectin receptors perhapsnot all of which have been found yet.

7 Conclusions

The present study investigated the role of PTP1B (protein tyrosine phosphatase 1B) gene, ADIPOQ (adiponectin gene) and the plasma adiponectin level in the metabolic syndrome. The conclusions of the study are as follows:

1. Two polymorphisms in PTP1B may have some, but not a strong, contribution to the development of type 2 diabetes in Finns. The genotype T/T of the Pro303Pro (C981T) polymorphism may be protective against the development of type 2 diabetes because it was found more frequently in the healthy control group compared to diabetics. The interaction effect between the PTP1B and LEPR led to a higher BMI in the PTP1B A82A + LEPR Arg223Arg genotype combination than in the other genotypes.
2. ADIPOQ polymorphisms are associated with obesity-related phenotypes. Caucasian carriers of rare His allele of Tyr111His polymorphism were more insulin resistant and in higher risk of type 2 diabetes. In African Americans the Gly15Gly and G276T polymorphisms associated with BMI and lipids. Thus, the effects of polymorphism on obesity related phenotypes seemed to differ in different ethnic groups.
3. Plasma adiponectin might be a useful biomarker of the metabolic syndrome. Lower adiponectin levels were associated with different components of the metabolic syndrome according to several definitions of the IDF. There was also a trend towards lowering of the plasma adiponectin when the individual had an increasing number of components of the metabolic syndrome.
4. Low adiponectin levels might be predictive of future impaired glucose regulation and type 2 diabetes. A low baseline adiponectin level was associated with a more than 2-fold risk for developing impaired glucose tolerance or type 2 diabetes during a mean follow-up of 5.1 years in a group of middle-aged Finnish subjects who were initially normoglycemic.
5. The reduction in adiponectin levels could be part of the “early harm” profile of the peroral estrogen replacement therapy detected in clinical trials on postmenopausal women. This study must be repeated with a larger sample since the complex effects of sex hormones on adiponectin levels need clarification

References

Adiels M, Taskinen MR, Packard C, Caslake MJ, Soro-Paavonen A, Westerbacka J, Vehkavaara S, Häkkinen A, Olofsson SO, Yki-Järvinen H & Boren J (2006) Overproduction of large VLDL particles is driven by increased liver fat content in man. Diabetologia 49(4): 755–765.

Ahmad F, Azevedo JL, Cortright R, Dohm GL & Goldstein BJ (1997a) Alterations in skeletal muscle protein-tyrosine phosphatase activity and expression in insulin-resistant human obesity and diabetes. J Clin Invest 100(2): 449–458.

Ahmad F, Considine RV, Bauer TL, Ohannesian JP, Marco CC & Goldstein BJ (1997b) Improved sensitivity to insulin in obese subjects following weight loss is accompanied by reduced protein-tyrosine phosphatases in adipose tissue. Metabolism 46(10): 1140–1145.

Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC,Jr, International Diabetes Federation Task Force on Epidemiology and Prevention, Hational Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society & International Association for the Study of Obesity (2009) 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 120(16): 1640–1645.

Alberti KG, Zimmet P, Shaw J & IDF Epidemiology Task Force Consensus Group (2005) The metabolic syndrome--a new worldwide definition. Lancet 366(9491): 1059–1062.

Alberti KG & Zimmet PZ (1998) 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 15(7): 539–553.

Alexander CM, Landsman PB, Teutsch SM, Haffner SM, Third National Health and Nutrition Examination Survey (NHANES III) & National Cholesterol Education Program (NCEP) (2003) NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. Diabetes 52(5): 1210–1214.

Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D, Brunner R, Brzyski R, Caan B, Chlebowski R, Curb D, Gass M, Hays J, Heiss G, Hendrix S, Howard BV, Hsia J, Hubbell A, Jackson R, Johnson KC, Judd H, Kotchen JM, Kuller L, LaCroix AZ, Lane D, Langer RD, Lasser N, Lewis CE, Manson J, Margolis K, Ockene J, O'Sullivan MJ, Phillips L, Prentice RL, Ritenbaugh C, Robbins J, Rossouw JE, Sarto G, Stefanick ML, Van Horn L, Wactawski-Wende J, Wallace R, Wassertheil-Smoller S & Women's Health Initiative Steering Committee (2004) Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 291(14): 1701–1712.

Andersson CX, Gustafson B, Hammarstedt A, Hedjazifar S & Smith U (2008) Inflamed adipose tissue, insulin resistance and vascular injury. Diabetes Metab Res Rev 24(8): 595–603.

Antoniades C, Antonopoulos AS, Tousoulis D & Stefanadis C (2009) Adiponectin: from obesity to cardiovascular disease. Obes Rev 10(3): 269–279.

Ardlie KG, Kruglyak L & Seielstad M (2002) Patterns of linkage disequilibrium in the human genome. Nat Rev Genet 3(4): 299–309.

Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T & Matsuzawa Y (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257(1): 79–83.

Asterholm IW & Scherer PE (2010) Enhanced Metabolic Flexibility Associated with Elevated Adiponectin Levels. Am J Pathol 176(3): 1364–76.

Bacha F, Saad R, Gungor N & Arslanian SA (2004) Adiponectin in youth: relationship to visceral adiposity, insulin sensitivity, and beta-cell function. Diabetes Care 27(2): 547–552.

Baker L, Meldrum KK, Wang M, Sankula R, Vanam R, Raiesdana A, Tsai B, Hile K, Brown JW & Meldrum DR (2003) The role of estrogen in cardiovascular disease. J Surg Res 115(2): 325–344.

Banga A, Bodles AM, Rasouli N, Ranganathan G, Kern PA & Owens RJ (2008) Calcium is involved in formation of high molecular weight adiponectin. Metab Syndr Relat Disord 6(2): 103–111.

Basu R, Pajvani UB, Rizza RA & Scherer PE (2007) Selective downregulation of the high molecular weight form of adiponectin in hyperinsulinemia and in type 2 diabetes: differential regulation from nondiabetic subjects. Diabetes 56(8): 2174–2177.

Bauche IB, Ait El Mkadem S, Rezsohazy R, Funahashi T, Maeda N, Miranda LM & Brichard SM (2006) Adiponectin downregulates its own production and the expression of its AdipoR2 receptor in transgenic mice. Biochem Biophys Res Commun 345(4): 1414–1424.

Bays HE (2009) ”Sick fat,” metabolic disease, and atherosclerosis. Am J Med 122(1 Suppl): S26–37.

Benjamini Y & Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Statist Soc B 57: 289–300.

Bento JL, Palmer ND, Mychaleckyj JC, Lange LA, Langefeld CD, Rich SS, Freedman BI & Bowden DW (2004) Association of protein tyrosine phosphatase 1B gene polymorphisms with type 2 diabetes. Diabetes 53(11): 3007–3012.

Berg AH, Combs TP, Du X, Brownlee M & Scherer PE (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 7(8): 947–953.

Berg AH, Combs TP & Scherer PE (2002) ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 13(2): 84–89.

Berneis KK & Krauss RM (2002) Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res 43(9): 1363–1379.

Biggerstaff KD & Wooten JS (2004) Understanding lipoproteins as transporters of cholesterol and other lipids. Adv Physiol Educ 28(1–4): 105–106.

Blakemore AI & Froguel P (2008) Is obesity our genetic legacy? J Clin Endocrinol Metab 93(11 Suppl 1): S51–6.

Boden G (2002) Interaction between free fatty acids and glucose metabolism. Curr Opin Clin Nutr Metab Care 5(5): 545–549.

Bostrom MA, Freedman BI, Langefeld CD, Liu L, Hicks PJ & Bowden DW (2009) Association of adiponectin gene polymorphisms with type 2 diabetes in an African American population enriched for nephropathy. Diabetes 58(2): 499–504.

Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH & Gagnon J (1995) The HERITAGE family study. Aims, design, and measurement protocol. Med Sci Sports Exerc 27(5): 721–729.

Bowden DW, Sale M, Howard TD, Qadri A, Spray BJ, Rothschild CB, Akots G, Rich SS & Freedman BI (1997) Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. Diabetes 46(5): 882–886.

Brooks NL, Moore KS, Clark RD, Perfetti MT, Trent CM & Combs TP (2007) Do low levels of circulating adiponectin represent a biomarker or just another risk factor for the metabolic syndrome? Diabetes Obes Metab 9(3): 246–258.

Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, Gentilcore E, Natale S, Cassader M, Rizzetto M, Pasquali R & Marchesini G (2005) Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. J Clin Endocrinol Metab 90(6): 3498–3504.

Cheung A, Kusari J, Jansen D, Bandyopadhyay D, Kusari A & Bryer-Ash M (1999) Marked impairment of protein tyrosine phosphatase 1B activity in adipose tissue of obese subjects with and without type 2 diabetes mellitus. J Lab Clin Med 134(2): 115–123.

Cheyssac C, Lecoeur C, Dechaume A, Bibi A, Charpentier G, Balkau B, Marre M, Froguel P, Gibson F & Vaxillaire M (2006) Analysis of common PTPN1 gene variants in type 2 diabetes, obesity and associated phenotypes in the French population. BMC Med Genet 7: 44.

Chow WS, Cheung BM, Tso AW, Xu A, Wat NM, Fong CH, Ong LH, Tam S, Tan KC, Janus ED, Lam TH & Lam KS (2007) Hypoadiponectinemia as a predictor for the development of hypertension: a 5-year prospective study. Hypertension 49(6): 1455–1461.

Chu MC, Cosper P, Nakhuda GS & Lobo RA (2006) A comparison of oral and transdermal short-term estrogen therapy in postmenopausal women with metabolic syndrome. Fertil Steril 86(6): 1669–1675.

Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD & Kahn SE (2003) Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 46(4): 459–469.

Collaku A, Rankinen T, Rice T, Leon AS, Rao DC, Skinner JS, Wilmore JH & Bouchard C (2004) A genome-wide linkage scan for dietary energy and nutrient intakes: the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study. Am J Clin Nutr 79(5): 881–886.

Combs TP, Wagner JA, Berger J, Doebber T, Wang WJ, Zhang BB, Tanen M, Berg AH, O'Rahilly S, Savage DB, Chatterjee K, Weiss S, Larson PJ, Gottesdiener KM, Gertz BJ, Charron MJ, Scherer PE & Moller DE (2002) Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. Endocrinology 143(3): 998–1007.

Committee on Diabetic Twins, Japan Diabetes Society. Diabetes mellitus in twins: a cooperative study in Japan. (1988) Diabetes Res Clin Pract 5(4): 271–280.

Comuzzie AG, Funahashi T, Sonnenberg G, Martin LJ, Jacob HJ, Black AE, Maas D, Takahashi M, Kihara S, Tanaka S, Matsuzawa Y, Blangero J, Cohen D & Kissebah A (2001) The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. J Clin Endocrinol Metab 86(9): 4321–4325.

Cooper BC, Burger NZ, Toth MJ, Cushman M & Sites CK (2007) Insulin resistance with hormone replacement therapy: associations with markers of inflammation and adiposity. Am J Obstet Gynecol 196(2): 123.e1–123.e7.

Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, Van Pelt RE, Wang H & Eckel RH (2008) The metabolic syndrome. Endocr Rev 29(7): 777–822.

Crawford DC & Nickerson DA (2005) Definition and clinical importance of haplotypes. Annu Rev Med 56: 303–320.

Dabelea D & Pettitt DJ (2001) Intrauterine diabetic environment confers risks for type 2 diabetes mellitus and obesity in the offspring, in addition to genetic susceptibility. J Pediatr Endocrinol Metab 14(8): 1085–1091.

Dallaire P, Bellmann K, Laplante M, Gelinas S, Centeno-Baez C, Penfornis P, Peyot ML, Latour MG, Lamontagne J, Trujillo ME, Scherer PE, Prentki M, Deshaies Y & Marette A (2008) Obese mice lacking inducible nitric oxide synthase are sensitized to the metabolic actions of peroxisome proliferator-activated receptor-gamma agonism. Diabetes 57(8): 1999–2011.

Danforth E,Jr (2000) Failure of adipocyte differentiation causes type II diabetes mellitus? Nat Genet 26(1): 13.

Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W & Auwerx J (1998) A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 20(3): 284–287.

Di Paola R, Frittitta L, Miscio G, Bozzali M, Baratta R, Centra M, Spampinato D, Santagati MG, Ercolino T, Cisternino C, Soccio T, Mastroianno S, Tassi V, Almgren P, Pizzuti A, Vigneri R & Trischitta V (2002) A variation in 3' UTR of hPTP1B increases specific gene expression and associates with insulin resistance. Am J Hum Genet 70(3): 806–812.

Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D & Purcell S (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316(5829): 1331–1336.

Dixon JB (2010) The effect of obesity on health outcomes. Mol Cell Endocrinol 316(2): 104–108.

Dong C, Wang S, Li WD, Li D, Zhao H & Price RA (2003) Interacting genetic loci on chromosomes 20 and 10 influence extreme human obesity. Am J Hum Genet 72(1): 115–124.

Doria A, Patti ME & Kahn CR (2008) The emerging genetic architecture of type 2 diabetes. Cell Metab 8(3): 186–200.

Dowse G & Zimmet P (1993) The thrifty genotype in non-insulin dependent diabetes. BMJ 306(6877): 532–533.

Dyck DJ (2009) Adipokines as regulators of muscle metabolism and insulin sensitivity. Appl Physiol Nutr Metab 34(3): 396–402.

Echwald SM, Bach H, Vestergaard H, Richelsen B, Kristensen K, Drivsholm T, Borch-Johnsen K, Hansen T & Pedersen O (2002) A P387L variant in protein tyrosine phosphatase-1B (PTP-1B) is associated with type 2 diabetes and impaired serine phosphorylation of PTP-1B in vitro. Diabetes 51(1): 1–6.

Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML & Kennedy BP (1999) Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 283(5407): 1544–1548.

Ferguson JF, Phillips CM, Tierney AC, Perez-Martinez P, Defoort C, Helal O, Lairon D, Planells R, Shaw DI, Lovegrove JA, Gjelstad IM, Drevon CA, Blaak EE, Saris WH, Leszczynska-Golabek I, Kiec-Wilk B, Riserus U, Karlstrom B, Miranda JL & Roche HM (2009) Gene-nutrient interactions in the metabolic syndrome: single nucleotide polymorphisms in ADIPOQ and ADIPOR1 interact with plasma saturated fatty acids to modulate insulin resistance. Am J Clin Nutr.

Fisher FF, Trujillo ME, Hanif W, Barnett AH, McTernan PG, Scherer PE & Kumar S (2005) Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. Diabetologia 48(6): 1084–1087.

Florez JC, Agapakis CM, Burtt NP, Sun M, Almgren P, Rastam L, Tuomi T, Gaudet D, Hudson TJ, Daly MJ, Ardlie KG, Hirschhorn JN, Groop L & Altshuler D (2005) Association testing of the protein tyrosine phosphatase 1B gene (PTPN1) with type 2 diabetes in 7,883 people. Diabetes 54(6): 1884–1891.

Florez JC, Hirschhorn J & Altshuler D (2003) The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. Annu Rev Genomics Hum Genet 4: 257–291.

Fontana L, Eagon JC, Trujillo ME, Scherer PE & Klein S (2007) Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes 56(4): 1010–1013.

Fortson J, Howe L, Harmon C & Sherrill WW (2008) Targeting cardiovascular risk: early identification of insulin resistance. J Am Acad Nurse Pract 20(6): 319–325.

Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE & Lodish HF (2001) Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A 98(4): 2005–2010.

Gable DR, Hurel SJ & Humphries SE (2006) Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic syndrome and cardiovascular disease. Atherosclerosis 188(2): 231–244.

Galgani JE, Moro C & Ravussin E (2008) Metabolic flexibility and insulin resistance. Am J Physiol Endocrinol Metab 295(5): E1009–17.

Gavrila A, Chan JL, Yiannakouris N, Kontogianni M, Miller LC, Orlova C & Mantzoros CS (2003) Serum adiponectin levels are inversely associated with overall and central fat distribution but are not directly regulated by acute fasting or leptin administration in humans: cross-sectional and interventional studies. J Clin Endocrinol Metab 88(10): 4823–4831.

Gerich JE (1998) The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. Endocr Rev 19(4): 491–503.

Girman CJ, Rhodes T, Mercuri M, Pyorala K, Kjekshus J, Pedersen TR, Beere PA, Gotto AM, Clearfield M & 4S Group and the AFCAPS/TexCAPS Research Group (2004) The metabolic syndrome and risk of major coronary events in the Scandinavian Simvastatin Survival Study (4S) and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). Am J Cardiol 93(2): 136–141.

Goldstein BJ, Ahmad F, Ding W, Li PM & Zhang WR (1998) Regulation of the insulin signalling pathway by cellular protein-tyrosine phosphatases. Mol Cell Biochem 182(1–2): 91–99.

Gouni-Berthold I, Giannakidou E, Muller-Wieland D, Faust M, Kotzka J, Berthold HK & Krone W (2005) The Pro387Leu variant of protein tyrosine phosphatase-1B is not associated with diabetes mellitus type 2 in a German population. J Intern Med 257(3): 272–280.

Grant SF & Hakonarson H (2008) Microarray technology and applications in the arena of genome-wide association. Clin Chem 54(7): 1116–1124.

Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF & Shulman GI (1999) Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes 48(6): 1270–1274.

Grundy SM (2008) Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biol 28(4): 629–636.

Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC,Jr, Spertus JA, Costa F, American Heart Association & National Heart, Lung, and Blood Institute (2005) Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 112(17): 2735–2752.

Gu HF, Abulaiti A, Ostenson CG, Humphreys K, Wahlestedt C, Brookes AJ & Efendic S (2004) Single nucleotide polymorphisms in the proximal promoter region of the adiponectin (APM1) gene are associated with type 2 diabetes in Swedish caucasians. Diabetes 53 Suppl 1: S31–5.

Gustafson B, Hammarstedt A, Andersson CX & Smith U (2007) Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. Arterioscler Thromb Vasc Biol 27(11): 2276–2283.

Gustafson B, Hammarstedt A, Andersson CX & Smith U (2007) Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. Arterioscler Thromb Vasc Biol 27(11): 2276–2283.

Hajer GR, van Haeften TW & Visseren FL (2008) Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J 29(24): 2959–2971.

Halperin F, Beckman JA, Patti ME, Trujillo ME, Garvin M, Creager MA, Scherer PE & Goldfine AB (2005) The role of total and high-molecular-weight complex of adiponectin in vascular function in offspring whose parents both had type 2 diabetes. Diabetologia 48(10): 2147–2154.

Hansen L & Pedersen O (2005) Genetics of type 2 diabetes mellitus: status and perspectives. Diabetes Obes Metab 7(2): 122–135.

Hansen L & Pedersen O (2005) Genetics of type 2 diabetes mellitus: status and perspectives. Diabetes Obes Metab 7(2): 122–135.

Havel PJ (2004) Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. Diabetes 53 Suppl 1: S143–51.

Hegele RA (2002) SNP judgments and freedom of association. Arterioscler Thromb Vasc Biol 22(7): 1058–1061.

Hivert MF, Manning AK, McAteer JB, Florez JC, Dupuis J, Fox CS, O'Donnell CJ, Cupples LA & Meigs JB (2008) Common variants in the adiponectin gene (ADIPOQ) associated with plasma adiponectin levels, type 2 diabetes, and diabetes-related quantitative traits: the Framingham Offspring Study. Diabetes 57(12): 3353–3359.

Hong Y, Weisnagel SJ, Rice T, Sun G, Mandel SA, Gu C, Rankinen T, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Bergman RN, Bouchard C, Rao DC & HERITAGE Family Study (2001) Familial resemblance for glucose and insulin metabolism indices derived from an intravenous glucose tolerance test in Blacks and Whites of the HERITAGE Family Study. Clin Genet 60(1): 22–30.

Hu E, Liang P & Spiegelman BM (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 271(18): 10697–10703.

Huang PL (2009) A comprehensive definition for metabolic syndrome. Dis Model Mech 2(5–6): 231–237.

Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS & Lodish HF (2004) T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proc Natl Acad Sci U S A 101(28): 10308–10313.

Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP & San Antonio Heart Study (2004) National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. Circulation 110(10): 1251–1257.

Hunt SC, Abkevich V, Hensel CH, Gutin A, Neff CD, Russell DL, Tran T, Hong X, Jammulapati S, Riley R, Weaver-Feldhaus J, Macalma T, Richards MM, Gress R, Francis M, Thomas A, Frech GC, Adams TD, Shattuck D & Stone S (2001) Linkage of body mass index to chromosome 20 in Utah pedigrees. Hum Genet 109(3): 279–285.

Ilanne-Parikka P, Eriksson JG, Lindstrom J, Hämälainen H, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Mannelin M, Rastas M, Salminen V, Aunola S, Sundvall J, Valle T, Lahtela J, Uusitupa M, Tuomilehto J & Finnish Diabetes Prevention Study Group (2004) Prevalence of the metabolic syndrome and its components: findings from a Finnish general population sample and the Diabetes Prevention Study cohort. Diabetes Care 27(9): 2135–2140.

Im JA, Lee JW, Lee HR & Lee DC (2006) Plasma adiponectin levels in postmenopausal women with or without long-term hormone therapy. Maturitas 54(1): 65–71.

Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR & Groop L (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 24(4): 683–689.

Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, Fu Y, Motone M, Yamamoto K, Matsuo A, Ohashi K, Kihara S, Funahashi T, Rakugi H, Matsuzawa Y & Ogihara T (2004) Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension 43(6): 1318–1323.

Kahn R, Buse J, Ferrannini E, Stern M, American Diabetes Association & European Association for the Study of Diabetes (2005) The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 28(9): 2289–2304.

Kanaley JA, Sames C, Swisher L, Swick AG, Ploutz-Snyder LL, Steppan CM, Sagendorf KS, Feiglin D, Jaynes EB, Meyer RA & Weinstock RS (2001) Abdominal fat distribution in pre- and postmenopausal women: The impact of physical activity, age, and menopausal status. Metabolism 50(8): 976–982.

Kang ES, Park SY, Kim HJ, Ahn CW, Nam M, Cha BS, Lim SK, Kim KR & Lee HC (2005) The influence of adiponectin gene polymorphism on the rosiglitazone response in patients with type 2 diabetes. Diabetes Care 28(5): 1139–1144.

Kaperonis EA, Liapis CD, Kakisis JD, Dimitroulis D & Papavassiliou VG (2006) Inflammation and atherosclerosis. Eur J Vasc Endovasc Surg 31(4): 386–393.

Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengard J & Kesäniemi YA (1992) Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia 35(11): 1060–1067.

Karjalainen A, Heikkinen J, Savolainen MJ, Bäckstrom AC & Kesäniemi YA (2000) Mechanisms regulating LDL metabolism in subjects on peroral and transdermal estrogen replacement therapy. Arterioscler Thromb Vasc Biol 20(4): 1101–1106.

Karjalainen A, Paassilta M, Heikkinen J, Bäckstrom AC, Savolainen M & Kesäniemi YA (2001) Effects of peroral and transdermal oestrogen replacement therapy on glucose and insulin metabolism. Clin Endocrinol (Oxf) 54(2): 165–173.

Kauma H, Savolainen MJ, Heikkila R, Rantala AO, Lilja M, Reunanen A & Kesäniemi YA (1996) Sex difference in the regulation of plasma high density lipoprotein cholesterol by genetic and environmental factors. Hum Genet 97(2): 156–162.

Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T, Durand JL, Li H, Li G, Jelicks LA, Mehler MF, Hui DY, Deshaies Y, Shulman GI, Schwartz GJ & Scherer PE (2007) Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest 117(9): 2621–2637.

Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, Marks JA, Krakower GR, Jacob HJ, Weber J, Martin L, Blangero J & Comuzzie AG (2000) Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci U S A 97(26): 14478–14483.

Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG & Kahn BB (2000) Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 20(15): 5479–5489.

Koren S & Fantus IG (2007) Inhibition of the protein tyrosine phosphatase PTP1B: potential therapy for obesity, insulin resistance and type-2 diabetes mellitus. Best Pract Res Clin Endocrinol Metab 21(4): 621–640.

Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M & Shulman GI (1999) Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. Diabetologia 42(1): 113–116.

Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T & Noda T (2002) Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem 277(29): 25863–25866.

Kubota N, Yano W, Kubota T, Yamauchi T, Itoh S, Kumagai H, Kozono H, Takamoto I, Okamoto S, Shiuchi T, Suzuki R, Satoh H, Tsuchida A, Moroi M, Sugi K, Noda T, Ebinuma H, Ueta Y, Kondo T, Araki E, Ezaki O, Nagai R, Tobe K, Terauchi Y, Ueki K, Minokoshi Y & Kadowaki T (2007) Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake (abstract). Cell Metab 6(1): 55–68.

Kusari J, Kenner KA, Suh KI, Hill DE & Henry RR (1994) Skeletal muscle protein tyrosine phosphatase activity and tyrosine phosphatase 1B protein content are associated with insulin action and resistance. J Clin Invest 93(3): 1156–1162.

Laaksonen DE, Niskanen L, Lakka HM, Lakka TA & Uusitupa M (2004) Epidemiology and treatment of the metabolic syndrome. Ann Med 36(5): 332–346.

Lahti-Koski M, Seppänen-Nuijten E, Männisto S, Härkanen T, Rissanen H, Knekt P, Rissanen A & Heliövaara M (2009) Twenty-year changes in the prevalence of obesity among Finnish adults. Obes Rev 11(3): 171–176.

Lango H, UK Type 2 Diabetes Genetics Consortium, Palmer CN, Morris AD, Zeggini E, Hattersley AT, McCarthy MI, Frayling TM & Weedon MN (2008) Assessing the combined impact of 18 common genetic variants of modest effect sizes on type 2 diabetes risk. Diabetes 57(11): 3129–3135.

Lara-Castro C, Fu Y, Chung BH & Garvey WT (2007) Adiponectin and the metabolic syndrome: mechanisms mediating risk for metabolic and cardiovascular disease. Curr Opin Lipidol 18(3): 263–270.

Laughlin GA, Barrett-Connor E & May S (2006) Sex-specific association of the androgen to oestrogen ratio with adipocytokine levels in older adults: the Rancho Bernardo Study. Clin Endocrinol (Oxf) 65(4): 506–513.

Lean ME (1989) Brown adipose tissue in humans. Proc Nutr Soc 48(2): 243–256.

Lembertas AV, Perusse L, Chagnon YC, Fisler JS, Warden CH, Purcell-Huynh DA, Dionne FT, Gagnon J, Nadeau A, Lusis AJ & Bouchard C (1997) Identification of an obesity quantitative trait locus on mouse chromosome 2 and evidence of linkage to body fat and insulin on the human homologous region 20q. J Clin Invest 100(5): 1240–1247.

Leung KC, Xu A, Craig ME, Martin A, Lam KS & O'Sullivan AJ (2009) Adiponectin isoform distribution in women--relationship to female sex steroids and insulin sensitivity. Metabolism 58(2): 239–245.

Li S, Shin HJ, Ding EL & van Dam RM (2009) Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 302(2): 179–188.

Lillioja S & Wilton A (2009) Agreement among type 2 diabetes linkage studies but a poor correlation with results from genome-wide association studies. Diabetologia 52(6): 1061–1074.

Ling H, Waterworth DM, Stirnadel HA, Pollin TI, Barter PJ, Kesaniemi YA, Mahley RW, McPherson R, Waeber G, Bersot TP, Cohen JC, Grundy SM, Mooser VE & Mitchell BD (2009) Genome-wide linkage and association analyses to identify genes influencing adiponectin levels: the GEMS Study. Obesity (Silver Spring) 17(4): 737–744.

Liu M & Liu F (2010) Transcriptional and post-translational regulation of adiponectin. Biochem J 425(1): 41–52.

Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y & Matsubara K (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 221(2): 286–289.

Martin LJ, Woo JG, Daniels SR, Goodman E & Dolan LM (2005) The relationships of adiponectin with insulin and lipids are strengthened with increasing adiposity. J Clin Endocrinol Metab 90(7): 4255–4259.

Mattsson N, Rönnemaa T, Juonala M, Viikari JS & Raitakari OT (2007) The prevalence of the metabolic syndrome in young adults. The Cardiovascular Risk in Young Finns Study. J Intern Med 261(2): 159–169.

McCarthy MI & Zeggini E (2009) Genome-wide association studies in type 2 diabetes. Curr Diab Rep 9(2): 164–171.

Menzaghi C, Ercolino T, Salvemini L, Coco A, Kim SH, Fini G, Doria A & Trischitta V (2004) Multigenic control of serum adiponectin levels: evidence for a role of the APM1 gene and a locus on 14q13. Physiol Genomics 19(2): 170–174.

Menzaghi C, Trischitta V & Doria A (2007) Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes 56(5): 1198–1209.

Meshkani R, Taghikhani M, Mosapour A, Larijani B, Khatami S, Khoshbin E, Ahmadvand D, Saeidi P, Maleki A, Yavari K, Nasoohi N & Adeli K (2007) 1484insG polymorphism of the PTPN1 gene is associated with insulin resistance in an Iranian population. Arch Med Res 38(5): 556–562.

Modena MG, Sismondi P, Mueck AO, Kuttenn F, Lignieres B, Verhaeghe J, Foidart JM, Caufriez A, Genazzani AR & TREAT (2005) New evidence regarding hormone replacement therapies is urgently required transdermal postmenopausal hormone therapy differs from oral hormone therapy in risks and benefits. Maturitas 52(1): 1–10.

Mohan V, Deepa R, Pradeepa R, Vimaleswaran KS, Mohan A, Velmurugan K & Radha V (2005) Association of low adiponectin levels with the metabolic syndrome--the Chennai Urban Rural Epidemiology Study (CURES-4). Metabolism 54(4): 476–481.

Mok A, Cao H, Zinman B, Hanley AJ, Harris SB, Kennedy BP & Hegele RA (2002) A single nucleotide polymorphism in protein tyrosine phosphatase PTP-1B is associated with protection from diabetes or impaired glucose tolerance in Oji-Cree. J Clin Endocrinol Metab 87(2): 724–727.

Moller DE & Kaufman KD (2005) Metabolic syndrome: a clinical and molecular perspective. Annu Rev Med 56: 45–62.

Mori Y, Otabe S, Dina C, Yasuda K, Populaire C, Lecoeur C, Vatin V, Durand E, Hara K, Okada T, Tobe K, Boutin P, Kadowaki T & Froguel P (2002) Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate Loci on 7p and 11p. Diabetes 51(4): 1247–1255.

Mousavinasab F, Tahtinen T, Jokelainen J, Koskela P, Vanhala M, Oikarinen J, Keinänen-Kiukaanniemi S & Laakso M (2006) Common polymorphisms (single-nucleotide polymorphisms SNP+45 and SNP+276) of the adiponectin gene regulate serum adiponectin concentrations and blood pressure in young Finnish men. Mol Genet Metab 87(2): 147–151.

Myers MG, Cowley MA & Munzberg H (2008) Mechanisms of leptin action and leptin resistance. Annu Rev Physiol 70: 537–556.

Nakano Y, Tobe T, Choi-Miura NH, Mazda T & Tomita M (1996) Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. J Biochem 120(4): 803–812.

Neel JV (1962) Diabetes mellitus: a ”thrifty” genotype rendered detrimental by ”progress”? Am J Hum Genet 14: 353–362.

Oda E (2008) The metabolic syndrome as a concept of adipose tissue disease. Hypertens Res 31(7): 1283–1291.

Okamoto M, Ohara-Imaizumi M, Kubota N, Hashimoto S, Eto K, Kanno T, Kubota T, Wakui M, Nagai R, Noda M, Nagamatsu S & Kadowaki T (2008) Adiponectin induces insulin secretion in vitro and in vivo at a low glucose concentration. Diabetologia 51(5): 827–835.

Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T & Matsuzawa Y (1999) Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 100(25): 2473–2476.

Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T & Matsuzawa Y (2001) Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation 103(8): 1057–1063.

Pääkko T, Ukkola O, Ikäheimo M & Kesäniemi YA (2010) Plasma adiponectin levels are associated with left ventricular hypertrophy in a random sample of middle-aged subjects. Ann Med 42(2): 131–137.

Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M & Scherer PE (2003) Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. J Biol Chem 278(11): 9073–9085.

Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA & Scherer PE (2004) Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 279(13): 12152–12162.

Palmer ND, Bento JL, Mychaleckyj JC, Langefeld CD, Campbell JK, Norris JM, Haffner SM, Bergman RN, Bowden DW & insulin resistance atherosclerosis study (IRAS) family study (2004) Association of protein tyrosine phosphatase 1B gene polymorphisms with measures of glucose homeostasis in Hispanic Americans: the insulin resistance atherosclerosis study (IRAS) family study. Diabetes 53(11): 3013–3019.

Paracchini V, Pedotti P & Taioli E (2005) Genetics of leptin and obesity: a HuGE review. Am J Epidemiol 162(2): 101–114.

Peake PW, Kriketos AD, Campbell LV, Shen Y & Charlesworth JA (2005) The metabolism of isoforms of human adiponectin: studies in human subjects and in experimental animals. Eur J Endocrinol 153(3): 409–417.

Peltonen L (2000) Positional cloning of disease genes: advantages of genetic isolates. Hum Hered 50(1): 66–75.

Perneger TV (1998) What's wrong with Bonferroni adjustments. BMJ 316(7139): 1236–1238.

Rabe K, Lehrke M, Parhofer KG & Broedl UC (2008) Adipokines and insulin resistance. Mol Med 14(11–12): 741–751.

Rajala U, Koskela P & Keinänen-Kiukaanniemi S (2001) Hyperglycemia as a risk factor of mortality in a middle-aged Finnish population. J Clin Epidemiol 54(5): 470–474.

Rajala U, Laakso M, Päivänsalo M, Pelkonen O, Suramo I & Keinänen-Kiukaanniemi S (2002) Low insulin sensitivity measured by both quantitative insulin sensitivity check index and homeostasis model assessment method as a risk factor of increased intima-media thickness of the carotid artery. J Clin Endocrinol Metab 87(11): 5092–5097.

Rantala AO, Kauma H, Lilja M, Savolainen MJ, Reunanen A & Kesäniemi YA (1999) Prevalence of the metabolic syndrome in drug-treated hypertensive patients and control subjects. J Intern Med 245(2): 163–174.

Rasouli N & Kern PA (2008) Adipocytokines and the metabolic complications of obesity. J Clin Endocrinol Metab 93(11 Suppl 1): S64–73.

Reaven GM (1997) Banting Lecture 1988. Role of insulin resistance in human disease. 1988. Nutrition 13(1): 64–66.

Richards JB, Waterworth D, O'Rahilly S, Hivert MF, Loos RJ, Perry JR, Tanaka T, Timpson NJ, Semple RK, Soranzo N, Song K, Rocha N, Grundberg E, Dupuis J, Florez JC, Langenberg C, Prokopenko I, Saxena R, Sladek R, Aulchenko Y, Evans D, Waeber G, Erdmann J, Burnett MS, Sattar N, Devaney J, Willenborg C, Hingorani A, Witteman JC, Vollenweider P, Glaser B, Hengstenberg C, Ferrucci L, Melzer D, Stark K, Deanfield J, Winogradow J, Grassl M, Hall AS, Egan JM, Thompson JR, Ricketts SL, Konig IR, Reinhard W, Grundy S, Wichmann HE, Barter P, Mahley R, Kesaniemi YA, Rader DJ, Reilly MP, Epstein SE, Stewart AF, Van Duijn CM, Schunkert H, Burling K, Deloukas P, Pastinen T, Samani NJ, McPherson R, Davey Smith G, Frayling TM, Wareham NJ, Meigs JB, Mooser V, Spector TD & GIANT Consortium (2009) A genome-wide association study reveals variants in ARL15 that influence adiponectin levels. PLoS Genet 5(12): e1000768.

Robertson SA, Leinninger GM & Myers MG,Jr (2008) Molecular and neural mediators of leptin action. Physiol Behav 94(5): 637–642.

Rosseneu M & Labeur C (1995) Physiological significance of apolipoprotein mutants. FASEB J 9(9): 768–776.

Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, Nagai M, Matsuzawa Y & Funahashi T (2004) Adiponectin as a biomarker of the metabolic syndrome. Circ J 68(11): 975–981.

Saiki A, Oyama T, Endo K, Ebisuno M, Ohira M, Koide N, Murano T, Miyashita Y & Shirai K (2007) Preheparin serum lipoprotein lipase mass might be a biomarker of metabolic syndrome. Diabetes Res Clin Pract 76(1): 93–101.

Salmenniemi U, Ruotsalainen E, Pihlajamäki J, Vauhkonen I, Kainulainen S, Punnonen K, Vanninen E & Laakso M (2004) Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. Circulation 110(25): 3842–3848.

Salmenniemi U, Zacharova J, Ruotsalainen E, Vauhkonen I, Pihlajamäki J, Kainulainen S, Punnonen K & Laakso M (2005) Association of adiponectin level and variants in the adiponectin gene with glucose metabolism, energy expenditure, and cytokines in offspring of type 2 diabetic patients. J Clin Endocrinol Metab 90(7): 4216–4223.

Saltiel AR (2001) You are what you secrete. Nat Med 7(8): 887–888.

Satoh H, Nguyen MT, Trujillo M, Imamura T, Usui I, Scherer PE & Olefsky JM (2005) Adenovirus-mediated adiponectin expression augments skeletal muscle insulin sensitivity in male Wistar rats. Diabetes 54(5): 1304–1313.

Sattar N (2008) Why metabolic syndrome criteria have not made prime time: a view from the clinic. Int J Obes (Lond) 32 (2): 30–34.

Scherer PE, Williams S, Fogliano M, Baldini G & Lodish HF (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270(45): 26746–26749.

Schneider JG, von Eynatten M, Schiekofer S, Nawroth PP & Dugi KA (2005) Low plasma adiponectin levels are associated with increased hepatic lipase activity in vivo. Diabetes Care 28(9): 2181–2186.

Seppälä-Lindroos A, Vehkavaara S, Häkkinen AM, Goto T, Westerbacka J, Sovijärvi A, Halavaara J & Yki-Järvinen H (2002) Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab 87(7): 3023–3028.

Shand B, Elder P, Scott R, Frampton C & Willis J (2006) Biovariability of plasma adiponectin. Clin Chem Lab Med 44(10): 1264–1268.

Southam L, Soranzo N, Montgomery SB, Frayling TM, McCarthy MI, Barroso I & Zeggini E (2009) Is the thrifty genotype hypothesis supported by evidence based on confirmed type 2 diabetes- and obesity-susceptibility variants? Diabetologia 52(9): 1846–1851.

Speakman JR (2008) Thrifty genes for obesity, an attractive but flawed idea, and an alternative perspective: the 'drifty gene' hypothesis. Int J Obes (Lond) 32(11): 1611–1617.

Staiger H, Machicao F, Fritsche A & Haring HU (2009) Pathomechanisms of type 2 diabetes genes. Endocr Rev 30(6): 557–585.

Sumino H, Takahashi T, Itoh T, Kusaka K, Yamakawa J, Ichikawa S, Kurabayashi M & Kanda T (2004) Plasma adiponectin levels in post-menopausal women receiving hormone replacement therapy. J Int Med Res 32(6): 639–645.

Szapary PO, Bloedon LT, Samaha FF, Duffy D, Wolfe ML, Soffer D, Reilly MP, Chittams J & Rader DJ (2006) Effects of pioglitazone on lipoproteins, inflammatory markers, and adipokines in nondiabetic patients with metabolic syndrome. Arterioscler Thromb Vasc Biol 26(1): 182–188.

Tabara Y, Osawa H, Kawamoto R, Tachibana-Iimori R, Yamamoto M, Nakura J, Miki T, Makino H & Kohara K (2008) Reduced high-molecular-weight adiponectin and elevated high-sensitivity C-reactive protein are synergistic risk factors for metabolic syndrome in a large-scale middle-aged to elderly population: the Shimanami Health Promoting Program Study. J Clin Endocrinol Metab 93(3): 715–722.

Tanko LB & Christiansen C (2005) Effects of 17beta-oestradiol plus different doses of drospirenone on adipose tissue, adiponectin and atherogenic metabolites in postmenopausal women. J Intern Med 258(6): 544–553.

Traurig M, Hanson RL, Kobes S, Bogardus C & Baier LJ (2007) Protein tyrosine phosphatase 1B is not a major susceptibility gene for type 2 diabetes mellitus or obesity among Pima Indians. Diabetologia 50(5): 985–989.

Trujillo ME & Scherer PE (2005) Adiponectin--journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. J Intern Med 257(2): 167–175.

Tschritter O, Fritsche A, Thamer C, Haap M, Shirkavand F, Rahe S, Staiger H, Maerker E, Haring H & Stumvoll M (2003) Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes 52(2): 239–243.

Tsubakio-Yamamoto K, Matsuura F, Koseki M, Oku H, Sandoval JC, Inagaki M, Nakatani K, Nakaoka H, Kawase R, Yuasa-Kawase M, Masuda D, Ohama T, Maeda N, Nakagawa-Toyama Y, Ishigami M, Nishida M, Kihara S, Shimomura I & Yamashita S (2008) Adiponectin prevents atherosclerosis by increasing cholesterol efflux from macrophages. Biochem Biophys Res Commun 375(3): 390–394.

Tsukinoki R, Morimoto K & Nakayama K (2005) Association between lifestyle factors and plasma adiponectin levels in Japanese men. Lipids Health Dis 4: 27.

Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M & Finnish Diabetes Prevention Study Group (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344(18): 1343–1350.

Ukkola O, Kervinen K, Salmela PI, von Dickhoff K, Laakso M & Kesaniemi YA (1993) Apolipoprotein E phenotype is related to macro- and microangiopathy in patients with non-insulin-dependent diabetes mellitus. Atherosclerosis 101(1): 9–15.

Ukkola O, Rankinen T, Lakka T, Leon AS, Skinner JS, Wilmore JH, Rao DC, Kesaniemi YA & Bouchard C (2005) Protein tyrosine phosphatase 1B variant associated with fat distribution and insulin metabolism. Obes Res 13(5): 829–834.

Ukkola O & Santaniemi M (2002) Protein tyrosine phosphatase 1B: a new target for the treatment of obesity and associated co-morbidities. J Intern Med 251(6): 467–475.

van Tilburg J, van Haeften TW, Pearson P & Wijmenga C (2001) Defining the genetic contribution of type 2 diabetes mellitus. J Med Genet 38(9): 569–578.

Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, Boutin P, Vaxillaire M, Lepretre F, Dupont S, Hara K, Clement K, Bihain B, Kadowaki T & Froguel P (2002) Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet 11(21): 2607–2614.

Vasunta RL, Kesäniemi YA & Ukkola O (in press) Plasma adiponectin concentration is associated with ambulatory daytime systolic blood pressure but not with the dipping status. J Hum Hypertens.

Vionnet N, Hani EH, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C & Froguel P (2000) Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am J Hum Genet 67(6): 1470–1480.

Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S & Nuutila P (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360(15): 1518–1525.

Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R & Kadowaki T (2003) Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem 278(41): 40352–40363.

Waki H, Yamauchi T, Kamon J, Kita S, Ito Y, Hada Y, Uchida S, Tsuchida A, Takekawa S & Kadowaki T (2005) Generation of globular fragment of adiponectin by leukocyte elastase secreted by monocytic cell line THP-1. Endocrinology 146(2): 790–796.

Wang J, Li H, Franco OH, Yu Z, Liu Y & Lin X (2008) Adiponectin and metabolic syndrome in middle-aged and elderly Chinese. Obesity (Silver Spring) 16(1): 172–178.

Wang ZV & Scherer PE (2008) Adiponectin, cardiovascular function, and hypertension. Hypertension 51(1): 8–14.

Weeks DE & Lathrop GM (1995) Polygenic disease: methods for mapping complex disease traits. Trends Genet 11(12): 513–519.

White MF & Kahn CR (1994) The insulin signaling system. J Biol Chem 269(1): 1–4.

Xydakis AM, Case CC, Jones PH, Hoogeveen RC, Liu MY, Smith EO, Nelson KW & Ballantyne CM (2004) Adiponectin, inflammation, and the expression of the metabolic syndrome in obese individuals: the impact of rapid weight loss through caloric restriction. J Clin Endocrinol Metab 89(6): 2697–2703.

Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R & Kadowaki T (2003) Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423(6941): 762–769.

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P & Kadowaki T (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7(8): 941–946.

Yang WS, Tsou PL, Lee WJ, Tseng DL, Chen CL, Peng CC, Lee KC, Chen MJ, Huang CJ, Tai TY & Chuang LM (2003) Allele-specific differential expression of a common adiponectin gene polymorphism related to obesity. J Mol Med 81(7): 428–434.

Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG & Kahn BB (2008) Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J Biol Chem 283(21): 14230–14241.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372(6505): 425–432.

Original articles

I Santaniemi M, Ukkola O & Kesäniemi YA (2004) Tyrosine phosphatase 1B and leptin receptor genes and their interaction in type 2 diabetes. J Intern Med 256(1): 48–55.

II Ukkola O, Santaniemi M, Rankinen T, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bergman R, Kesäniemi YA & Bouchard C (2005) Adiponectin polymorphisms, adiposity and insulin metabolism: HERITAGE family study and Oulu diabetic study. Ann Med 37(2): 141–50.

III Santaniemi M, Kesäniemi YA & Ukkola O (2006) Low plasma adiponectin concentration is an indicator of the metabolic syndrome. Eur J Endocrinol 155(5): 745–50.

IV Jalovaara K, Santaniemi M, Timonen M, Jokelainen J, Kesäniemi YA, Ukkola O, Keinänen-Kiukaanniemi S & Rajala U (2008) Low serum adiponectin level as a predictor of impaired glucose regulation and type 2 diabetes mellitus in a middle-aged Finnish population. Metabolism 57(8): 1130–4.

V Kunnari A, Santaniemi M, Jokela M, Karjalainen AH, Heikkinen J, Ukkola O & Kesäniemi YA (2008) Estrogen replacement therapy decreases plasma adiponectin but not resistin in postmenopausal women. Metabolism 57(11): 1509–15.

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