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JUHA SALTEVO

# Low-Grade Inflammation and Adiponectin in the Metabolic Syndrome

Doctoral dissertation

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## ABSTRACT

The metabolic syndrome (MetS) is an established risk factor cluster for cardiovascular disease (CVD) and type 2 diabetes (T2DM). A rapid increase in the prevalence of obesity worldwide is also associated with an increase of the MetS. Abdominal obesity and insulin resistance are probably the key elements of the syndrome. The role of low-grade inflammation and hypoadiponectinemia remains unclear in the MetS.

The aim of this study was to evaluate the association and significance of low-grade inflammation, measured by high-sensitivity(hs)-CRP and IL-1 receptor antagonist (IL-1 Ra), and adiponectin with the MetS in a population-based study.

The study population consisted of subjects from five different age groups (mean age 46 years) living in the city of Pieksämäki. Altogether 923 of 1 294 (71.3%) individuals participated in the cross-sectional studies in 1997-98. The prevalence of the MetS according to the International Diabetes Federation (IDF) definition was 38% in men and 34% in women. The corresponding figures according to the National Cholesterol Education Program (NCEP) definition were 34% and 27%.

The levels of pro-inflammatory markers, hs-CRP and IL-1 Ra, were significantly higher among women with the MetS compared to men with the MetS, independently of the definition used. In contrast, no gender difference in these markers between men and women was observed in subjects without the MetS.

The relative change in BMI from childhood to adulthood and the levels of adiponectin and markers of a low-grade inflammation were related. The association was particularly strong among women. Insulin sensitivity correlated significantly with adiponectin and IL-1 Ra levels, independently of confounding factors, but did not correlate with hs-CRP. The levels of adiponectin, hs-CRP and IL-1 Ra were similarly and linearly correlated with the number of components of the MetS in both sexes according to the IDF and NCEP definitions.

In conclusion, the MetS was associated with hypoadiponectinemia and low-grade inflammation measured by hs-CRP and IL-1 Ra in this cross-sectional population-based study. An association was found between the relative change in BMI between childhood and adulthood, insulin sensitivity, the number of components of the MetS and, above all, female gender. Low-grade inflammation could be one explanation why prediabetic women tend to have a more atherogenic risk profile than males years before the diagnosis of diabetes. This long-lasting inflammatory stress may in part explain why T2DM is related to relatively higher CVD mortality in women than in men.

National Library of Medicine Classification: WB 286, WD 210, WD 200.5.H8, WG 120, WK 810  
Medical Subject Headings: Adiponectin; Adult; Body Mass Index; C-Reactive Protein; Cross-Sectional Studies; Diabetes Mellitus, Type 2; Cardiovascular Diseases; Child; Female; Finland; Humans; Inflammation; Insulin Resistance; Interleukin 1 Receptor Antagonist Protein; Male; Metabolic Syndrome X; Obesity; Risk Factors



**Tiedon määrä on rajaton, mutta sen totuus rajallinen.**

**"Hiljaa pitää miehen kairassa kulkea ja nostaa hattua kelopuulle"  
(Alpiini, Lapin mies)**

*To my wife Sirke and,  
children Ilona, Inari, Saana, Onni and  
grandchildren Veeti, Aslak and Hilla*



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**Jyväskylä, 13 th May 2008**

**Juha Saltevo**



## LIST OF ABBREVIATIONS

AACE	American Association of Clinical Endocrinologists
ACE	angiotensin-converting enzyme
ADA	American Diabetes Association
AHA	American Heart Association
ANOVA	analysis of variance
BMI	body mass index
CB	cannabinoid
CHD	coronary heart disease
CI	confidence interval
CRP	C-reactive protein
CVD	cardiovascular disease
DM	diabetes mellitus
DPP	Diabetes Prevention Program
DPS	Diabetes Prevention Study
EASD	European Association for the Study of Diabetes
ECG	electrocardiogram
EGIR	European Group for the Study of Insulin Resistance
ELISA	enzyme-linked immunosorbent assay
ER	endoplasmic reticulum
FFA	free fatty acid
HDL	high-density lipoprotein
HMW	high-molecular weight
HOMA	homeostasis model assessment
IDF	International Diabetes Federation
IGT	impaired glucose tolerance
IL	interleukin
IL-1 Ra	interleukin-1 receptor antagonist
LDL	low-density lipoprotein
LMW	low-molecular weight
MetS	metabolic syndrome
MI	myocardial infarction
MMW	middle-molecular weight
mRNA	messenger ribonucleid acid
NCEP	National Cholesterol Education Program
NEFA	non-esterified fatty acid
NGT	normal glucose tolerance
NHANES	National Health and Nutrition Examination Survey
NO	nitric oxide
NS	non-significant
OGTT	oral glucose tolerance test
OR	odds ratio
PAI	plasminogen activator inhibitor
PCOS	polycystic ovary syndrome
PVD	peripheral vascular disease
QUICKI	quantitative insulin sensitivity check index
ROS	reactive oxyxen species
SD	standard deviation
SNP	single nucleotide polymorphism
TNF	tumor necrosis factor
TRL	triglyceride rich lipoprotein
TZD	thiazolidinedione
T2DM	type 2 diabetes mellitus

WHO  
VLDL  
WHR

World Health Organization  
very-low-density lipoprotein  
waist-to-hip ratio

## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which will be referred to their Roman numerals I-IV:

I Saltevo J, Vanhala M, Kautiainen H, Laakso M. Levels of adiponectin, C-reactive protein and interleukin-1 receptor antagonist are associated with the relative change in body mass index between childhood and adulthood. *Diabetes and Vascular Disease Research*. 2007;4:328-31

II Saltevo J, Laakso M, Jokelainen J, Keinänen-Kiukaanniemi S, Kumpusalo E, Vanhala M. Levels of adiponectin, C-reactive protein and interleukin-1 receptor antagonist are associated with insulin sensitivity: a population-based study. *Diabetes/Metabolism Research and Reviews*. 2008;2Apr ([http://www3.interscience.wiley.com/cgi-bin/fulltext/117952371/main.html,ftx\\_abs](http://www3.interscience.wiley.com/cgi-bin/fulltext/117952371/main.html,ftx_abs))

III Saltevo J, Vanhala M, Kautiainen H, Kumpusalo E, Laakso M. Association of C-reactive protein, interleukin-1 receptor antagonist and adiponectin with the metabolic syndrome. *The Mediators of Inflammation*. 2007; Article ID 93573:1-8

IV Saltevo J, Vanhala M, Kautiainen H, Kumpusalo E, Laakso M. Gender differences in C-reactive protein, interleukin-1 receptor antagonist and adiponectin levels in the metabolic syndrome: a population-based study. *Diabetic Medicine* 2008;23 Apr:e-pub ahead a print (DOI.10.1111/j.1464-5491.2008.02440.x)

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## 1. INTRODUCTION

The metabolic syndrome (MetS) is the clustering of multiple cardiovascular risk factors in an individual. The constellation of metabolic abnormalities includes glucose intolerance (type 2 diabetes, impaired glucose tolerance, or impaired fasting glycemia), insulin resistance, central obesity, dyslipidemia, and hypertension, all well-documented risk factors for CVD. These conditions co-occur in an individual more often than expected by chance and are associated with increased risk for cardiovascular disease (CVD) (1,2) and type 2 diabetes mellitus (T2DM) (3,4).

Over the past two decades, there has been a striking increase in the number of people with the MetS worldwide. This increase is associated with a global epidemic of obesity and diabetes (5), and the syndrome is seen more often in young adults and children (6).

The pathophysiology of the MetS is complex and not completely understood. The detrimental effects of visceral adipose tissue accumulation and the active endocrine role of adipose tissue have been acknowledged in recent years. Abdominal obesity is believed to be the cause for the MetS, as it clusters with diabetogenic, atherogenic, prothrombotic and proinflammatory metabolic abnormalities (7). Adipose tissue secretes a variety of bioactive substances called adipocytokines, which are closely linked to the MetS and its complications (8). A recently discovered protein, adiponectin, is the most abundant adipocytokine (9), and its expression is highly specific to adipose tissue. Adiponectin has insulin-sensitising properties (10). Reduced adiponectin levels are observed in viscerally obese subjects which contribute to an atherogenic and diabetogenic metabolic risk factor profile (11). In obesity, there is evidence of macrophage infiltration in adipose tissue, leading to an inflammatory condition characterised by elevation of the Interleukin (IL) cytokine superfamily, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and C-reactive protein (CRP). Adipose tissue produces, presumably as an adaptive response to chronic stress, anti-inflammatory factors such as interleukin-1 receptor antagonist (IL-1 Ra) (12). Insulin resistance is increasingly recognized as a chronic, low-grade, inflammatory state. Atherosclerosis and insulin resistance share similar pathophysiological mechanisms. This low-grade inflammation could be the link between T2DM and atherosclerosis ("common soil hypothesis") (13). Thus, understanding of the pathophysiology of the MetS and identification of subjects with MetS is highly important. There is urgent need for effective strategies to prevent this emerging global epidemic to be able to prevent the increase of CVD and T2DM (5).

In this population-based study the association and the role of adiponectin, CRP and IL-1 Ra applying two different definitions of the MetS were examined. The association of the above-mentioned proinflammatory markers and adiponectin were also analyzed with relative change in BMI between childhood and adulthood, insulin sensitivity and gender differences in the

prevalence of the MetS in a cohort of 5 age groups born in 1942, 1947, 1952, 1957 and 1962 (N=1294) in the town of Pieksämäki (population about 20 000), in eastern Finland.

The review of literature will discuss the evolution of the concept, different definitions, epidemiology, and the closely related components of the MetS, especially low-grade inflammation and hypoadiponectinemia. The genetics, lifestyle factors, treatment, early life aspects and gender differences with the MetS will also be discussed.

## **2. REVIEW OF LITERATURE**

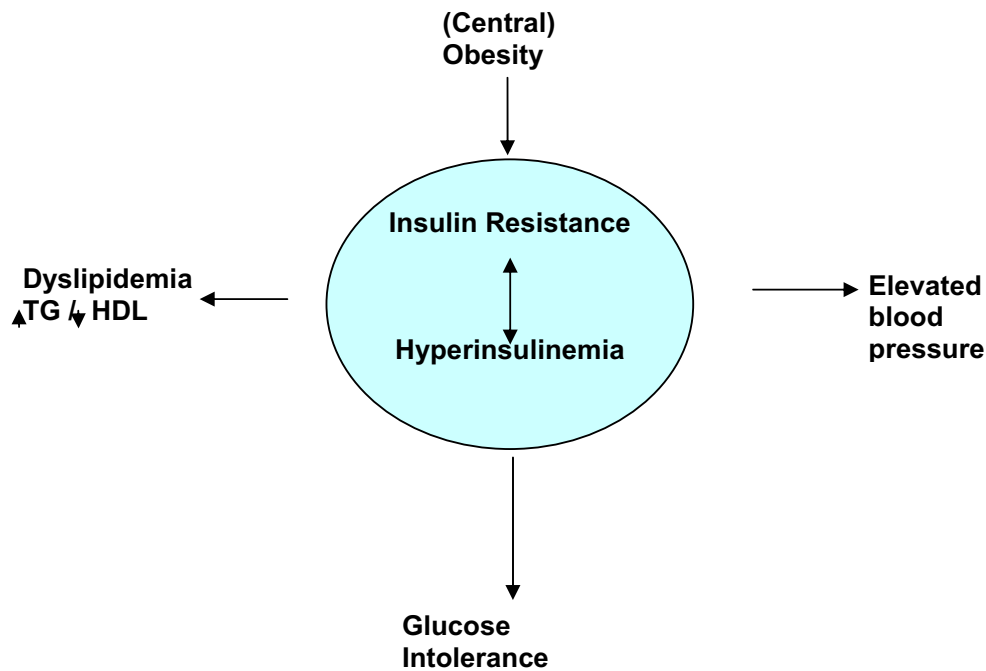
### **2.1. Metabolic syndrome**

#### **2.1.1. Evolution of the concept of the metabolic syndrome**

Some 250 years ago in the 18<sup>th</sup> century Joannes Baptista Morgagni introduced the mechanistic concept in human physiology and pathology. With the help of only knife for anatomical dissection he was able to identify the intra-abdominal and mediastinal fat accumulation in android obesity. He described the association between visceral obesity, hypertension, hyperuricemia, atherosclerosis and obstructive sleep apnea (14). In modern medical literature the same clustering of cardiovascular risk factors, hypertension, hyperglycemia and hyperurikemia, was first described in 1923 by Kylin, a Swedish physician (15). Later, in 1947, the French physician, Vague, drew attention to upper body adiposity (android or male type obesity) as the obesity phenotype that was often associated with metabolic abnormalities, diabetes and cardiovascular disease (16).

In his Banting Lecture professor Gerald Reaven in 1988 described "Syndrome X" (17). This syndrome was "a cluster of risk factors for diabetes and cardiovascular disease" and tightly associated with insulin resistance. Subsequently, the MetS was called "the deadly quartet"(18) or "the insulin resistance syndrome" (19). However, the etiology of the syndrome has remained unclear. In 1998, the World Health Organization (WHO) proposed the first internationally accepted criteria for the MetS (20). The term "metabolic syndrome" was preferred over "insulin resistance syndrome"(Figure 1).





**Figure 1.** The classical features of metabolic syndrome. Insulin resistance and hyperinsulinemia are the core of the syndrome. Central obesity may also have an etiological role. Dyslipidemia, elevated blood pressure and glucose intolerance are included in the criteria and they might result from the underlying insulin resistance.

### 2.1.2. Definitions of the metabolic syndrome

Despite general recognition of the syndrome, the lack of knowledge of its etiology and uniform diagnostic criteria complicated epidemiological studies of the MetS for many years. The WHO diagnostic criteria was the first attempt to achieve some agreement on the definition, particularly for research purposes (20). Insulin resistance was the primary abnormality of this diagnosis. It had to be coupled with any two other features of the syndrome (central obesity, elevated blood pressure, dyslipidemia and microalbuminuria). The report clearly stated that the definition did not imply causal relationships and that the definition could be modified as new information was gathered.

The WHO criteria have been criticized because of the inclusion of microalbuminuria as a component of the syndrome. Microalbuminuria in non-diabetic individuals is uncommon (21) and its association with other components of the syndrome is not consistent (22,23). Furthermore, the requirement that insulin resistance should be measured with the hyperinsulinemic euglycemic clamp technique (24) in glucose tolerant subjects made the criteria

impractical for epidemiological studies. For these reasons the European Group for the Study of Insulin Resistance (EGIR) presented a simpler definition, particularly for epidemiological studies (25). Insulin resistance was still the central element of the diagnosis, but it was defined as the presence of fasting hyperinsulinemia (25,26). Other differences compared to the WHO criteria were the cut-off points for hypertension and dyslipidemia (Table 1). Furthermore, instead of the waist-to-hip ratio (WHR), waist circumference was proposed as a measure of abdominal obesity because it showed a stronger correlation with intra-abdominal adipose tissue mass (27). Microalbuminuria was not included in the EGIR criteria.

The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) published their diagnostic criteria for the MetS in 2001 (28). This definition was based on fasting triglycerides, high-density lipoprotein (HDL) cholesterol, plasma glucose, waist circumference and blood pressure with equal weight on each component. Insulin resistance was not included in the definition of the syndrome. American Association of Clinical Endocrinologists (AACE) published their slightly different modified NCEP criteria in 2002 to refocus on insulin resistance as the primary cause of metabolic risk factors. Like the EGIR, they used the name insulin resistance syndrome. Major criteria were IGT, elevated triglycerides, reduced HDL cholesterol, elevated blood pressure, and obesity. No specified number of factors qualified for diagnosis, which was left to clinical judgment (29).

Recently, the International Diabetes Federation (IDF) revised the definition of the MetS (30) to be globally more useful. In the IDF criteria abdominal obesity is recognized as an underlying factor for the syndrome. Therefore, a large waist circumference was defined as a compulsory component for the diagnosis of the MetS. For the first time ethnic-specific cut-points for waist circumference were given. For Caucasian men the cut-off point is  $\geq 94$  cm and for European women  $\geq 80$  cm, based on the sensitivity and specificity of these cut-off points to identify subjects with body mass index (BMI)  $> 25$  kg/m<sup>2</sup> or WHR  $\geq 0.9$  for men and  $\geq 0.85$  for women (31). The cut-off points for waist circumference for Asian populations are lower since their risk for T2DM and CVD is apparent at lower levels of adiposity than in Europeans (32). The criteria for dyslipidemia and elevated blood pressure are identical to those of the NCEP definition, but the limit for hyperglycemia has been lowered to  $\geq 5.6$  mmol/l according to the new definition of impaired fasting glucose by the American Diabetes Association (ADA) (33).

**Table 1.** Definitions of the MetS by the WHO, EGIR, NCEP, ACE, IDF, and updated NCEP criteria (modified from 34).

	WHO	EGIR	NCEP	AACE	IDF	Updated NCEP
Required	f-insulin in top 25%; f-glucose $\geq 6.1$ mmol/L; 2 h glucose $\geq 7.8$ mmol/L and $\geq 2$ of:	f-insulin in top 25% and $\geq 2$ of: $\geq 6.1$ mmol/L <1.0	— $\geq 3$ of: $\geq 6.1$ mmol/L <1.03 men or <1.29 women	High risk of being insulin resistant and $\geq 2$ of: $\geq 6.1$ mmol/L <1.03 men or <1.29 women	Waist $\geq 94$ cm in men or $\geq 80$ cm in women and $\geq 2$ of: $\geq 5.6$ mmol/L <1.03 men or <1.29 women	— $\geq 3$ of: $\geq 5.6$ mmol/L <1.03 men or <1.29 women
Fasting glucose						
HDL cholesterol (mmol/L)	<0.9 in men or <1.0 in women	<1.0	<1.03 men or <1.29 women	<1.03 men or <1.29 women	<1.03 men or <1.29 women	<1.03 men or <1.29 women
	or	or				
Triglycerides	$\geq 1.7$ mmol/L	>2.0 mmol/L	$\geq 1.7$ mmol/L	$\geq 1.7$ mmol/L	$\geq 1.7$ mmol/L	$\geq 1.7$ mmol/L
Obesity	Waist/hip ratio > 0.90 in men or > 0.85 in women; BMI $\geq 30$ kg/m <sup>2</sup>	Waist $\geq 94$ cm in men or $\geq 80$ cm in women	Waist $\geq 102$ cm in men or $\geq 88$ cm in women		Waist $\geq 102$ cm in men or $\geq 88$ cm in women	Waist $\geq 102$ cm in men or $\geq 88$ cm in women
Hypertension (mmHg)	$\geq 140/90$ or drug treatment	$\geq 140/90$ or drug treatment	$\geq 130/85$ or drug treatment	$\geq 130/85$ or drug treatment	$\geq 130/85$ or drug treatment	$\geq 130/85$ or drug treatment
Microalbuminuria	u-albumin $\geq 20$ $\mu$ g/min					

f=fasting, HDL= high-density cholesterol, u= urinary.

### 2.1.3. Epidemiology of the metabolic syndrome

#### 2.1.3.1. Prevalence of the metabolic syndrome

The prevalence and consequences of the MetS were difficult to estimate before more generally accepted definitions of the syndrome were published. It has become obvious that simultaneously with an increase in obesity there has been an increase in the prevalence of the MetS. Analysis of data on a representative sample of 8814 men and women in the United States (Third National Health and Nutrition Examination Survey from 1988-1999), estimated that the age-adjusted prevalence of the MetS according to the NCEP criteria was 23.6% (35). The prevalence of the MetS increased from 6.7% among participants aged from 20 to 29 years to 43.5% for participants aged from 60 to 69 years. There were also considerable differences in the prevalence of MetS among different ethnic groups (35). The prevalence was highest among Mexican Americans (31.9%) and lowest among Caucasians (21.6%), African Americans (21.6%) and people reporting "another" race or ethnicity (20.3%). Comparison of the WHO and the NCEP definitions showed that the age-adjusted prevalence of the MetS was quite similar, 25.1% and 23.9%, respectively, but 15-20% of the individuals were classified as having the syndrome based on one definition but not on the other, with equal discordance (36).

In a Finnish study of 1 209 middle-aged men (42-60 years) the prevalence of MetS in 1984-1989 was 14.2%, according to the WHO (insulin resistance estimated as hyperinsulinemia), and 8.8 % according to the NCEP definitions. The prevalence numbers are rather low, but subjects with diabetes and CVD were excluded from this cohort (2). More recent data from Finland show that according to the modified WHO criteria, the prevalence numbers in the 1992 FINRISK survey, conducted as part of the FINMONICA cardiovascular risk factor study, were much higher in men (38.8 %) and in women (22.2 %). The prevalence rates were also analyzed in different categories of glucose tolerance in this cross-sectional, population-based sample of 2 049 individuals. The prevalence of the MetS was 14.4% in men and 10.1% in women with normal glucose tolerance (NGT), 84.8% and 65.4% among those with impaired glucose tolerance (IGT) and 91.1% and 82.7% in subjects with type 2 diabetes, respectively (37). In the same publication, the Finnish Diabetes Prevention Study (DPS) cohort was analyzed and the prevalence of the MetS in 522 participants with IGT was 78.4% for men, and 72.2% for women (37).

The prevalence of the MetS in a random sample of 207 middle-aged subjects in Tampere and 1 148 subjects in Pieksämäki in 1993-94 were 17% in men and 8% in women. In this study the MetS was defined as a clustering of dyslipidemia (hypertriglyceridemia, low HDL cholesterol or both) and insulin resistance (abnormal glucose tolerance, hyperinsulinemia, or both) (38,39).

Recent data on 1 099 healthy Finnish male military conscripts around 19 years of age showed prevalence numbers of the MetS to be 6.8% according to the IDF and 3.5% by the NCEP (40).

A recent study of 9 669 subjects representative of the Greek population reported a prevalence of the MetS 24.5% according to the NCEP criteria, which is very similar to the prevalence in US and Finnish adults (41). According to the new IDF definition, the age-adjusted prevalence of the MetS in the same Greek population was 43.4% and thus much higher. The significantly higher prevalence of the MetS by the IDF definition was attributable to the lower cut-off points for waist circumference and fasting glucose since most subjects with only one or two NCEP diagnostic criteria had the MetS according to the IDF definition (41).

Little is known about the prevalence of the MetS among children and adolescents. Data from the U.S. on adolescents aged 12-17 years in 1999-2004 showed that the prevalence of the MetS using the IDF definition was about 4.5%. It increased with age, was higher among males (6.7%) than females (2.1%), and was highest among Mexican-American adolescents (7.1%) (42). These figures are possibly higher nowadays, because of the rapid rise in adiposity among youth.

#### 2.1.3.2. Metabolic syndrome and the risk of cardiovascular disease and type 2 diabetes

The main reason for identifying subjects with the metabolic syndrome is the fact that these individuals are at a high risk for developing CVD and T2DM (43).

Several prospective population-based studies have investigated the association between metabolic risk factors and CVD. In a large cohort of about 20 000 men and women, followed for an average of 7 years, the all-cause and CVD mortality increased with increasing number of abnormalities associated with the MetS (44).

In the Helsinki policemen study cohort, the insulin resistance factor during the 22-year follow-up had an age-adjusted hazard ratio of 1.28 for CVD risk and 1.64 for stroke risk. In the same study the lipid factor predicted the risk of CVD, but not that of stroke (45). The MetS also predicted coronary heart disease events in elderly (65-74 years) non-diabetic Finnish men followed up for 7 years (hazard ratio 1.33) (46).

In the Botnia study 4 483 subjects aged 35-70 years were evaluated for cardiovascular risk associated with the MetS applying the WHO definition (insulin resistance estimated by HOMA). The prevalence of CHD and stroke was 3-fold higher in subjects with the syndrome and their mortality markedly increased (12.0%) during the 7-year prospective follow-up compared to subjects without the syndrome (2.2%) (1).

In the Kuopio Ischaemic Heart Disease Risk Factor Study, a population-based, prospective cohort study of 1 209 Finnish men aged 42 to 60 years at baseline (1984-1989) who were initially without CVD, cancer, or diabetes were followed up until December 1998. Those with the

MetS defined applying the NCEP criteria (waist > 102 cm) had 4.2 times and those with the MetS defined by the other NCEP criteria (waist > 94 cm) 2.9 times higher risk to death from CHD than men without the MetS after adjustment for conventional cardiovascular risk factors. The corresponding figures, according to the WHO definition of the MetS were from 2.9 (waist-hip ratio >0.90 or BMI  $\geq$  30) to 3.3 (waist  $\geq$  94cm) (2).

The predictive value of the WHO and NCEP criteria were compared in the San Antonio Heart Study (2 815 subjects aged 25-64 years, average follow-up time 12.7 years), where the prevalence rate of the MetS in the general population was 25.2% using both definitions. In the general population the NCEP criteria were predictive of all-cause mortality (hazard ratio 1.5) and CVD mortality (hazard ratio 2.5). The WHO criteria only predicted CVD mortality (hazard ratio 1.6) (47).

The Hoorn Study comprised 615 men and 749 women aged 50 to 75 years without diabetes and a history of CVD at baseline in 1989-1990. The prevalence of the MetS ranged from 17-32% using different definitions (NCEP, WHO, EGIR). All the definitions increased the risk for incident cardiovascular morbidity and mortality in 10 years about 2-fold (48).

In the ARIC study population men and women fulfilling the NCEP definition of the MetS had a 1.5 to 2 times higher risk for developing CHD or stroke after adjustments, but having the MetS did not improve CHD risk prediction beyond the level achieved by the Framingham Risk Score (49). In contrast in the Scandinavian Simvastatin Survival Study(4S) and Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) placebo-treated patients with MetS defined by the NCEP criteria showed increased risk for major coronary events irrespective of their Framingham-calculated 10-year-risk score category (50).

In a prospective 4-year follow-up study of 750 coronary patients who underwent coronary angiography, the NCEP-definition of the MetS conferred a significantly higher risk for vascular events than the IDF definition (51). The MetS, as defined by all the main criteria, also predicted incident end-stage peripheral vascular disease (PVD) in a prospective population based study in the elderly, but only when adjusted for diabetes status. Two of the single components of the MetS, elevated fasting plasma glucose and microalbuminuria, predicted PVD (52).

The other major complication of the MetS is T2DM (3,4). Overall, the risk for type 2 diabetes in patients with the MetS is 3-5-fold higher (53). In the Insulin Resistance Atherosclerosis Study the IDF and NCEP definitions were shown to predict type 2 diabetes equally well (54). A close relationship between the MetS and glucose tolerance was demonstrated in the Botnia study, where the prevalence of the MetS was 10% in women and 15% in men with normal glucose tolerance compared to 42 and 62% in subjects with impaired glucose tolerance (IGT) and 78 and 84% in subjects with T2DM (1). The predictive power of the MetS for incident diabetes in a Chinese high-risk population was tested during a 5-year follow-up. The NCEP, WHO, EGIR and

AACE definitions identified men as having a 3.7-4.5-fold and women a 1.6-2.8-fold risk of developing diabetes during the 5-year follow-up (55).

Finally, in the San Antonio Heart Study, the WHO, NCEP and IDF definitions showed a similar ability to predict incident diabetes, which ability was not fully explained by glucose intolerance. All definitions predicted incident diabetes independently of age, sex, ethnic origin or family history of diabetes (56).

#### **2.1.4. Inflammation and metabolic disorders**

The metabolic and immune systems are among the most fundamental requirements for survival. Many metabolic and immune response pathways or nutrient-pathogen-sensing systems have been evolutionarily conserved throughout species. As a result, immune response and metabolic regulation are highly integrated and the proper function of each is dependent on the other. This interface can be viewed as a central homeostatic mechanism, dysfunction of which can lead to a cluster of chronic metabolic disorders, particularly obesity, type 2 diabetes and cardiovascular disease (57).

The finding in 1993 that tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) is overexpressed in the adipose tissue of obese mice provided the first clear link between obesity, diabetes and chronic inflammation (58). With regard to inflammation, the traditional features of this state do not apply to the diseases in question. In the classic literature, inflammation is described as the principal response of the body invoked to deal with injuries, the hallmarks of which include swelling, redness, pain and fever (tumor, rubor, dolor and calor) (59). This, often short-term, adaptive response is a crucial component of tissue repair and involves the integration of many complex signals in distinct cells and organs. However, the long-term consequences of prolonged inflammation are often not beneficial. Although many of the same mediators are involved in obesity and diabetes, few, if any, of the classic features of inflammation have been observed. Therefore, this new distinct form of injury response is called low-grade or chronic inflammation. This condition is principally triggered by nutrients and metabolic surplus, and engages a similar set of molecules and signalling pathways to those involved in classical inflammation (57).

Both adipose tissue and the liver have an architectural organization in which metabolic cells (adipocytes or hepatocytes) are in close proximity to immune cells (Kupffer cells or macrophages) and both have immediate access to a vast network of blood vessels. This interface might contribute to the emerging importance of these two organs in the initiation and development of metabolic diseases, particularly in the context of obesity and type 2 diabetes. This chronic inflammation is characterized by abnormal cytokine production, increased acute-

phase reactants and other mediators, and activation of a network of inflammatory signalling pathways (60,61).

### **2.1.5. Future challenges for diagnostics and definition**

Although the diagnosis of the MetS has been established and accepted by international organizations, the reasons for using the diagnosis have recently been questioned (63). Diagnosis is warranted if a syndrome reflects a unifying pathological process or predicts future adverse event(s) better than the sum of its components. According to its critics, the current data on the MetS does not fulfill either of these criteria, and thus the labeling of subjects with the term MetS should be avoided. In their critical joint statement the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) expressed concerns regarding the MetS that the criteria are ambiguous or incomplete and the rationale for thresholds are ill defined. The value of including diabetes in the definition is questionable and insulin resistance as the unifying etiology is uncertain. It is stated that there is no clear basis for excluding other CVD risk factors as smoking and elevated LDL cholesterol, and therefore the CVD risk associated with the syndrome appears to be no greater than the sum of its parts. The treatment of the syndrome is not different from the treatment of each of its components (62).

Furthermore, the separate components of the syndrome may show different risk profiles (49,63,64), suggesting that the different combinations do not carry equal risk. According to a recent review by Richard Kahn, entitled “The metabolic syndrome (emperor) wears no clothes”, the dichotomous nature of the diagnosis of the MetS is problematic, because the criteria included in the definition are continuous variables with no upper limits given for any of them (65).

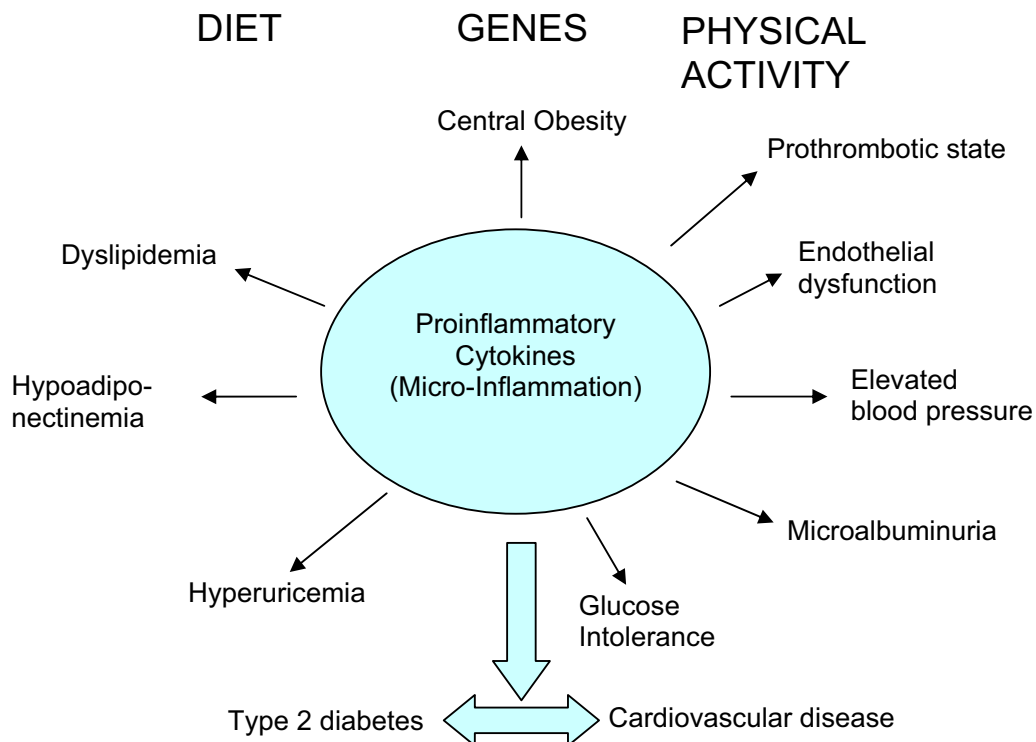
The high intercorrelation between the components of the MetS complicates the use of traditional statistical methods and therefore, confirmatory factor analysis, a multivariate correlation method, has been used to investigate the relationship of the variables included in the syndrome (66,67). Factor analysis identifies statistically independent latent factors underlying the associations of the included variables. If an analysis reveals only a single factor this supports the hypothesis that the MetS has a common etiology e.g. insulin resistance. The other case is that several factors represent distinct physiological phenotypes, which suggests a more complex etiology. However, if a variable is associated with more than one factor, this overlap indicates unifying commonalities between physiological domains and provides an insight into the common factor underlying the syndrome (66). Factor analysis has consistently yielded from 2 to 4 factors for the MetS with a separate obesity-hyperinsulinemia factor (46,68-72) that in many cases also included dyslipidemia. Blood pressure has most consistently loaded on a separate factor (68,69,71,72).



Confirmatory factor analysis supports the current clinical definition of the MetS, as well as the existence of a single factor that links all of the core components (73). The same one factor model was found also in adolescents and the model appeared to be consistent across sex and racial/ethnic subgroups (74). Further investigation is needed to determine whether the core factor is genetic or environmental (73).

## 2.2. Etiology and components of the metabolic syndrome

The original description of the MetS was based on the assumption that the syndrome is a clinical manifestation of insulin resistance. However, it is unlikely that insulin resistance can explain all the components of the MetS. As more data have been published and many new features have been proposed as being part of the syndrome (low-grade inflammation, hypoadiponectinemia, endothelial dysfunction and prothrombotic state), abdominal obesity has been nominated as a marker of “dysfunctional adipose tissue”, and is of central importance in the clinical diagnosis of the MetS (7). Also environmental, possibly preventive, intrauterine and early life factors are involved along with genetics in the pathophysiology of the MetS (Figure 2).



**Figure 2.** Components of the metabolic syndrome. Diet, genes and physical inactivity contribute to the pathophysiology of the MetS.

### 2.2.1. Abdominal obesity and fat distribution

The dramatic worldwide increase in the prevalence of obesity, defined as BMI > 30 kg/m<sup>2</sup>, is the most important underlying cause for the increasing prevalence of the MetS and T2DM (5). Basically, obesity results from an imbalance between energy intake and energy expenditure. However, this balance is modified by numerous genetic, environmental and psychosocial factors (75). In 1956, Vaque reported for the first time that android-type upper body fat deposition is associated with increased risk for chronic diseases, including diabetes, atherosclerosis and gout (76).

In the NHANES study conducted in the U.S between 1989 and 1994 according to the NCEP criteria 30.1% of men had abdominal obesity, while 5 years later it had increased to 36.0%. A similar increase was observed in women during the same period from 45.7% to 51.9% (77). In a recent Finnish population-based study of 45 to 65-year old persons, the prevalence of abdominal obesity was 68.8% in men and 76.4 % in women, according to the IDF criteria ( $\geq 94$  cm for men and  $\geq 80$  cm for women), and 36.4% and 51.8%, according to the NCEP criteria ( $\geq 102$  for men and  $\geq 88$  cm for women) (78).

Randomly chosen primary care physicians in 63 countries recruited consecutive patients aged 18 to 80 years on 2 prespecified half days. Waist circumference and BMI were measured and the presence of CVD and diabetes mellitus recorded. Overall, 24% of 69 409 men and 27% of 98 750 women were obese (BMI > 30 kg/m<sup>2</sup>). A further 40% and 30 % of men and women, respectively, were overweight. Increased waist circumference ( $>102$  cm for men and  $> 88$  cm for women) was noted in 29% and 48%, CVD in 16% and 13%, and diabetes mellitus in 13% and 11% of men and women, respectively. A statistically significant graded increase existed in the frequency of CVD and diabetes mellitus for both BMI and waist (79).

While BMI provides an indicator of overall obesity for epidemiological purposes, a more accurate assessment of total body composition can be obtained by bioelectrical impedance (80), underwater weighing (81) or dual energy X-ray absorptiometry (DEXA) (82). Waist circumference is probably the most important anthropometric measure, because the impact of the distribution of body fat is crucial for the metabolic consequences. Waist circumference correlates with abdominal obesity better than waist-to-hip ratio (WHR) (28). The most reliable assessment of abdominal fat distribution can be obtained with computed tomography (83) or magnetic resonance imaging (84).

Adipose tissue is distributed throughout the body as large homogeneous discrete compartments and as small numbers of cells "marbling" or adjacent to other tissues. Most adipose tissue (about 85% of total adipose tissue mass) is located under the skin (subcutaneous fat) in both lean and obese persons (85). In both sexes, older men and women had a significantly greater increase in visceral fat measured by MRI for a given waist

circumference than younger men and women (< 50 years) (86). The term "visceral fat" is commonly used to describe intra-abdominal fat, and includes both intraperitoneal fat (mesenteric and omental fat), which drains directly into portal circulation, and retroperitoneal fat, which drains into the systemic circulation. The relative contribution of intra-abdominal fat mass to total body fat is influenced by sex, age, race/ethnicity, physical activity, and total adiposity (87). At any given level of waist circumference, the prevalence of diabetes is consistently higher in Asians than in Caucasians (88,89).

Since Vague's work a large number of both cross-sectional and prospective studies have assessed the impact of body fat distribution and confirmed the link of abdominal obesity with insulin resistance (90,91), the metabolic syndrome (92,93), T2DM (94,95), CVD (96,97) and mortality independently of BMI (98). An increase in visceral fat mass, despite similar total fat mass, has been demonstrated in subjects with family history of T2DM compared with subjects without. This suggests that increased visceral fat mass may be an early sign of predisposition to the development of insulin resistance and T2DM (99).

In addition to its function as a store of surplus energy, adipose tissue has multiple regulatory functions in metabolism. Changes in biological properties of adipocytes are thought to contribute to the adverse metabolic effects of abdominal fat tissue. In the normal state the balance between adipose tissue, lipolysis and triglyceride synthesis is carefully governed by energy status, various hormones and the autonomic nervous system. This balance is disrupted in abdominal obesity due to an increase in both fasting and postprandial free fatty acid (FFA) levels (100,101). Furthermore, visceral adipose tissue has been shown to be more sensitive to  $\beta$ -adrenergic lipolysis than subcutaneous adipose tissue because of the larger numbers of  $\beta$ -adrenergic receptors on the cell surface (102). The antilipolytic effect of insulin has shown to be impaired in visceral adipose tissue, resulting in an excess supply of FFAs and causing multiple adverse events closely associated with insulin resistance (103,104). Viscerally obese subjects are also characterized by an exaggerated postprandial triglyceride response (105,106).

However, it is not known whether the storage of an absolute or relative excess amount of triglycerides in abdominal fat depots is directly responsible for increased disease risk or whether such deposition is simply associated with other processes that cause risk (87). In one of the earliest hypotheses, Björntorp proposed that intra-abdominal fat mass was the result of activation of the hypothalamic-hypopituitary-adrenal axis by environmental stress (107-109). More recently, it has been suggested that the limited ability of subcutaneous fat depots to store excess energy results in "overflow" of chemical energy to intra-abdominal fat tissue and "ectopic" sites, causing insulin resistance in the liver (110) and skeletal muscle (111), whereas fat accumulation in the liver rather than in skeletal muscle is associated more with features of the MetS (112). Liver fat content has been found to be 4-fold higher in subjects with the MetS than without, independently of age, gender, or body mass index (113), and type 2 diabetic patients

have 80% more liver fat, underestimated by ALT level, than age-, weight-, and gender-matched non-diabetic subjects (114). Even in obese adolescents high visceral and low abdominal subcutaneous fat stores, measured in many different ways are the main determinants of an adverse metabolic phenotype (115).

The biological activity of an adipocyte changes as its lipid storage increases. Compared with small adipocytes, large cells are more insulin-resistant and lipolytic, release more inflammatory cytokines and less adiponectin (116,117), and are more frequently found in people with obesity-related metabolic disorders (118).

These hypotheses are not mutually exclusive, and it is possible that all, including genetic factors, are involved in the association between abdominal fat mass and adverse metabolic consequences.

#### **2.2.1.1. Adipokines secreted by adipose tissue**

Adipose tissue is not only involved in the storage and mobilization of lipids but is also an important endocrine organ releasing numerous polypeptides, collectively termed adipokines. The term adipokine is generally applied to biologically active substances found in the adipocytes of adipose tissue (119). Adipokines include a variety of proinflammatory peptides: TNF- $\alpha$ , Interleukin(IL)-6, CRP, adiponectin, leptin, resistin, visfatin, PAI-1, apelin, hepcidin, vaspin and IL-1 Ra (120) (Table 2).

TNF- $\alpha$  is a paracrine mediator in adipocytes and appears to act locally to reduce insulin sensitivity of adipocytes (121). This leads to high release of FFA:s and atherogenic dyslipidemia (122). TNF- $\alpha$  also increases the secretion of other inflammatory mediators (121).

IL-6 is a systemic adipokine, which not only impairs insulin sensitivity, but is also a major determinant of the hepatic production of C-reactive protein (CRP), the most important source of this inflammatory marker (123). The role of CRP in the MetS is discussed later in section 2.2.5.2. During the last decade, an accumulating amount of data has suggested that IL-6 plays a pivotal role as a multifaceted pleiotropic cytokine. IL-6 produced in the working muscle during physical activity could act as an energy sensor by activating AMP-activated kinase and enhancing glucose disposal, lipolysis and fat oxidation (124).

Adiponectin is the most abundant adipokine in the plasma (125). In contrast to other adipokines, adiponectin level is inversely associated with insulin resistance, obesity, the MetS and type 2 diabetes (8,126), and women have higher levels of adiponectin compared to men (127). The role of adiponectin is discussed later in section 2.2.5.1.

Leptin was first identified in 1994 as a hormone secreted by adipocytes. It is a 16 kDa non-glycosylated peptide hormone encoded by the gene *obes(ob)*, the murine homolog of the human gene *LEP* (128). Adipose tissue is the only known source of leptin, and its secretion is proportional to adipocyte size (129). Leptin participates in the regulation of appetite and energy

expenditure (130), acts as a potential modulator of the hypothalamic-pituitary-adrenal-axis (131), and is considered a hormone with pleiotropic actions (132). Leptin acts as a fundamental signal for the brain to modulate food intake as a function of energy status. Loss of leptin function results in obesity (133). In men (134) and patients with type 2 diabetes (135), plasma leptin levels are associated with the occurrence of cardiovascular diseases. Because the level of leptin increases with obesity and that of adiponectin decreases with obesity, the ratio of leptin to adiponectin can be used as an index of insulin resistance (136). In a recent study with young Finnish adults it was found that the ratio between hs-CRP and leptin was independent of obesity and cardiovascular risk factors. Because the effect of leptin was not restricted to obesity, it was suggested that leptin might regulate development of chronic low-grade inflammation at all levels of body weight (137). In a prospective population-based study leptin levels significantly predicted the development of the MetS, independently of baseline BMI (138).

Resistin is a dimeric protein that is highly expressed in mouse adipose tissue (139). In humans resistin is mainly produced by macrophages and is involved in an inflammatory process that may be related to atherosclerosis (140). Abdominal fat depots showed a 418% increase in resistin mRNA expression compared with thigh fat (141).

Visfatin, originally identified as a pre-B-cell colony-enhancing factor, is highly expressed in visceral adipose tissue, and plasma visfatin levels correlated with obesity (142). Visfatin lowers blood glucose levels in mice, and *in vitro* visfatin directly activates the insulin-receptor signalling cascade (142). However, in humans no correlation was found between plasma visfatin levels and parameters of insulin sensitivity (143).

High levels of plasminogen activator inhibitor (PAI)-1 contribute to the procoagulant state in the MetS. Although PAI-1 is synthesized in many cell types, adipose tissue is the major source of PAI-1, and circulating PAI-1 levels correlate with visceral obesity (144). In obese individuals PAI-1 levels are increased in those with MetS (145). The fibrinolytic system could play a role in the regulation of adipose tissue development and insulin signaling in adipocytes (146).

Apelin is a bioactive peptide that was originally identified as the endogenous ligand of orphan G-protein-coupled receptor APJ (147). It has been suggested that production of apelin in the obese might be an adaptive response to obesity-related disorders such as mild chronic inflammation (148).

The most recently discovered adipokines are hepcidin and vaspin. Hepcidin was discovered in 2001 as a urinary antimicrobial peptide synthesized in the liver, and was later identified as an adipokine. It has a function as a key regulator of iron homeostasis, hypoxia and inflammatory stimuli (149,150). Vaspin was discovered in 2005 as a serpin (serine protease inhibitor) produced in visceral adipose tissue. It might also constitute a compensatory mechanism in response to obesity and inflammation (151). Vaspin concentrations are higher in female

compared to male subjects. In the normal glucose-tolerant (NGT) group, circulating vaspin significantly correlated with BMI and insulin sensitivity (152).

**Table 2.** Overview of key adipokines (modified from 120, 154).

<b>Adipokine</b>	<b>Key Properties</b>	<b>Secretion in obesity</b>
Tumour Necrosis-Factor (TNF)- $\alpha$	Pro-atherogenic, diabetogenic, paracrine role in adipocyte Decreased insulin signalling Increased secretion of other inflammatory mediators	Increased
Interleukin (IL)-6	Promotes inflammation, pro-atherogenic, diabetogenic Increased vascular inflammation Increased hepatic C-reactive protein production Decreased insulin signalling Possible enhances glucose disposal and lipolysis	Increased
C-reactive protein	Promotes inflammation, pro-atherogenic, diabetogenic Marker of chronic low-grade inflammation Predicts adverse cardiovascular outcomes	Increased
Adiponectin	Anti-atherogenic, anti-diabetogenic Decreased differentiation of macrophages into foam cells Decreased atherogenic vascular remodelling Decreased hepatic glucose output Increased insulin sensitivity	Decreased
Leptin	Inhibits appetite and weight gain in hypothalamus Decreased insulin signalling Pleiotropic actions	Increased
Resistin	Exarberates insulin resistance Decreased insulin signalling Decreased endothelial function Increased vascular smooth muscle proliferation	Increased
Visfatin	Activates insulin signalling in mice	Increased
Plasminogen Activator-Inhibitor (PAI)-1	Pro-atherogenic, pro-coagulant Increased atherothrombotic risk	Increased
Apelin	Adaptive response to obesity (?) Increased by TNF	Increased
Hepcidin	Regulator of iron homeostasis and inflammatory stimulus	Increased
Vaspin	Compensatory mechanism to obesity and inflammation (?)	Increased
Interleukin-1 Receptor antagonist (IL-1 Ra)	Adaptive response to inflammation Prevents IL-1 responses Acute phase reactant Increases insulin resistance	Increased

Adipose tissue produces, presumably as an adaptive response, anti-inflammatory factors such as interleukin-1 receptor antagonist (IL-1 Ra), which binds competitively to the type 1

receptor without triggering activity within the cell (153). The role of IL-1 Ra is discussed later in section 2.2.5.3.

Accumulating evidence suggests that an increase in visceral abdominal fat mass is the primary perturbation in the pathogenesis of the MetS, providing mediators, adipokines, for cross-talk with other tissues and finally leading to insulin resistance and vascular disorders (154).

### **2.2.2. Insulin resistance and glucose intolerance**

Insulin is a hormone with important metabolic functions. By stimulating the uptake of glucose into skeletal muscle cells and adipocytes and inhibiting hepatic glucose production, insulin can be regarded as the most important regulator of the plasma glucose level. In addition, insulin stimulates lipogenesis and glycogen and protein synthesis in adipose tissue, liver and skeletal muscle cells and inhibits glycogenolysis, lipolysis and protein breakdown (155).

Insulin resistance can be defined as an insufficient response of the target organs (liver, skeletal muscle cells and adipose tissue) to physiological plasma insulin levels. A disruption in the insulin signalling transduction pathway is considered to be the most important underlying mechanism. Insulin resistance has different effects in different organs and leads to a compensatory increase in the production of insulin by pancreatic beta-cells, resulting in hyperinsulinemia (155). The impact of genetic factors has been demonstrated by reduced insulin sensitivity in certain ethnic groups (156) and in first-degree relatives of T2DM patients (157). Also free fatty acids and TNF- $\alpha$  and other adipokines play a central role. Insulin resistance is a strong predictor of the risk for developing T2DM (158). In large prospective population studies hyperinsulinemia (a surrogate marker of insulin resistance) has also predicted CVD and mortality (159-161).

The hyperinsulinemic euglycemic clamp is widely recognized as the gold standard for measuring insulin sensitivity (24). However, it is complex, costly and time consuming, and therefore not suitable for population-based studies. The homeostasis model assessment (HOMA-IR) of insulin resistance is a simple and less expensive method and therefore widely used in epidemiological studies. HOMA-IR gives an estimate of basal insulin resistance from the mathematical modeling of fasting plasma glucose and insulin concentrations. The correlation with clamp-measured insulin sensitivity is generally around -0.80 (162). Fasting insulin level has also been used as a marker of insulin resistance in epidemiological studies. The correlation of insulin levels and clamp measured insulin sensitivity is around -0.70 in subjects with normal glucose tolerance (26). In the large DECODE study, hyperinsulinemia, defined by the highest quartile for fasting insulin, was significantly associated with cardiovascular mortality in both men and women independently of other risk factors (163). Hyperinsulinemia and insulin

resistance may partly represent different phenotypes (164). The quantitative insulin sensitivity check index (QUICKI) is an alternative method of measuring insulin sensitivity in large population studies, and is determined by fasting glucose and insulin levels. QUICKI correlates significantly with the glucose clamp method (165,166), and predicts better than fasting insulin the onset of type 2 diabetes (167).

The biological action of insulin is mediated via the insulin receptor, which is widely expressed in human tissues. Insulin resistance is believed to result from a defect inherent in the insulin signalling pathway, which is common to many insulin-responsive cell types. Individuals who lack the insulin receptor or have antibodies against it develop severe insulin resistance (168). However, this intrinsic receptor defect does not explain the link between obesity and insulin resistance. As insulin is anabolic and enhances fat storage, insulin resistance in adipose tissue might be expected to mitigate obesity (169).

An alternative theory postulates that insulin resistance arises when pathological levels of humoral factors disrupt insulin signalling in responsive tissues. This humoral theory emerged from the recently recognized role of adipose tissue as a secretory organ (170), and provided an obvious link between obesity and insulin resistance. In 2005 this was confirmed and extended by demonstrating that inflammation in the liver can be the primary source of systemic factors that lead to the development of insulin resistance (171). Insulin has been found to be an anti-inflammatory hormone. It has been shown to suppress several proinflammatory transcription factors, such as nuclear factor- $\kappa$ B, EGR-1 and activating protein-1 and the corresponding genes regulated by them, which mediate inflammation (172,173). Impairment in the action of insulin because of insulin resistance would thus result in the activation of these proinflammatory transcription factors and an increase in the expression of the corresponding genes (174).

The integration of all these mechanisms might lead to so called endoplasmic reticulum (ER) stress. This theory was presented by Hotamisligil (175). It is believed that inflammatory responses in obesity can be triggered in the adipocyte or macrophages by extracellular mediators such as cytokines or lipids, or initiated through ER stress. Signals from all these mediators converge on inflammatory signalling pathways. The mediators include kinases: c-Jun NH2-terminal kinase and protein kinase C (PKC), and reactive oxygen species (ROS). Once activated they lead to the production of additional inflammatory mediators through transcriptional regulation and this starts a vicious circle. They also directly inhibit insulin receptor signalling (175).

Glucose intolerance develops when insulin secretion from pancreatic  $\beta$  cells fails to compensate for insulin resistance in target tissues. Insufficient insulin action in the liver leads to increased gluconeogenesis and to glycogenolysis of stored glycogen. An increase in FFA levels, due to adipose tissue insulin resistance, contributes to glucose production by stimulating gluconeogenesis (176). Rising hyperglycemia per se also contributes via so-called glucose



toxicity to impaired insulin secretion (177). Diminished insulin secretion patterns, which might be genetically determined, are also observed in first-degree relatives of T2DM even during euglycemia (178,179).

In many subjects, atherosclerosis precedes the development of type 2 diabetes, and cardiovascular complications are often present in newly diagnosed type 2 diabetes patients. In the Euro Heart Survey 31% of patients with acute or chronic CHD had diabetes. In the patients with acute CHD, 36% had IGT, and 22% had newly detected diabetes. In the stable group these proportions were 37% and 14%. Only about 30% of the CHD patients had normal glucose tolerance (180). On the other hand diabetes without prior myocardial infarction was CHD equivalent in an 18-year follow-up study of Finnish subjects, if prior CHD was defined as myocardial infarction. When less stringent criteria for prior CHD were used (myocardial infarction or ischemic ECG changes or angina pectoris), type 2 diabetes carried a larger risk than prior CHD, especially in women (181). The metabolic syndrome and diabetes carry increased risk of stroke (182). These findings indicate that diabetes and cardiovascular disease may share an underlying cause (13,183). It is possible that this common link between T2DM and CVD is insulin resistance (184).

### **2.2.3. Dyslipidemia**

Dyslipidemia in the MetS is characterized by elevated triglycerides (TG) and low levels of HDL-C (20,25,29,30). Plasma LDL cholesterol (LDL-C) levels are often normal in the MetS. A common finding, however, is that LDL particles are smaller and denser than normal (185), a state believed to be associated with increased cardiovascular risk.

The predominant mechanism in the insulin resistance setting is increased influx of FFAs into the liver, which increases hepatic production of triglyceride-rich VLDL particles (186). Insulin resistance may also lead to impaired LPL activity, and thus reduced triglyceride clearance may contribute to elevated triglyceride levels in the MetS (187). A recent study links postprandial elevation of triglyceride-rich lipoproteins (TRL) to high hepatic fat content and low adiponectin levels. Elevation of postprandial TRL exposes the liver to excess FFA and can influence hepatocellular lipid metabolism, leading to hepatic steatosis and disturbance in the insulin signaling pathways (188). Hypertriglyceridemia is an excellent reflection of the insulin resistant condition and one important criteria for diagnosis of the MetS.

In the presence of hypertriglyceridemia, a decrease in the cholesterol content of HDL results from decreases in the cholesterol ester content of the lipoprotein core along with variable increases in triglycerides making the particle small and dense. This process is in part a function of the cholesteryl ester transfer protein (189). This change in lipoprotein composition also results in increased clearance of HDL from the circulation (190). The relation of these changes

in HDL to insulin resistance are probably indirect, arising in concert with the changes in triglyceride-rich lipoprotein metabolism (53).

In addition to HDL, the composition of LDL is modified. With fasting serum triglycerides > 2,0 mmol/l, almost all individuals have a predominance of small dense LDL (191), which might be more atherogenic than buoyant LDL. The propensity towards small dense LDL is attributable to its facilitated transit through the endothelium and tight adherence to intimal proteoglycans, in addition to its susceptibility to oxidation (192). All these changes are not independent, but related to concomitant changes in other lipoproteins and risk factors (193).

#### **2.2.4. Blood pressure**

The association of elevated blood pressure and insulin resistance or hyperinsulinemia was first reported more than 40 years ago (194). Insulin resistance is found to be directly correlated with the severity of hypertension (195). About a half of hypertensive subjects are insulin-resistant (196,197). The association of hyperinsulinemia and hypertension has also been confirmed in prospective studies. In the San Antonio Heart Study (198), and in Swedish middle-aged men (199) and women (200), fasting insulin levels were associated with hypertension 8-12 years later.

The underlying mechanisms connecting insulin resistance and hypertension are not clear. Insulin is a vasodilator when given intravenously to normal-weight subjects (201), with secondary effects on sodium re-absorption in the kidney (202). In insulin-resistant subjects the vasodilatory effect of insulin is lost, possibly impairing endothelium-derived nitric oxide (NO) production (201), but the renal effect of sodium reabsorption is preserved (202). Hyperinsulinemia also increases the activity of the sympathetic nervous system (203) and modifies transmembrane cation transport (204). Furthermore, locally enhanced glucocorticoid activity in the adipose and muscle tissue of obese subjects contributes to metabolic abnormalities associated with the MetS, and this may lead to hypertension via the activation of the renin-angiotensin system (205). The role of adipokines and cytokines in hypertension and the MetS have not yet been established (206), but adiponectin concentrations have been found to be low in subjects with essential hypertension (207).

Insulin resistance and hyperinsulinemia may in the long term promote increases in blood pressure, complementing many other mechanisms in the pathogenesis of hypertension, or they might even be the primary defect (208).

### **2.2.5. Low-grade inflammation and adiponectin in the metabolic syndrome**

The finding that TNF- $\alpha$  is overexpressed in the adipose tissue of obese mice provided the first clear link between obesity, diabetes and chronic low-grade inflammation (58). However, the first indication of this pathophysiological connection between diabetes and inflammation was presented over 130 years ago, when Ebstein reported that high doses of salicylate improved glucosuria in diabetic patients (209).

This idea was then forgotten until a group of epidemiologists in the mid-1990s discussed the possibility that diabetes and atherosclerosis have common antecedents (the "common soil hypothesis") (13). In 1998 Pickup and Crook (210) finally laid out a more specific pathophysiological hypothesis. Based on the observation that dyslipidemia, common to people with T2DM, is also a feature of the experimental disease and naturally occurring acute-phase reactions, Pickup and Crook proposed that in individuals with an innately hypersensitive acute-phase response, long-term lifestyle and environmental stressors, such as high-fat diet, produce disease (T2DM) instead of repair. They further explained that the innate immune system is primarily responsible for the acute-phase response, a self-limiting process induced by a variety of stressors (infection, tissue injury, and malignancy) causing a number of cells (macrophages, adipocytes, and endothelial cells) to secrete cytokines (interleukin (IL)-1, IL-6 and TNF- $\alpha$ ), which act on the liver to synthesize acute-phase proteins (fibrinogen, C-reactive protein, serum amyloid A, and others). Due to its self-limiting nature, the acute-phase response is aimed at restoring the homeostasis disturbed by an acute stressor. However, in response to chronic stressors, the system may become allostatic, i.e., the sustained effort to acutely battle challenges may ultimately result in overload of the system's resources, and thus the system breaks down (211) and type 2 diabetes develops (212). Adipose tissue produces, presumably as an adaptive response to chronic stress, anti-inflammatory factors such as interleukin-1 receptor antagonists (IL-1 Ra) (153).

#### **2.2.5.1. Adiponectin**

Adiponectin is an anti-inflammatory cytokine produced exclusively by adipocytes. Physiological plasma adiponectin concentrations are very high (5-10  $\mu\text{g/ml}$ , higher in women than in men, accounting for about 0.05% of total serum proteins) and visceral obesity has been shown to be negatively correlated with plasma adiponectin levels (213). The mechanism by which (visceral) obesity leads to a reduction in plasma adiponectin levels has not yet been elucidated (155), but reduced adiponectin levels can be caused by interactions of genetic factors such as single nucleotide polymorphism 276 in the adiponectin gene itself and environmental factors, i.e., lifestyle changes that cause obesity, such as a high-fat diet and sedentary lifestyle (214).

Adiponectin, also termed Acrp30 (adipocyte complement-related protein of 30 kDa) (9), AdipoQ (215), apM1 (adipose most abundant gene transcript 1) (216) or gelatin binding protein of 28 kDa (217), was originally identified independently by 4 groups using different approaches in 1995-96. Adiponectin exists in a wide range of multimer complexes in plasma and combines via its collagen domain to create 3 major oligomeric forms: a low-molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer, and high-molecular weight (HMW) 12- to 18-mer adiponectin (218).

Several mechanisms for the metabolic effects of adiponectin have been described (169). In the liver, adiponectin enhances insulin sensitivity, decreases the influx of NEFAs, increases fatty acid oxidation, and reduces hepatic glucose output. In muscle, adiponectin stimulates glucose use and fatty acid oxidation. Within the vascular wall, adiponectin inhibits monocyte adhesion by decreasing the expression of adhesion molecules, inhibits macrophage transformation to foam cells by inhibiting scavenger receptors, and decreases proliferation of migrating smooth cells in response to growth factors. Adiponectin has a sticky nature, binding to collagen I, III and V, which are present in vascular intima. Hence the name adiponectin. In addition, adiponectin increases nitric oxide production in endothelial cells and stimulates angiogenesis. These effects are mediated via increased phosphorylation of the insulin receptor, activation of AMP-activated protein kinase, and modulation of the nuclear factor  $\kappa$ B pathway.

White adipose tissue is the only source of adiponectin, and in contrast to other adipokines, adiponectin level is inversely associated with insulin resistance, obesity, the MetS (219,220), T2DM (221), and atherosclerosis (222,223). In prospective studies a low level of adiponectin has been predictive of the development of insulin resistance, T2DM, hypertension and cardiovascular disease (224-228). High adiponectin levels have been found to be protective against development of T2DM (229,230). Pima Indians with high concentrations of adiponectin were 40% less likely to develop T2DM than those with low concentrations after adjustment for BMI, indicating that adiponectin could be used as a predictor of the future development of T2DM (229).

In both humans and rodents females have higher plasma adiponectin levels than males, suggesting that hormones regulate the production of adiponectin, although it is controversial how these hormones, such as estrogen and testosterone, are involved (125,214). Relatively little is known about how plasma adiponectin is metabolized and cleared from the circulation, but it is detected in urine in T2DM subjects and healthy males, and shows elevated levels in patients with macro-albuminuria (231).

According to the adiponectin hypothesis, a therapeutic strategy for the treatment of insulin resistance, type 2 diabetes, the MetS, and cardiovascular disease may include the elevation of plasma adiponectin levels by the upregulation of adiponectin receptors, Adipo R1 and Adipo R2 (232), or the development of adiponectin receptor agonists (214). The increase in the level of

adiponectin is observed after administration of insulin-sensitizing thiazolidinediones (TZD) (243), which serve as agonist for the transcription factor PPAR, and angiotensin II receptor blockers (234) or angiotensin-converting enzyme inhibitors (235). Also weight loss (236), Roux-en-Y gastric bypass surgery (237) and some dietary factors such as soy protein (238) and fish oils (239) increase adiponectin levels. Physical training resulted in increased adiponectin receptors 1 and 2 mRNA expression in subcutaneous fat, and thus may mediate the improvement of insulin resistance in response to exercise (240).

The exact role of adiponectin in health, the MetS, insulin resistance, T2DM and cardiovascular disease is not clear, but it is assumed to play a key role in these contexts and can be called an endogenous insulin sensitizer. It has been suggested that a low level of circulating adiponectin represents an independent risk factor and a possible biomarker for the metabolic syndrome (241). The idea of increasing the level of adiponectin by upregulation of receptors makes adiponectin an even more promising treatment option in the future (11).

#### **2.2.5.2. C-reactive protein**

C-reactive protein was first described in 1944. An increase in the concentration of serum capsular swelling protein (later named as CRP) was seen in a patient with acute myocardial infarction accompanied by a rise in body temperature and white blood cell count (242). It was four decades later that this increase in CRP in unstable coronary disease was recognized as potentially reflecting more than an inflammatory response to necrosis (243). Since then many epidemiological studies have demonstrated a significant association between elevated CRP and the prevalence of underlying CVD, the risk of recurrent CVD events among those with established disease, or the incidence of first CVD event among those at risk (244). In particular it has been shown that there is a consistent and robust relationship between high-sensitivity CRP (hs-CRP) and the risk of future CV events (245,246). High CRP levels are associated with an increased risk of CVD among subjects with the MetS or diabetes, even independently of currently established lifestyle risk factors, blood lipids, and glycemic control (247,248), although in the Framingham offspring study discrimination of subjects at risk of CVD events using both MetS and CRP was not better than using either alone (249).

After the findings of the association between CRP and CHD, a relationship with obesity and elevated CRP and IL-6 was observed in healthy subjects in 1999 (12). Obesity was an important determinant of CRP concentration in monozygotic twins, independent of genetic influences (250). Higher BMI (251,252) and central obesity was seen as a major determinant of increased CRP in the MetS (253) and in T2DM (254).

The association between CRP and a number of the components of the MetS have been described, supposing that the MetS is associated with a systemic inflammatory response (255). An increment of 1 mg/L in CRP over 12 years in elderly Finnish women was associated with a

37% increase in the risk of developing the MetS during the same period after adjustment for baseline age, smoking, the use of drugs for lipids, hormone replacement therapy, and prevalent CVD (256). The optimal CRP cut-off point for the MetS was 0,65 mg/L in a Japanese healthy population (257). An association between CRP and insulin resistance (258,259), fasting and post-load glucose (260,261) and risk for developing diabetes (262-264) has been found in many studies.

Because of all these data linking CRP and cardiovascular disease it has been suggested that CRP could be added to the definition of the MetS. The cut-off point is suggested to be 3 mg/L (265). However, in a Mendelian randomisation study no evidence was found of a causal link between CRP and pathology of the components of the MetS (266). Nevertheless reciprocal association between hs-CRP and adiponectin has been observed in many studies (267,268), suggesting that decreased adiponectin levels might be fundamentally associated with the early stage of low-grade inflammation. In contrast, the KORA S4 study did not show the association between hypoadiponectinemia and proinflammation (269).

### **2.2.5.3. Interleukin-1 Receptor antagonist**

Inflammation in autoimmune diseases is characterized by an imbalance between pro- and anti-inflammatory cytokines. The members IL-1 $\alpha$  and IL-1 $\beta$  of the IL-1 cytokine superfamily are strong inducers of inflammation (270,271). IL-1 Receptor antagonist (IL-1 Ra) acts in an antagonist manner and serves as a natural compensatory mechanism for the IL-1-induced disease process (272). When IL-1 occupies its receptor, various proinflammatory events are initiated, but when IL-1 Ra occupies the receptor, no such events are initiated, because IL-1 cannot bind to cells (153).

In healthy individuals, IL-1 Ra is detectable in plasma, in contrast to usually undetectable levels of IL-1 $\beta$  (271). White adipose tissue is an important source of IL-1 Ra (273). IL-1 Ra levels are increased in human obesity (274) and may contribute to the development of insulin resistance (275). In nondiabetic offspring of diabetic probands with the MetS IL-1 Ra and IL-1  $\beta$  were found to be highly elevated, whereas in the same study population TNF- $\alpha$  did not differ between the factor score tertiles (276). IL-1 Ra has also been found to be the most sensitive marker of cytokine response in the prediabetic state (277); however when diabetes develops, the levels of IL-1 Ra fall (278).

IL-1 Ra was also found to be a sensitive marker of clinical instability in CAD patients (279), levels of IL-1 Ra correlated with extent of myocardial loss in patients with acute myocardial infarction (280), and IL-1 Ra genotype was associated with coronary atherosclerosis in T2DM patients (281).

A recent preliminary study with anakinra (a recombinant human IL-1 Ra), lasting for 13 weeks, showed that anakinra did not affect insulin resistance in type 2 diabetic patients. Also BMI remained stable, but glycemic control improved slightly, most likely through enhanced beta-cell secretory function (282). This may indicate that the effects of IL-1 Ra are different in prediabetes compared to established type 2 diabetes, but further studies are needed to confirm these findings.

### **2.2.6. Other features in the metabolic syndrome**

Insulin resistance is accompanied by many other alterations that are not included in the diagnostic criteria for the metabolic syndrome. Increases in uric acid, pro-thrombotic factors (fibrinogen, plasminogen activator inhibitor (PAI)-1), serum viscosity, asymmetric dimethylarginine (an endogenous inhibitor of NO synthase, which plays an important role in endothelial dysfunction), homocysteine, leucocyte count, pro-inflammatory cytokines, the presence of microalbuminuria, non-alcoholic fatty liver disease, polycystic ovarian disease, obstructive sleep apnea, cholesterol gallstones and a decrease in adiponectin are all associated with insulin resistance (283). *Cigarette smoking* (284) and a *sedentary lifestyle* (285) can also produce many of the major criteria of the syndrome and beyond. Genetics also plays an important role between environmental factors and the components of the MetS.

#### **2.2.6.1. Prothrombotic state**

The prothrombotic state present in the MetS is due to numerous changes in the coagulation pathway, fibrinolytic pathway and platelet function (286). Increased plasma levels of fibrinogen reflect the activation of the coagulation pathway, and increased levels of fibrinogen are associated with low-grade inflammation and insulin resistance in the MetS (287), and with impaired fibrinolysis (288). Fibrinolytic dysfunction, as a consequence of increased levels of PAI-1, also has independent value for future cardiovascular disease (289,290). Insulin resistance has been associated with increased levels of coagulation factors VII-IX (291) and increased red cell blood count (292). Platelet function is also disturbed in insulin resistance. Platelets from obese insulin-resistant subjects have reduced sensitivity to the known anti-aggregatory effect of insulin (293). All these might contribute to the increased risk for the development of cardiovascular disease in people with the MetS.

#### **2.2.6.2. Hyperuricemia**

Serum uric acid is the major product of the purine metabolism. In cross-sectional studies, uric acid correlates with the components of metabolic syndrome: hypertension, obesity, low HDL

cholesterol, hypertriglyceridemia, and insulin resistance (294-296). In the Finnish Diabetes Prevention Study (DPS) baseline uric acid and changes in it during the follow-up were related to corresponding changes in fasting and post-load glucose and insulin levels (297). In the Rotterdam population-based study serum uric acid was a strong and independent risk factor for diabetes during a follow-up of 10.1 years (298). Although hyperuricemia and hyperinsulinemia are closely linked, the mechanism behind this is obscure. The most promising hypothesis is that this occurs at renal level: renal tubular function is influenced by hyperinsulinemia, urinary uric acid clearance decreasing with decreasing insulin-mediated glucose disposal (295). The importance of hyperuricemia as a possible treatment target is not known.

#### **2.2.6.3. Endothelial dysfunction**

The endothelium is involved in the regulation of vascular tone, platelet adhesion, coagulation, fibrinolysis and leukocyte adherence, but the term endothelial dysfunction specifically refers to impaired endothelium-dependent relaxation caused by a loss of NO bioactivity in the vessel wall (299). Endothelial cells synthesize a wide variety of other vasoactive mediators, and the balance between them may be an important contributing factor to the vascular function of blood vessels. Endothelin-1 is the most powerful vasoconstrictive factor yet discovered (300), and has been found to be elevated in postmenopausal type 2 diabetic women (301). Endothelial dysfunction has been shown to predict future adverse CVD events (302) and type 2 diabetes (303). Endothelial dysfunction characterizes all insulin-resistant conditions and is thought to link insulin resistance to CVD (299). Reduction of inflammatory cytokine concentrations and improvement of endothelial functions have been found at the same time in obese women after weight loss (304). In a study with offspring of type 2 diabetic patients, the levels of adhesion molecules, relating to endothelial dysfunction, were not elevated. Instead, a correlation was found between inflammatory markers and adhesion molecules, suggesting that low-grade inflammation may precede elevated levels of adhesion molecules (305).

Endothelial dysfunction is linked to insulin resistance and other associated features of the MetS. It has been suggested that dysfunction of the endothelium in large and medium-sized arteries plays a central role in atherogenesis, whereas dysfunction at the peripheral vascular endothelial level plays the primary role in the pathogenesis of insulin resistance (306).

#### **2.2.6.4. Microalbuminuria**

Microalbuminuria is a component of the MetS as defined by the WHO (20). Microalbuminuria is a well established marker for incipient nephropathy in patients with diabetes (307). It is also associated with increased CVD in both diabetic and non-diabetic subjects (308,309). Previous studies reported that microalbuminuria in non-diabetic subjects was associated with MetS, and



could be a component of it (310,311). However, multiple logistic regression analysis in diabetic and non-diabetic subjects separately showed that microalbuminuria was independently associated only with hypertension, diabetes and waist-hip-ratio, but not with the other variables of the MetS. It is therefore likely that microalbuminuria is more a complication of hypertension and diabetes, showing vascular disturbances, and not an integral part of the MetS (22,23).

#### **2.2.6.5. Polycystic ovary syndrome**

Polycystic ovary syndrome (PCOS) is a heterogeneous clinical entity characterized by signs and symptoms of hyperandrogenism and anovulatory disorders often associated with infertility and obesity. The underlying pathogenesis remains uncertain, although key components in the syndrome may be insulin resistance and hyperinsulinemia (312, 313). However, in a Finnish population-based study surprisingly few women with the MetS had symptoms suggestive of PCOS, in comparison with obese and lean women. These results suggest that at the population level PCOS only accounts for a distinct subgroup of the MetS (314).

#### **2.2.6.6. Depression**

In 1674, Thomas Willis speculated that diabetes was caused by "long sorrow and other depressions"(315). Since then many studies have found similarities in the pathophysiology of depressive disorders and alterations in the metabolic network. It has been proposed that depressive symptoms be classified as "metabolic syndrome type II" (316). In women with suspected CHD, the MetS was independently associated with depression but explained only a small portion of the association between depression and incident CVD (317). In a recent population-based 7-year follow-up study nondepressed women and men with the MetS at baseline were twice as likely to have depressive symptoms at follow-up compared with the non-depressed members of this cohort without the MetS at baseline (318). In the Diabetes Prevention Program (DPP) the risk of developing diabetes was associated more often with the use of antidepressants than other metabolic factors (319). The high rate of depressive symptoms in the MetS suggests that the MetS may be an important predisposing factor to the development of depression. Effective prevention and treatment of the MetS could also be important for the prevention of depression.

### **2.3. Genetics of the metabolic syndrome**

Genetic factors influence the components of the MetS and predispose subjects to the MetS together with changing environmental and behavioral factors. The genetic factors contributing to the syndrome are still largely unknown. However, it is likely that a large number of genes interact with each other and the environment, and thus contribute to the risk for the MetS (320).

According to the "thrifty gene" hypothesis, the genes that were beneficial to the survival for our ancestors during famines by maximizing the ability to store energy as fat, predispose nowadays to obesity, MetS and T2DM when exposed to a sedentary lifestyle and high caloric intake (321). One recently found candidate for this thrifty gene is adipose (Adp), which appears to be involved in an ancient pathway that regulates fat accumulation (322).

Racial differences in the risk (323), familial clustering of the MetS (324,325) and twin studies (326) have given grounds for the argument that genetic factors contribute to the MetS. Insulin resistance also clusters in families. In the Botnia study 45% of the first degree relatives of patients with T2DM were insulin resistant compared with 20% of individuals without a family history of diabetes (157). The estimates of the heritability of obesity vary greatly, but generally about 40-70% of the variation in body weight can be explained by genetic factors (327,328). Genes are believed to explain about 60% of the variance in abdominal fat in menopausal women (329). In the Botnia-study, first-degree relatives of T2DM had a higher waist-hip-ratio than their spouses without a family history of T2DM (157). Other components of the MetS also exhibit strong genetic components: heritability levels ranging from 25 to 45% for TGs, 50-60% for total cholesterol, 30-55% for HDL-cholesterol, 30% for systolic blood pressure, 20-30% for diastolic blood pressure, 50% for hypertension (330) and about 30% for microalbuminuria (331).

Two approaches have been used in the search for the thrifty genes: the candidate gene approach and genome-wide scanning (332). The candidate gene approach aims to identify genes on the basis of information about their function. Genes affecting body weight and fat distribution, lipolysis, fuel oxidation or skeletal muscle glucose metabolism could predispose to the MetS (333). Such candidate genes include  $\beta$ 2- and  $\beta$ 3-adrenergic receptors, hormone sensitive lipase, uncoupling proteins, peroxisome proliferator-activated receptor gamma-2, TNF- $\alpha$ , insulin receptor substrates and glycogen synthase, as well as many others (334).

Growing interest is being shown in the genes controlling adiponectin and CRP. The ADIPO $\beta$  gene encoding adiponectin has been mapped to chromosome 2q27 (335). There are data that genetic variation within the ADIPO $\beta$  gene may be part of the genetic determinants of risk for type 2 diabetes and insulin resistance via the modulation of adiponectin levels (336,337). CRP concentration in old people may in part be determined genetically by the CRP gene (338). CRP may also suppress adiponectin gene expression partially through the PI-3 kinase pathway, and thus might represent a possible mechanism by which CRP regulates insulin sensitivity (339).

The growing epidemic of obesity and MetS may be explained by gene-environment interactions (340). Alterations in physical activity can have different effects on abdominal fat (341) and even diastolic blood pressure, as seen in the HERITAGE Family Study (342). In the Finnish DPS it was also found that genetic variation may modify the magnitude of the beneficial effects of physical activity on characteristics of the MetS in persons with IGT (343).

An increasing understanding of the role of genes in the development of the MetS may reveal genetic variants that, in combination with conventional risk factors, may help to predict the risk for an individual to developing the MetS and to find different preventive methods and therapy for genetically different subjects.

#### **2.4. Life style and other factors in the metabolic syndrome**

During the last century in the western world, industrialization has provided increasing access to large quantities of mass-produced, high caloric foods and many labour saving and transportation devices, abolishing starvation and heavy manual work. As stated earlier, our genes are ancestral energy-conserving, and the result of this combination of genes and industrialization is an epidemic of obesity, MetS, type 2 diabetes and CVD.

Caloric-restricted diet extends the life-span of laboratory rodents up to 50%. This finding has been represented in organisms ranging from yeast to primates, and is the most robust method currently known of extending the lifespan of higher organisms. Caloric restriction even slows or prevents major ageing related diseases in rodent models (344). One mechanism behind this might be sirtuins, which have some unknown role in regulating  $\beta$ -cell function in mammals (345). Theoretically, the same finding has recently been reported in Cuba, where the economic crises of 1989-2005 resulted in reduced energy intake, increased physical activity, and sustained population-wide weight loss. The prevalence of obesity declined from 14% to 7%. During 1997-2002, there were declines in death attributed to diabetes of 51%, to CHD of 35%, to stroke of 20%, and to all causes of 18%. However, a modest increase in the all-cause death rate among elderly subjects, was observed (346).

Sedentary lifestyle is clearly associated with several features, especially abdominal obesity, of the MetS (285,347), but no good prospective studies have shown that a lifestyle intervention can prevent the evolution of the MetS and its complications T2DM and CVD. The most well-known preventive studies (both dietary and increased physical activity) have involved subjects with IGT and the end-point has been the prevention of diabetes. Both the DPS (348) and DPP (349) studies showed the same 58% reduced risk of diabetes in the lifestyle intervention group compared with the control group. The intervention goals in DPS were to reduce body weight (a reduction of 5% or more of baseline weight), limit dietary fat (<30%) and saturated fat (<10%) of total energy consumed, and to increase dietary fibre intake (15 g/100 kcal or more) and physical activity ( $\geq 30$  minutes/day) (348). In the secondary analysis of the DPS study, the effects of the lifestyle intervention on MetS and its components were a significant reduction in the prevalence of the MetS (OR of 0.62) and especially the component of abdominal obesity (OR 0.48). Other components of the MetS did not show significant effects between the groups (350). In many studies it is difficult to distinguish between physical activity and dietary changes, but all the

studies and systematic reviews published thus far show a constant positive effect of physical activity on the prevention of T2DM and MetS (351-355).

Exercise has anti-inflammatory effects by suppressing TNF- $\alpha$  and IL-6 production (356). Inflammatory markers, such as baseline CRP, were found in the DPS to be the best immunological marker of lifestyle changes for progression to overt diabetes (357). In the HERITAGE Family Study CRP levels reduced in response to exercise with high initial CRP levels (358). Exercise-induced increase in adiponectin levels was not found despite decreased abdominal adiposity and improved insulin sensitivity in T2DM men (359).

Dietary aspects are also involved in the inflammatory processes. It is believed that a macronutrient (energy-dense, high fast carbohydrate) diet results in the inability to suppress the inflammation generated by the meal (174). A 900-kcal AHA step 2 diet-based meal rich in fruit and fiber does not cause significant oxidative stress or inflammation, in contrast to the effect of an isocaloric fast food meal (360). Postprandial increases in circulating IL-6 levels, but not plasma CRP levels, were seen after a high-fat meal in abdominally obese men (361). A carbohydrate-rich diet was associated with lower adiponectin concentrations in men (362). Weight loss was associated with a decline in CRP levels in many studies, showing that each 1 kg loss in weight results in a mean decrease in CRP of 0.13 mg/L (363). The mediterranean diet has been shown to be inversely associated with the incidence of the MetS in a prospective study cohort (364).

Smoking is a well recognized health hazard. In addition to its effects on cardiovascular and cancer morbidity, it induces central obesity (365) and increases the risk of developing hypertension, independently of inflammation (366) and type 2 diabetes (367).

Epidemiological studies have shown an association between moderate alcohol consumption and reduced risk for the MetS and T2DM. The mechanisms reported to explain this observation include improvement of the lipid profile, especially increased levels of HDL cholesterol (368) and increased insulin sensitivity (369). This positive effect may be mediated through alcohol-induced increases in adiponectin (362,370,371), also in women (372). The positive cardioprotective effect of moderate alcohol consumption was seen, in particular, in men with the MetS (373).

The intake of coffee may lower the risk of type 2 diabetes. However, although the explanation for this coffee-induced increased insulin sensitivity and type 2 diabetes risk reduction is not known (374-376), it may be caused by increased levels of adiponectin, at least in women (377). Higher magnesium intake, mainly from whole grain consumption, in young adults was also associated with lower risk of MetS (378). On the other hand, elevation in the markers of the iron metabolism increased the incidence of the MetS in both sexes (379). Another new lifestyle feature is the voluntary restriction of sleep duration. Self-reported sleep duration has decreased, while during the same time obesity, MetS and T2DM have increased (380). Both short (<6h)

and long (>8h) sleep duration is related to increased risk (381,382). Psychosocial, stressful life events (383) and low socioeconomic status (384) also predict the risk for developing the MetS. A positive association has been found between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults. The expected association between obesity and diabetes was absent in people with low concentrations of persistent organic pollutants, but association became much stronger along with a higher concentration of pollutants (385).

## **2.5. Treatment and clinical aspects of the metabolic syndrome**

Prevention of the MetS and its complications, diabetes and CVD, is an important public health goal. Lifestyle modification to reduce obesity, increase physical activity and quit smoking, is primary on both the population and individual levels. After the successful DPS (348) and DPP (349) studies, population-based programs to prevent diabetes, and MetS at the same time, have been launched by the International Diabetes Federation (IDF) (386), and also at national level, as in the Finnish implementation program to prevent type 2 diabetes (FIN-D2D) (387). Lifestyle modification, especially increased physical activity, is the only known treatment without side-effects which affects positively all components of the MetS. This is important, as cardiovascular mortality is higher, when more components of the MetS are present (388).

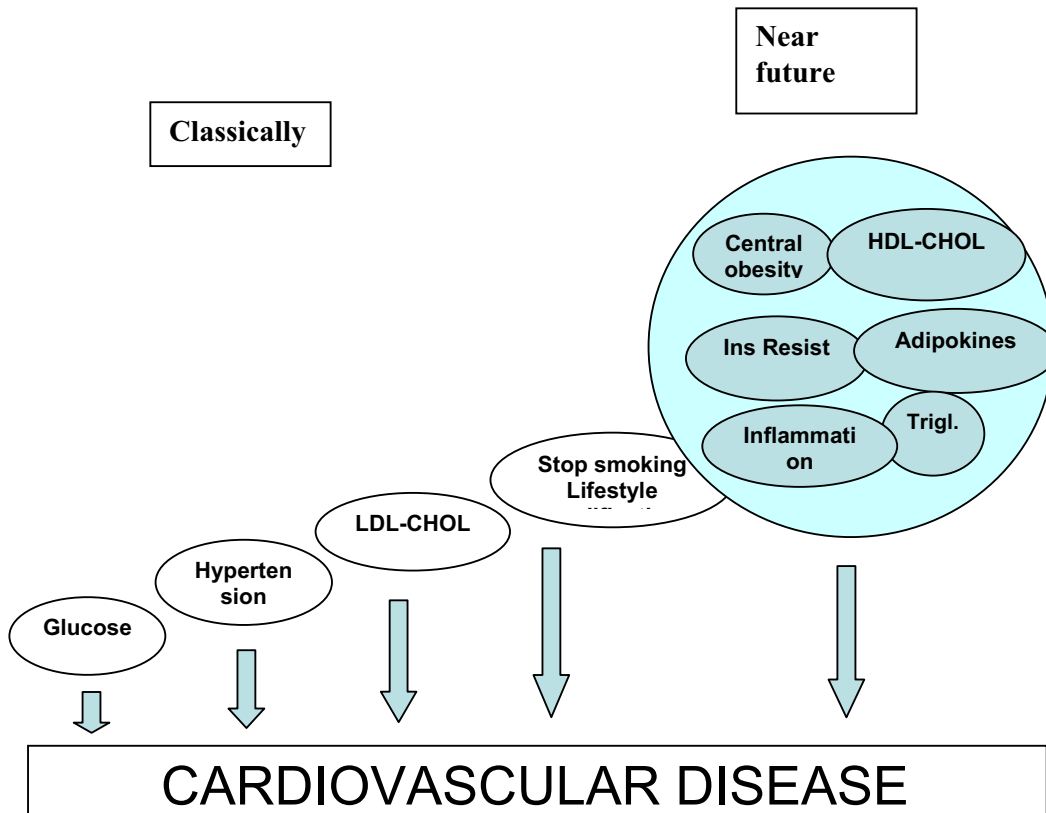
If therapeutic lifestyle modification fails, or if the 10-year cardiovascular risk is high, drug therapy to modify different components might be required in addition (33).

Classically, as described below in Figure 3, antihypertensive drugs (389), such as angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers, and lipid-lowering statins, are required along with lifestyle changes (390,391). Low-dose aspirin is needed to prevent MetS-related increase in arterial thrombosis formation (392).

The metabolic abnormalities clustering around abdominal obesity should be the main target for the drugs used in the treatment of the MetS. The recent discovery of the endocannabinoid-CB1 receptor system and its impact on the regulation of the energy metabolism represents a significant advance (393). Studies with rimonabant, the first CB1 blocker, show that in overweight/obese subjects 20 mg/day was associated with clinically meaningful weight loss, a reduction in abdominal obesity, and improvements in insulin resistance, lipid profile, and glucose metabolism. Overall, LDL cholesterol levels remained unchanged. These positive effects may be mediated by increased levels of adiponectin. Rimonabant was generally quite well tolerated, but increase in depression requires special attention (394,395).

Inflammation is strongly associated with the features of the MetS, and elevated CRP level is one marker of this. Lifestyle therapies will reduce concentrations of CRP and thus mitigate an underlying inflammatory state (363). No specific anti-inflammatory drugs are available to treat

the proinflammatory state. However, statins are reported to reduce the concentration of CRP (396), and patients who have low CRP levels after statin therapy have better clinical outcomes than those with higher CRP levels, regardless of the level of LDL cholesterol (397).



**Figure 3.** Treatment of the metabolic syndrome.

Also, thiazolidinediones (e.g. rosiglitazone and pioglitazone) a class of insulin-sensitizing agents currently used in the treatment of diabetic hyperglycemia, are known to lower CRP levels and have positive effects on endothelial dysfunction (398). They were believed to be ideal candidates for the early treatment of many components associated with the MetS, as they also increase adiponectin levels (399); however, recent associations, especially in connection with rosiglitazone, with increased cardiological problems have made this class less promising (400). Antagonist of TNF- $\alpha$ , etanercept, has also been found to lower CRP in the MetS (401). The strong association between hypoadiponectinemia and metabolic dysfunction provides the perfect scenario for adiponectin replacement therapy. Cellular and animal studies have shown evidence for the benefit of adiponectin therapy (402). This may include the increase of plasma

adiponectin levels by the upregulation of adiponectin receptors, or the development of adiponectin receptor agonists (214). Metformin, which have been used more than 50 years, may be the safest drug to accompany lifestyle modification in the MetS (355).

## **2.6. Early life and the metabolic syndrome in adulthood**

The discovery that individuals who develop coronary heart disease grow differently during early life has led to the recognition of new developmental models for the disease. In 1995 David Barker wrote: "The fetal origins hypothesis states that fetal undernutrition in middle to late gestation, which leads to disproportionate fetal growth, programmes later coronary heart disease" (403). Barker's hypothesis is also known as the "Thrifty phenotype hypothesis". In humans, birth size serves as a marker of the intrauterine environment. Considering that birth size is just one snapshot of the trajectory of fetal growth, it is intriguing that long-term health outcomes are predicted by the body size of the newborn (404).

The association between birth size and cardiovascular morbidity is largely modified by growth later in life. The highest risk of coronary disease was found among individuals who were born small and became heavier during childhood (405). This association was especially noticeable in women who crossed from the low centile of weight at birth to the high centile of BMI in adulthood (406). Low weight gain during infancy increases the risk for IGT and type 2 diabetes. The effect was greater in people who had low birth weight (407). Low BMI at 2 years of age and increased BMI from 2 to 11 years of age were also associated with insulin resistance later in life (408). Also hypertension (409,410) later in life and its complication, stroke (411), is associated with infant and childhood growth.

These epidemiological findings identify the phenomena of fetal programming without explaining the underlying mechanisms that establish the causal link. Animal models have demonstrated that reduction in the availability of nutrients during fetal development programs the endocrine pancreas and insulin-sensitive tissues. The molecular mechanisms responsible for intrauterine programming of the  $\beta$ -cells remain elusive, but two hypotheses have emerged: programming of mitochondrial dysfunction (412) and epigenetic regulation, which means the interaction of genes and uterine environment (413). Strong evidence for epigenetic programming comes from monozygotic twin studies (414).

These theories are problematic with respect to explaining the growing epidemic of obesity and MetS in westernized countries, as the relative proportion of underweight newborn children is decreasing (415). Obesity at the age of seven is seen to predict the MetS in adulthood (416,417), and it is possible that the Ala12 allele of the PPAR $\gamma$ 2 gene is associated with high weight and ponderal index at birth, and weight gain and high waist circumference in adulthood (418). Hypoadiponectinemia, observed even in visceral adiposity in adolescents (419), may

partly explain the fact that overweight in childhood (420) and adolescence (421) increases the risk of CHD in adulthood.

## **2.7. Gender differences in the metabolic syndrome, diabetes and cardiovascular disease**

In the general population CHD morbidity and mortality vary widely with a distinctive male-preponderance: a male-female ratio of approximately 2,5 has been found in different countries (422).

Diabetes abolishes the normal gender-related difference in the risk for CVD. In the Framingham Study the risk for acute MI was 150% greater in diabetic than nondiabetic women, but only 50% greater in diabetic than nondiabetic men when adjusted for hypertension, dyslipidemia and obesity among individuals aged from 50 to 59 years (423). In the Framingham study diabetic women were at a similar absolute risk as their men counterparts, at a 2-fold risk in comparison to male nondiabetic subjects, and at a 4-fold risk in comparison to female nondiabetic subjects for CVD (424). In a Finnish study diabetes-related risk for CHD mortality was 4-fold in men and 24-fold in women, and for CHD incidence 3- and 14-fold, respectively (425). After adjusting for conventional risk factors the proportion of diabetes-related CHD risk remained more unexplained in women than in men (425). In Finnish women the association between diabetes and mortality was stronger than that between myocardial infarction and mortality, whereas the opposite was true among men (426). Recent data from NHANES show that the reduction in mortality rates among diabetic patients during last decades has been limited to men (427).

Low-grade inflammation, especially increased CRP levels, have been shown to predict CHD (428), and development of the MetS and type 2 diabetes in men (429) and women (430,431). In the Hoorn study a significant association of hs-CRP with incident DM was observed in men, but not in women (432). In Japanese subjects this association was observed in both sexes (433), but solely in women in the Mexico City Diabetes Study (434) and in the MONICA/KORA study (435).

Women have higher levels of adiponectin than men (127). In the Hoorn Study a high level of adiponectin was strongly associated with a low risk for IGT and T2DM, especially in women (230). Lower plasma adiponectin concentrations characterize women with previous gestational diabetes mellitus independently of their prevailing level of insulin sensitivity or degree of obesity, and are associated with subclinical inflammation and atherogenic parameters (436).

Endogenous sex hormones may, at least in part, explain the gender differences in the risk for diabetes in the prediabetic state and CHD. A recent meta-analysis demonstrated that high testosterone levels are associated with higher risk for T2DM in women but with lower risk in men (437). Low sex hormone-binding globulin is associated with the MetS in women (438).



Testosterone replacement therapy has been found to improve insulin resistance, visceral adiposity and hypercholesterolemia in hypogonadal men with T2DM (439), and rapid weight loss in abdominally obese men with the MetS resulted in a sustained increase in free testosterone levels (440).

### 3. AIMS OF THE STUDY

This study was undertaken to investigate the associations of adiponectin and low-grade inflammation, measured by hs-CRP and IL-1 Ra, with the metabolic syndrome in a population-based study.

The specific aims were:

1. To examine the association of IL-1 Ra, hs-CRP and adiponectin with relative weight gain between childhood and adulthood.
2. To investigate the relationship between insulin sensitivity with adiponectin and inflammatory markers (hs-CRP and IL-1 Ra) levels.
3. To evaluate the differences and associations between inflammatory markers (hs-CRP and IL-1 Ra) and adiponectin with the MetS according to the NCEP and IDF definitions.
4. To study the gender difference in the association of the levels of adiponectin, hs-CRP and IL-1 Ra with the MetS as defined by the IDF and NCEP criteria.

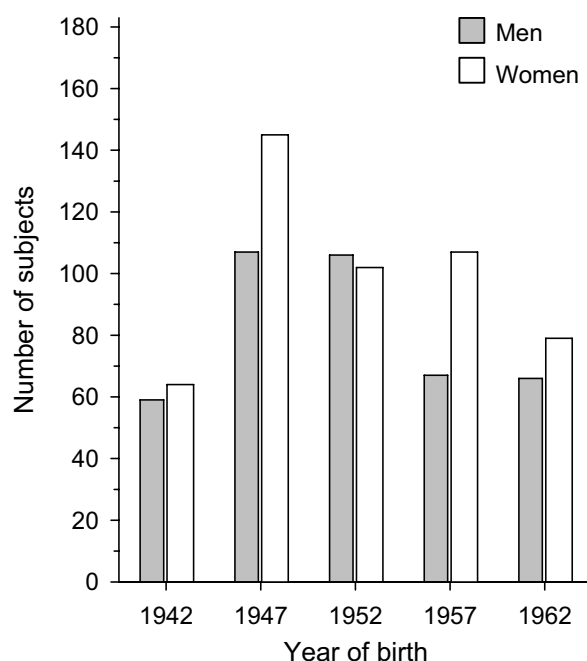
## 4. SUBJECTS AND METHODS

### 4.1. Subjects

The study population consisted of middle-aged, Caucasian subjects (n=1 294) born in 1942, 1947, 1952, 1957 and 1962 in Pieksämäki, Finland. The gender and age distribution of the study population is shown in Figure 4. The gender distribution in different age groups was not statistically significant. The entire age group was recruited from the local population records by three separate invitations. No exclusion criteria were applied. Altogether 923 (411 men and 512 women) of 1 294 subjects (71.3 %) participated in this cross-sectional study in 1997-98.

For the statistical analysis we excluded 18 subjects (7 men and 11 women) whose hs-CRP concentration was  $\geq 10.0$  mg/L in order to eliminate possible cases of acute infections and other occult diseases, leaving a total of 905 subjects. Additional three women were lost from statistical analysis because of missing data for the components of the MetS. In Studies III and IV analysis was done for 902 participants. In study II analysis was done for 907 subjects, because we excluded all the 15 subjects with known diabetes and 1 subject whose hs-CRP concentration was  $> 30.0$  mg/L.

The study protocol was approved by the Ethics Committee of the Kuopio University Hospital and the University of Kuopio. All the participants gave an informed written consent.



**Figure 4.** The whole study population by age group and gender.

## 4.2. Clinical methods

All the measurements were done by two trained nurses at Pieksämäki. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. Waist was measured at the midpoint between the lateral iliac crest and the lowest rib to the nearest 1.0 cm. Blood pressure was measured twice with subjects in a sitting position after a 15-minute rest with a mercury sphygmomanometer. The latter value was used in statistical analysis. No physical examination was done by a physician.

All participants filled a standard questionnaire, including questions about smoking habits and physical activity. A current smoker was a subject who smoked at least once a day. Physical activity was defined as exercise that makes a person sweat at least mildly. Subjects exercising more than three times a week for at least 30 minutes per session were defined as physically active.

Fasting blood samples were drawn after 12 hours of fasting. Plasma was separated by centrifugation, and samples frozen immediately at -20° C and sended weekly from Pieksämäki to Kuopio and stored there at -70° C until analyses in 2003.

## 4.3. Assays and calculations

Insulin was determined by the Phadeseph Insulin Radio-immunoassay (RIA) 100 method (Pharmacia Diagnostics AB, Uppsala, Sweden). Glucose concentration was measured by the automated colorimetric method (Peridochrom Glucose GOD-PAP, Boehringer, Germany). Insulin sensitivity was calculated as follows: QUICKI =  $1/(\log \text{FPI} + \log \text{FPG})$ , where FPI = fasting plasma insulin level expressed as mU/l, and FPG = fasting plasma glucose level expressed as mg/dL (165).

Serum cholesterol and triglycerides were measured at Pieksämäki from fresh serum samples with enzymatic colorimeter methods (CHOD-PAP, GPO-PAP, Boehringer Mannheim GmbH, Germany). Serum HDL cholesterol was measured using the same methods after the precipitation of low-density cholesterol and very-low-density lipoprotein cholesterol by phosphotungstic acid and magnesium.

Serum adiponectin was determined with an enzyme immunoassay (human Adiponectin ELISA Kit, B-Bridge International Inc., Mountains View, CA, USA). Plasma concentration of IL-1 Ra was measured with high-sensitivity assay kits from R&D Systems (Minneapolis, MN, USA). C-reactive protein (CRP) was measured with an Immulite 2000 High Sensitivity CRP assay (DPC, Los Angeles, CA, USA). All these adiponectin, IL-1 Ra and hs-CRP measurements were done at the same time (2003) in Kuopio University.

#### 4.4. Statistical analyses

The results in the studies I, III and IV are expressed as means and standard deviation (SD), with 95% confidence intervals (95% CI). Statistical significance between groups was evaluated by the chi-square test or t-test. When the assumptions of parametric tests were violated permutation test (Monte Carlo p-value), bootstrap t-test or analysis of variance (ANOVA) were used. Confidence intervals for means were obtained by bias-corrected and accelerated bootstrapping (5000 replications), because of the skewed distribution of the variables. The normality of the variables was tested by using the Shapiro-Wilk W test. Agreement between definitions was determined by the kappa statistic ( $\kappa$ ) or Jaccard similarity index (Chamberlain's positive agreement). The level of agreement is considered to be moderate with  $\kappa=0.41$  to 0.60, substantial with  $\kappa=0.61$  to 0.80, and very good with  $\kappa>0.80$  (441). Bootstrap-based multiplicity adjustment was applied to correct levels of significance for multiple testing when appropriate (IV).

Statistical analysis in the Study II was performed (unlike in the other studies) using the SAS version 9.1 for Windows (SAS Institute Inc., Cary, NC, USA). The results are shown as means and standard deviations (SD), except for variables with a skewed distribution (insulin, adiponectin, hs-CRP, IL-1 Ra), which are given as medians and interquartile ranges, unless stated differently. The levels of serum plasma adiponectin and inflammatory markers (hs-CRP and IL-1 Ra) were logarithmically transformed to obtain a normal distribution. The comparison between men and women was performed with Student's t-test for continuous variables and with the chi-square test for categorical variables. Simple and partial Pearson correlations were calculated to examine the association of QUICKI with adiponectin and inflammatory markers without adjustment and after adjustment for age, gender, BMI, smoking status, and physical activity. The  $\alpha$  level was set at 0.05 for all tests.

## 5. RESULTS

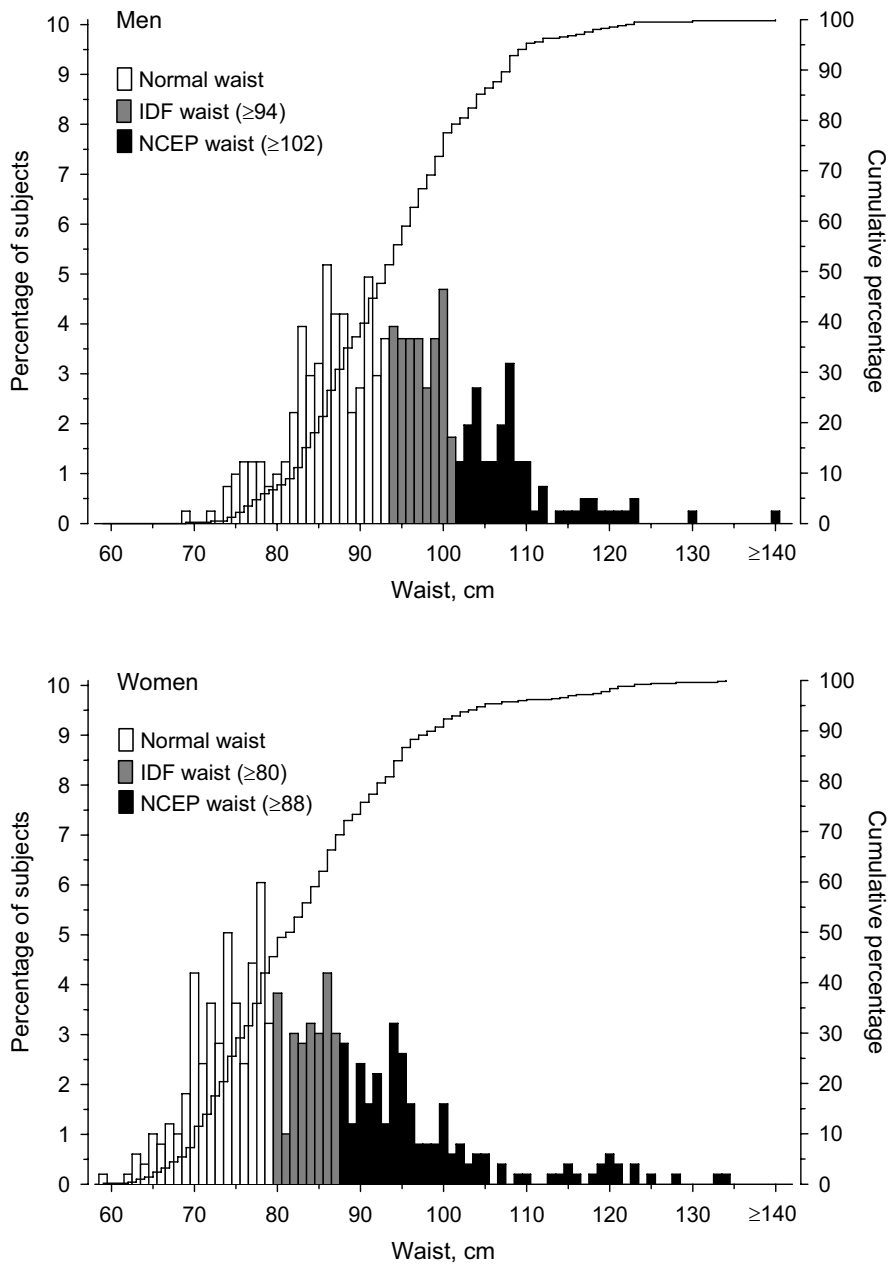
### 5.1. Characteristics of the study subjects

The basic demographic, clinical and biochemical characteristics of study subjects regarding the components of the MetS and adiponectin and pro-inflammatory markers (hs-CRP and IL-1 Ra) are shown in Table 3 and Figure 5.

**Table 3.** Demographic, clinical and biochemical characteristics of the whole study population.

Variables	Men (N=411) Mean (SD)	Women (N=512) Mean (SD)	P-value
<i>Demographic</i>			
Age, years	46 (6)	46 (6)	
Body mass index, kg/m <sup>2</sup>	26.7 (3.8)	26.3 (4.9)	0.15
Waist, cm	93.8 (10.6)	83.3 (12.2)	<0.001
<i>Clinical</i>			
Blood pressure, mmHg			
Systolic	137 (17)	131 (17)	<0.001
Diastolic	84 (10)	79 (9)	<0.001
<i>Biochemical</i>			
HDL cholesterol, mmol/l	1.3 (0.3)	1.5 (0.3)	<0.001
Triglycerides, mmol/l	1.7 (1.3)	1.2 (0.6)	<0.001
Fp-glucose, mmol/l	5.9 (0.6)	5.6 (0.5)	<0.001
Fp-insulin, mU/l	10.7 (5.9)	9.8 (6.5)	0.033
hs-CRP, pg/ml	1.3 (1.5)	1.5 (1.7)	0.035
IL-1 Ra, pg/ml	172 (131)	192 (167)	0.16
Adiponectin, µg/ml	4.9 (2.7)	7.9 (4.4)	<0.001

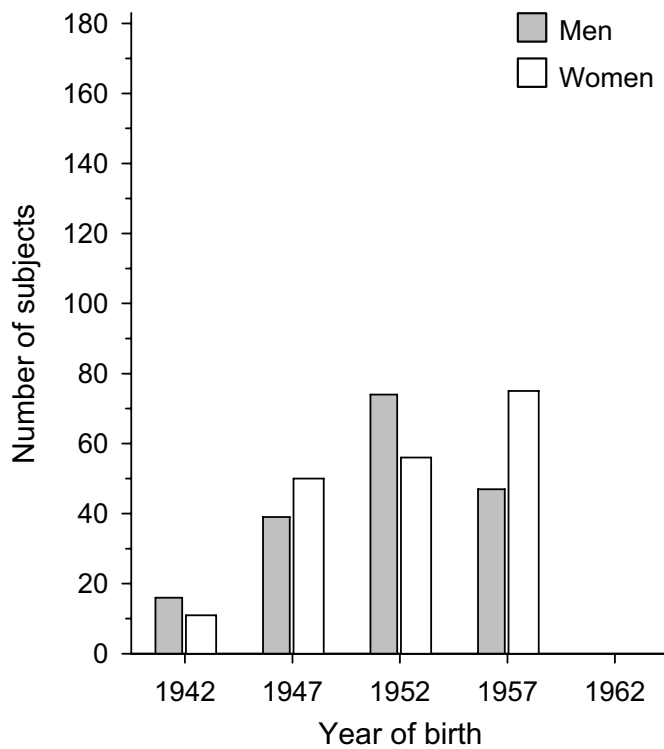
HDL = high density lipoprotein, hs-CRP = high-sensitivity C-Reactive Protein  
IL-1 Ra= Interleukin-1 Receptor antagonist.



**Figure 5.** Waist circumference is shown for the entire study population. According to the IDF definition of the MetS, waist circumference was normal in 51% of men and 45% of women. According to the NCEP criteria, waist circumference was normal in 79% of men and 69% of women.

## 5.2. Associations of adiponectin and pro-inflammatory markers with relative change in BMI between childhood and adulthood (Study I)

The study population comprised all those for whom information on weight and height at the age of 7 years (the start of primary school) was available from their local health registries. The levels of cytokines were lower in men than in women (Table 4). These data were available for 368 subjects, who did not differ in their clinical characteristics and laboratory values from subjects lacking weight and height data at the age of 7 years. The age group born in 1962 was not included in this study (Figure 6). The relative change in BMI was calculated as adult BMI divided by childhood BMI.



**Figure 6.** The population (N=368) in Study I by age group and gender.



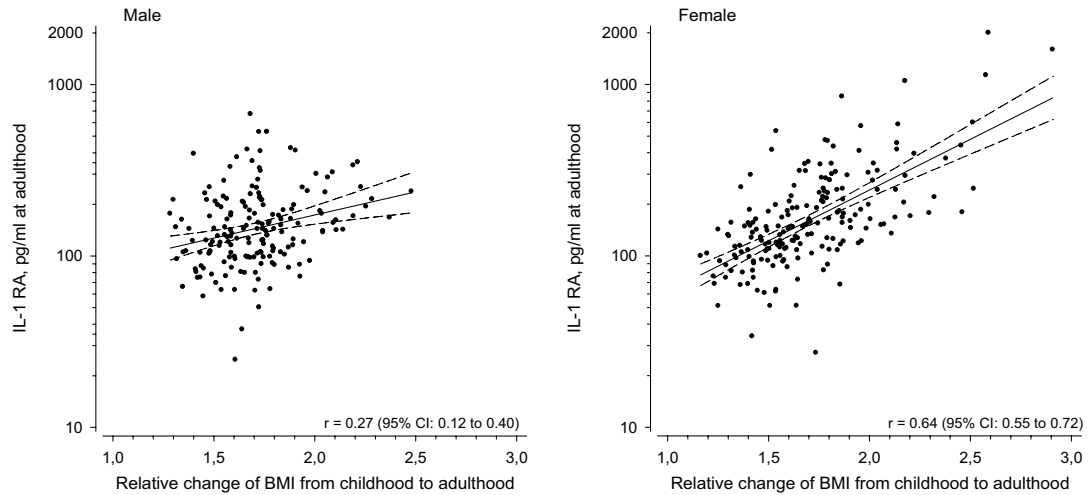
**Table 4.** Demographic data in childhood and adulthood and clinical and biochemical characteristics of the subjects in Study I in adulthood.

Characteristics	Men N=176 Mean (SD)	Women N=192 Mean (SD)	P-value
<i>Demographic</i>			
At 7 years			
Height, cm	122 (5)	121 (5)	NS
Weight, kg	23 (4)	23 (4)	0.22
Body mass index, kg/m <sup>2</sup>	15.4 (1.3)	15.4 (1.7)	0.91
Adulthood			
Age, years	46 (4)	46 (5)	0.11
Height, cm	177 (6)	163 (6)	<0.001
Weight, kg	82 (12)	70 (13)	<0.001
Body mass index, kg/m <sup>2</sup>	26.3 (3.4)	26.2 (4.9)	0.81
<i>Clinical</i>			
Blood pressure, mmHg			
Systolic	135 (16)	130 (17)	0.002
Diastolic	83 (10)	79 (9)	<0.001
<i>Biochemical</i>			
Total cholesterol, mmol/l	5.9 (1.0)	5.6 (1.0)	0.009
HDL cholesterol, mmol/l	1.3 (0.3)	1.5 (0.3)	<0.001
Total triglycerides, mmol/l	1.6 (0.9)	1.3 (0.8)	0.002
Fasting plasma glucose, mmol/l	6.0 (1.0)	5.7 (1.0)	<0.001
Adiponectin, µg/ml	4.6(2.2)	8.0(4.8)	<0.001
hs-CRP, pg/ml	1.4(2.4)	2.0(2.8)	0.03
IL-1 Ra, pg/ml	166(97)	209(223)	0.02

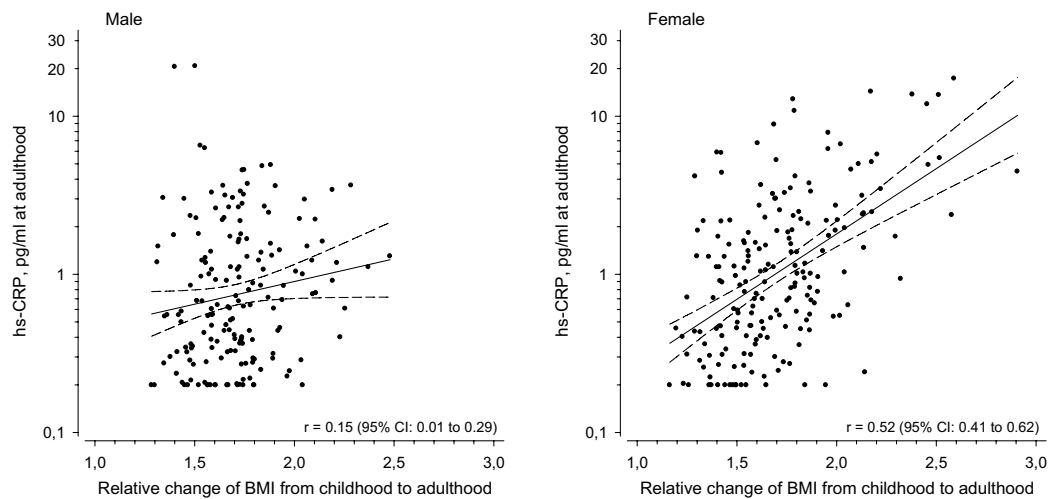
NS = non-significant; HDL = high-density lipoprotein.

Figures 7-8 show the significant positive relationship between the inflammatory markers (hs-CRP and IL-1 Ra) at adulthood and the relative change in BMI from childhood to adulthood. Figure 9 shows the significant inverse relationship between adiponectin measured at adulthood and the relative change in BMI from childhood to adulthood. In both sexes the strongest

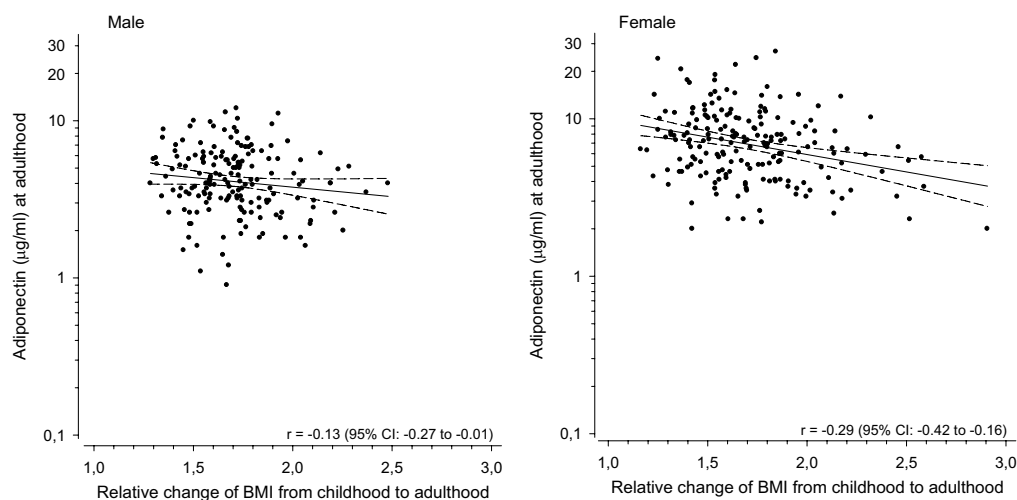
correlation was found between the relative change in BMI from childhood to adulthood and IL-1 Ra: for women ( $r = 0.64$  (95% CI: 0.55 to 0.72)) and for men ( $r = 0.27$  (95% CI: 0.12 to 0.40)).



**Figure 7.** Relationship between IL-1 Ra levels at adulthood and the relative change of BMI between childhood and adulthood in males and females.



**Figure 8.** Relationship between hs-CRP levels at adulthood and the relative change of BMI between childhood and adulthood in males and females.



**Figure 9.** Relationship between adiponectin levels at adulthood and the relative change of BMI between childhood and adulthood in males and females.

### 5.3. Associations of adiponectin, C-reactive protein and interleukin-1 receptor antagonist with insulin sensitivity in a population-based cohort (Study II)

Insulin sensitivity in this study was measured by QUICKI-index. About 50% of subjects were physically active, and 34% of men and 22 % of women were current smokers. The median adiponectin concentration was lower in men than in women (4.2 vs. 6.9 µg/ml,  $p < 0.001$ ). Levels of hs-CRP were higher in women than in men (0.9 vs. 0.7 pg/ml,  $p = 0.040$ ). There was no statistically significant difference between the sexes in the levels of IL-1 Ra (men vs. women, 141 vs. 149 pg/ml,  $p = 0.071$ ). The QUICKI index was higher in women than in men (0.340 vs. 0.334,  $p < 0.001$ ) (Table 5).

The Pearson correlation between QUICKI and adiponectin level was 0.334 (95% CI, 0.275 to 0.392) and partial correlation adjusted for gender, age, BMI, smoking status and physical activity was 0.247 (95% CI, 0.185 to 0.308). IL-1 Ra and CRP correlated inversely with QUICKI. The correlation between QUICKI and IL-1 Ra, adjusted for gender, BMI, smoking status, physical activity and age was statistically significant (Table 6).

**Table 5.** Clinical and laboratory characteristics of the Study II population.

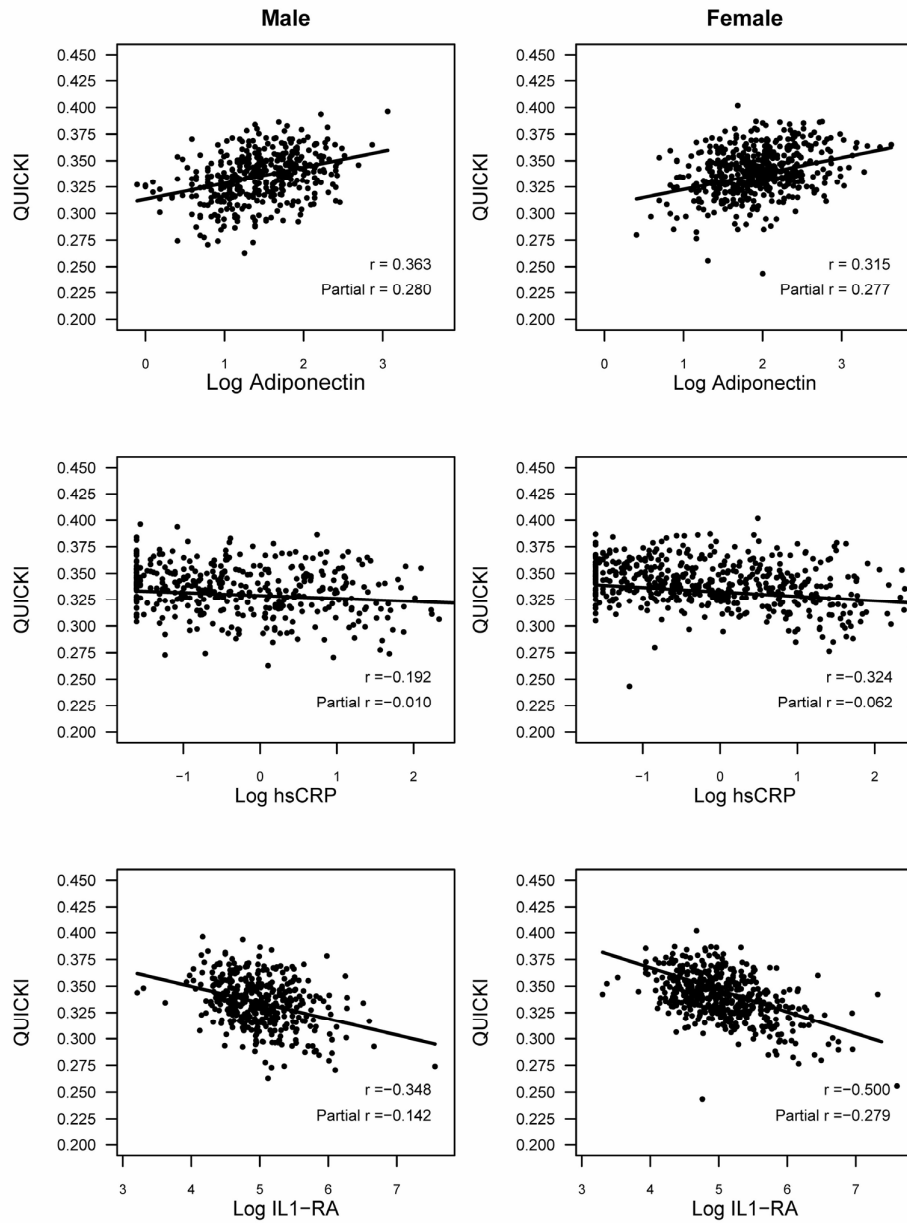
Variables	Males	Females	P-value
	N=400	N=507	
Age, years	47 ± 6	46 ± 6	0.58
Current smokers (%)	34	22	<0.001
Physically active (%)	46	54	0.43
BMI (kg/m <sup>2</sup> )	26.6 ± 3.7	26.3 ± 5.0	0.27
Waist (cm)	93.6 ± 10.2	83.4 ± 12.3	<0.001
Quicki	0.334 ± 0.023	0.340 ± 0.022	<0.001
Fp-glucose (mmol/l)	6.0 ± 0.9	5.7 ± 0.6	<0.001
Fp-insulin (mU/l)	8.9 (7.0-12.3)	8.7(6.8-11.1)	0.11
Adiponectin (ug/ml)	4.2(3.0-6.0)	6.9(4.8-9.7)	<0.001
hs-CRP (pg/ml)	0.7(0.3-1.8)	0.9(0.4-2.2)	0.040
IL1–Ra (pg/ml)	141(104-202)	149(105-219)	0.071

Data are mean ± SD, medians (interquartile ranges), or percent. BMI=body mass index, hs-CRP = high-sensitivity C-reactive protein; IL-1 Ra= interleukin 1 receptor antagonist,

**Table 6.** Association of insulin sensitivity, measured by QUICKI, with adiponectin, high-sensitivity C-reactive protein (hs-CRP) and interleukin 1 receptor antagonist (IL-1 Ra) levels.

Inflammation cytokines	Quantitative insulin sensitivity check index (QUICKI)	
	Pearson correlation	Partial correlation <sup>§</sup>
	r (95 % CI)	r (95 % CI)
Adiponectin <sup>§</sup> (ug/ml)	0.334 ( 0.275 to 0.392)	0.247 ( 0.185 to 0.308)
hsCRP <sup>§</sup> (pg/ml)	-0.241 (-0.302 to -0.178)	-0.004 (-0.070 to 0.062)
IL1–Ra <sup>§</sup> (pg/ml)	-0.385 (-0.440 to -0.328)	-0.178 (-0.240 to -0.113)

<sup>§</sup> Data logarithmically transformed. <sup>§</sup> Adjusted for sex, BMI, smoking status, physical activity, and age.



**Figure 10.** A scatter-plot between insulin sensitivity, measured by QUICKI, and log-transformed adiponectin, hs-CRP), and IL-1 Ra levels. Pearson and partial Pearson correlation coefficients (adjusted for age, gender, body mass index, smoking status and physical activity) and regression line are shown.

The correlation between QUICKI and adiponectin, CRP and IL-1 Ra and the partial correlation (adjusted for age, gender, BMI, smoking status and physical activity) are shown in Figure 10 for both sexes. The gender difference was statistically significant only for IL-1 Ra ( $p=0.017$ ). In women the correlation of QUICKI with IL-1 Ra was  $-0.500$  and partial correlation  $-0.279$ , and in men the corresponding correlations were  $-0.348$  and  $-0.142$ .

#### **5.4. The associations of CRP, IL-1 Ra and adiponectin with the metabolic syndrome defined by the NCEP and the IDF criteria (Study III)**

Among the 923 participants, the prevalence of the MetS according to the IDF definition was 38% in men and 34% in women. According to the NCEP criteria the corresponding numbers were 34% in men, and 27% in women. In women the agreement ( $\kappa$ ) between the IDF and NCEP criteria was 0.75 (95% CI 0.68 to 0.81) and in men 0.60 (95% CI 0.52 to 0.68).

Insulin sensitivity index measured by QUICKI was significantly higher in both men and women without the MetS than with the MetS according to both definitions (the IDF criteria:  $0.35\pm 0.18$  vs.  $0.32\pm 0.02$ ,  $p<0.001$ ; the NCEP criteria:  $0.35\pm 0.02$  vs.  $0.32\pm 0.02$ ,  $p<0.001$ ).

When the MetS was present according to both IDF and NCEP definitions, waist and triglyceride levels were significantly higher and HDL cholesterol lower in both men and women compared to those who had the MetS according to the IDF but not NCEP criteria, or the MetS according to the NCEP but not IDF criteria (Table 7).

In men the mean hs-CRP level was 1.00 pg/ml in those having the MetS only according to the NCEP definition, 1.52 pg/ml in those having the MetS only according to the IDF definition, and 1.73 pg/ml in those having the MetS according to both definitions (IDF and NCEP) ( $p=0.033$  between groups and linearity 0.020). In women the corresponding mean hs-CRP levels were 0.81, 1.45 and 2.62 pg/ml ( $p<0.001$  between the groups, linearity 0.010). The corresponding levels of adiponectin and IL-1 Ra for both sexes are also shown in Table 8.

Figure 11 shows the correlation of adiponectin, hs-CRP and IL-1 Ra with the number of components of the MetS present (0-1, 2-3, 4-5) according to the IDF and the NCEP criteria for both men and women ( $p$  for linearity  $< 0.001$  in all definitions).

**Table 7.** Demographic, clinical and biochemical characteristics of subjects with the metabolic syndrome (Study III).

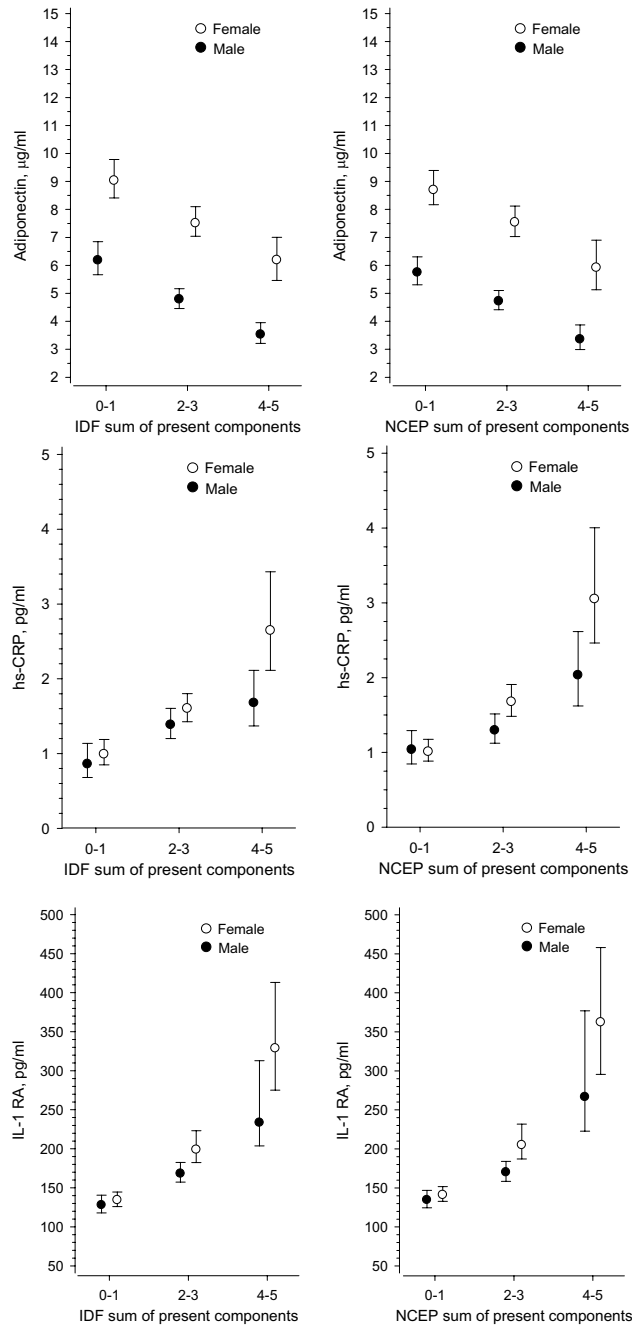
Variables	Definitions of the metabolic syndrome									
	Only NCEP				Only IDF				Both IDF and NCEP	
	Male (N=28)	Female (N=10)	Male (N=46)	Female (N=44)	Male (N=109)	Female (N=125)	Male (N=109)	Female (N=125)	Mean (SD)	Mean (SD)
<i>Demographic</i>										
Age, years	48 (7)	49 (5)	48 (6)	47 (6)	47 (6)	48 (6)	47 (6)	48 (6)	47 (6)	48 (6)
Body mass index, kg/m <sup>2</sup>	25.7 (1.9)	24.1 (1.9)	27.1 (1.7)	27.2 (2.3)	30.8 (3.4)	31.5 (5.1)	30.8 (3.4)	31.5 (5.1)	30.8 (3.4)	31.5 (5.1)
Waist, cm	89 (4)	76 (4)	97 (2)	85 (2)	106 (9)	97 (11)	106 (9)	97 (11)	106 (9)	97 (11)
<i>Clinical</i>										
Blood pressure, mmHg										
Systolic	141 (12)	134 (6)	142 (19)	134 (16)	144 (16)	141 (16)	144 (16)	141 (16)	144 (16)	141 (16)
Diastolic	87 (11)	80 (10)	87 (9)	82 (7)	89 (9)	85 (7)	89 (9)	85 (7)	89 (9)	85 (7)
<i>Biochemical</i>										
HDL cholesterol, mmol/l	1.2 (0.3)	1.2 (0.2)	1.4 (0.3)	1.5 (0.4)	1.1 (0.2)	1.3 (0.3)	1.1 (0.2)	1.3 (0.3)	1.1 (0.2)	1.3 (0.3)
Triglycerides, mmol/l	2.5 (1.5)	1.6 (0.6)	1.6 (0.9)	1.3 (0.6)	2.4 (1.8)	1.7 (0.8)	2.4 (1.8)	1.7 (0.8)	2.4 (1.8)	1.7 (0.8)
Fp-glucose, mmol/l	6.1 (0.6)	5.8 (0.4)	6.1 (0.5)	5.9 (0.6)	6.3 (0.7)	6.0 (0.6)	6.3 (0.7)	6.0 (0.6)	6.3 (0.7)	6.0 (0.6)

**Table 8.** Levels of adiponectin, interleukin 1 receptor antagonist (IL-1 Ra) and high-sensitivity C-reactive protein (hs-CRP) according to the IDF and the NCEP definitions of the metabolic syndrome and according to gender.

Variables	Definitions of metabolic syndrome			P-value †	
	Only NCEP	Only IDF	Both IDF and NCEP	Between group	Linearity
<i>Men</i>					
Number	28	46	109		
Adiponectin, µg/ml, mean (SD)	4.91 (2.54)	4.94 (3.05)	3.81 (2.10)	0.021	0.034
IL-1Ra, pg/ml, mean (SD)	140 (73)	189 (118)	228 (203)	0.032	0.018
Hs-CRP, pg/ml, mean (SD)	1.00 (0.98)	1.52 (1.40)	1.73 (1.70)	0.033	0.020
<i>Women</i>					
Number	10	44	125		
Adiponectin, µg/ml, mean (SD)	8.48 (5.08)	7.62 (4.18)	6.63 (3.52)	0.25	0.14
IL-1Ra, pg/ml, mean (SD)	146 (84)	222 (216)	305 (248)	0.004	0.044
hs-CRP, pg/ml, mean (SD)	0.81 (1.06)	1.45 (1.18)	2.62 (2.26)	<0.001	0.010

† Bootstrap type ANOVA.





**Figure 11.** Relationship between adiponectin, hs-CRP, IL-1 RA and the number of components of the metabolic syndrome according to the IDF and NCEP criteria (mean and 95% confidence intervals obtained by bias-corrected and accelerated bootstrapping).

### **5.5. Gender differences in CRP , IL-1 Ra and adiponectin levels in the metabolic syndrome defined by the NCEP and the IDF definitions (Study IV)**

Waist circumference, blood pressure, triglyceride and glucose levels were higher and HDL cholesterol concentrations lower in men than in women ( $p < 0.001$ ). Mean adiponectin (7.9(4.4) vs. 4.9(2.7)  $\mu\text{g/ml}$ ,  $p < 0.001$ ) and mean hs-CRP levels were higher in women than in men (1.5(1.7) vs. 1.3(1.5)  $\text{mg/L}$ ,  $p=0.035$ ), but no gender difference was found in IL-1 Ra levels. No gender difference was found in the use of drugs for treatment of hypertension and dyslipidemia. The prevalences of the MetS were the same as in Study III. Adiponectin levels were significantly lower ( $p < 0.001$ ) and hs-CRP and IL-1 Ra concentrations higher ( $p < 0.001$ ) in subjects with the MetS compared to subjects without the MetS.

In subjects with the MetS defined by the IDF criteria the gender difference in the levels of hs-CRP (higher in women) was 0.65  $\text{mg/L}$  [(95% CI, 0.23 to 1.06),  $p < 0.001$ ] and in the levels of IL-1 Ra 67  $\text{pg/ml}$  [(95% CI, 21 to 113),  $p=0.0028$ ]. Similar gender differences were observed in subjects with the MetS according to the NCEP criteria (the gender difference in hs-CRP levels was 0.92  $\text{mg/L}$  [(95% CI 0.47 to 1.41),  $p < 0.001$ ] and in IL-1 Ra levels 83  $\text{pg/ml}$  [(95% CI 32 to 135),  $p=0.0016$ ]). In subjects without the MetS, hs-CRP and IL-1 Ra levels did not differ between the sexes (Table 9).

**Table 9.** Adiponectin, interleukin 1 receptor antagonist (IL-1 Ra) and high sensitivity C-reactive protein (hs-CRP) levels according to the IDF and the NCEP definitions of the metabolic syndrome in men and women.

	Definitions of the metabolic syndrome					
	IDF			NCEP		
	Not present	Present	P-value <sup>†</sup>	Not present	Present	P-value <sup>†</sup>
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
<i>Men</i>						
No of subjects ( %)	250 (62)	155 (38)		268 (66)	137 (34)	
Adiponectin, µg/ml	5.31 (2.75)	4.15 (2.46)	<0.001	5.29 (2.83)	4.04 (2.23)	<0.001
IL-1 Ra, pg/ml	145 (72)	217 (182)	<0.001	153 (84)	210 (187)	<0.001
hs-CRP, mg/L	1.10 (1.39)	1.67 (1.62)	<0.001	1.18 (1.44)	1.57 (1.61)	0.04
<i>Women</i>						
No of subjects, %	328 (66)	169 (34)		362 (73)	135 (27)	
Adiponectin, µg/ml	8.44 (4.69)	6.89 (3.72)	<0.001	8.34 (4.63)	6.77 (3.67)	<0.001
IL-1 Ra, pg/ml	145 (77)	284 (242) <sup>1</sup>	<0.001	154 (106)	294 (243) <sup>3</sup>	<0.001
hs-CRP, mg/L	1.10 (1.26)	2.32 (2.09) <sup>2</sup>	<0.001	1.15 (1.26)	2.49 (2.24) <sup>4</sup>	<0.001

<sup>†</sup> Bootstrap type t-test with bootstrap-based multiplicity adjustments (5000 replications). 95% confidence interval obtained by bias-corrected and accelerated bootstrapping (5000 replications). Statistical differences between the sexes: <sup>1</sup> p=0.0028, <sup>2</sup> p<0.001, <sup>3</sup> p=0.0016 and <sup>4</sup> p<0.001.

## 6. DISCUSSION

### 6.1. Study population and design

The study population consisted of middle aged (mean age about 46 years) Caucasian subjects from the city of Pieksämäki, eastern Finland. Entire age groups, born in 1942, 1947, 1952, 1957 and 1962, were recruited from the local comprehensive population data records by three separate invitations. No exclusion criteria were applied. Altogether 923 of 1294 subjects (71.3%) participated in this cross-sectional study conducted in 1997-98 at Pieksämäki health center.

This is a representative population-based age-cohort study. The gender distribution did not differ between the age groups. The strength of this study is that the number of male participants (44.6%) was high compared to many other population-based studies (52). The prevalence of the MetS in the study population (IDF men 38%, women 34% and NCEP men 34% and women 27%) was about the same as in the Oulu project Elucidating the Risk of Atherosclerosis study, which is a population-based study with participants randomly selected from Finnish national population register (220). In that study population (mean age about 50 years) the prevalence of the MetS was, on the IDF definition, 37.6% and on the NCEP definition 29.8% of all study subjects.

Because of the rather low mean age of our study population, there were only few cases of diagnosed CHD (4/0.4%) and DM (15/1.6%). Of the total population 43 (4.7%) of men and 41 (4.4%) of women were on antihypertensive medication, and 14 (1.5%) and 6 (0.7%) on lipid lowering medication.

Due to the nature of the study there is no data about the missing 28.7% of the study population who did not attend despite three invitations. The missing subjects were, anyhow, evenly distributed to all age groups. It is unlikely that this will effect the study results.

### 6.2. Study methods

The adiponectin, hs-CRP and IL-1 Ra analyses were determined at the scientific laboratory of Kuopio University. To minimize the inter-assay variations all the analysis of cytokines and adiponectin were performed at the same time in 2003. Fresh plasma samples were immediately frozen to  $-20^{\circ}\text{C}$  and stored at  $-70^{\circ}\text{C}$  after transportation to the central laboratory. As far as known, the analyses are reliably done after at least 3-5 years storage in  $-70^{\circ}\text{C}$  (442). One limitation of our study may be that we measured total adiponectin and not the high molecular weight (HMW) multimer of adiponectin, which has been shown to be a better marker for the

MetS than total adiponectin (443), especially in early diagnosis of insulin resistance in metabolically obese, normal weight subjects (444).

In Studies III and IV 18 subjects (7 men and 11 women) were excluded whose hs-CRP concentrations were >10,0 mg/L in order to eliminate possible cases of acute infections and other occult diseases. In the insulin resistance study (Study II) 15 (1.6%) persons were excluded from all statistical analyses due to known diabetes and one person with CRP >30 mg/L, as treatment for diabetes and acute infections could effect our results. However, when these subjects were included in the statistical analysis, the results remained essentially the same.

A limitation of these studies is the cross-sectional design, which does not give answers regarding the exact predictive values of adiponectin and cytokines for diabetes and cardiovascular disease, which are the main future complications of the MetS.

### **6.3. Associations of cytokines and adiponectin with growth between childhood and adulthood**

The novel finding in this study is an association between the relative change in BMI from childhood to adulthood and levels of adiponectin and markers of a low-grade inflammation measured in adulthood. This might mean that decreased levels of adiponectin and elevated levels of IL-1 Ra and hs-CRP are indicators of relative BMI change. The association was particularly strong among women, but it was also present in men. This may suggest that in females the growth pattern, i.e., fat distribution, is different from that in males. The gender difference is, however, unclear, as in one study intra-abdominal fat tended to be higher in men than in women (445), while in another women and men had similar amounts of liver and intra-abdominal fat, but women had more subcutaneous fat (446). In general women have a higher percentage of body fat than men, and markers of inflammation correlate strongly with measures of adiposity (447).

The elevated levels of the low-grade inflammatory markers used (IL-1 Ra and hs-CRP) and low levels of adiponectin reflected BMI gain retrospectively. IL-1 Ra had the strongest correlations with relative weight change. The level of IL-1 Ra has been shown to be markedly and reversibly elevated in human obesity and predicted by lean body mass and insulin levels (274). IL-1 Ra has also been shown to be the most sensitive marker of cytokine response in the prediabetic state in the offspring of type 2 diabetic patients (277). Later on the levels of IL-1 Ra decrease as type 2 diabetes develops (278). This might indicate that it protects cells from being overloaded by glucose uptake in the diabetic state.

Levels of adiponectin are decreased in obesity. Inflammation and the influx of fat into the cells associated with obesity could explain this observation by inhibiting adiponectin expression

(448). These study results support the hypothesis that adiponectin may be the link between over nutrition, weight gain, insulin resistance and cardiovascular disease (126, 228). The recent finding that obesity at the age of 18 years increased lifetime risk for diabetes (449) may reflect the effect over many years of lower levels of adiponectin and higher levels of cytokines. In agreement with this notion a Swedish 6-year follow-up study of 2 821 non-diabetic healthy men demonstrated a significant correlation between positive inflammation-sensitive plasma proteins (fibrinogen, orosomucoid,  $\alpha$ 1-antitrypsin, haptoglobin and ceruloplasmin) and weight gain (450). Therefore, the inflammatory process, possibly genetically determined, could be the driving force for weight change from childhood to adulthood, and not just a marker of current obesity. However, a prospective study, with an admittedly short follow-up period (2-3 years), in Pima Indians failed to demonstrate an association between hypoadiponectinemia and weight gain (451).

A limitation of this study is cross-sectional and retrospective design. For this reason we used the relative change in BMI from childhood to adulthood. Prospective studies from childhood to adulthood are needed to verify this finding and to show whether the inflammation comes first and, if so, whether it predicts future fat mass.

It can be concluded that elevated levels of inflammatory markers IL-1Ra and hs-CRP and decreased levels of adiponectin are related to relative change in BMI from childhood to adulthood in both females and males.

#### **6.4. Associations of adiponectin, hs-CRP and IL-1 Ra with insulin sensitivity**

The finding of this population-based study (n=923) was that QUICKI correlated significantly with adiponectin and IL-1 Ra levels. However, the correlation between QUICKI and hs-CRP was not statistically significant after adjustment for age, gender, BMI, smoking status and physical activity. Therefore, it is possible that inflammation is more a consequence than a cause of obesity and insulin resistance.

This data agrees with previous results indicating that a low level of adiponectin is associated with decreased insulin sensitivity and may lead to type 2 diabetes (229). The new finding of the present study was that the negative correlation between insulin sensitivity and IL-1 Ra remained significant after adjustment for gender, BMI, smoking, age and physical activity. The members of the IL-1 cytokine superfamily, IL-1  $\alpha$  and IL-1  $\beta$ , are strong inducers of inflammation (270). Previous studies also show that insulin resistance may precede the elevation of CRP levels in the development of obesity and the metabolic syndrome. Similarly to these findings, in one study hs-CRP levels were not significantly associated with insulin sensitivity, but CRP was related to adiposity in a study of 10-to 16-year-old children (452). In contrast, in young people hypoadiponectinemia has been shown to correlate independently with insulin resistance and

visceral obesity (419). Similar strong correlations of adiponectin with insulin sensitivity and CRP with adiposity have been demonstrated in studies of adult obese and non-obese people with and without disorders of glucose metabolism (453).

In this study, the association between QUICKI and hs-CRP was similar in both sexes, but it was not as strong as the association between QUICKI and IL-1 Ra. The correlation between QUICKI and hs-CRP was not statistically significant after adjustment for confounding factors. Generally, hs-CRP is considered to be more a risk factor for coronary heart disease than for insulin resistance (248,249).

Correlations of adiponectin and CRP with QUICKI were very similar in both sexes. The strongest correlation was observed between insulin sensitivity and IL-1 Ra, and this was observed especially in women ( $r=-0.500$  Pearson and  $-0.279$  partial correlation). It is not clear why there is a gender difference between IL-1 Ra and QUICKI. This may be a consequence of differential fat distribution or hormonal differences between the sexes.

The other anti-inflammatory markers, especially IL-10, have high correlations with metabolic parameters. Previous studies have shown that IL-10 level is elevated in obese women without the metabolic syndrome, and that IL-10 level is low in women who have the metabolic syndrome or type 2 diabetes (454). A positive correlation of IL-10 level and insulin sensitivity has recently been demonstrated in young healthy individuals (455). In the study of the offspring of type 2 diabetic subjects the level of IL-1 Ra was the most sensitive marker of cytokine response in the prediabetic state (277). In that study, the level of IL-1  $\beta$  was increased in the normal glucose tolerance group, whereas it was decreased in the impaired glucose tolerance (IGT) group. Eizirik et al. (456) have shown that 10- to 100-fold excess of IL-1 Ra over IL-1  $\beta$  suffices to block the effects of IL-1  $\beta$  on pancreatic islets. In the above-mentioned study (277) the ratio of IL-1 Ra to IL-1  $\beta$  was found to be >100-fold higher in the IGT group, indicating decreased biological activity of IL-1  $\beta$ . Therefore, it is unlikely that IL-1  $\beta$  would mediate  $\beta$ -cell failure during the progression from IGT to type 2 diabetes.

All the statistical analyses in this study were adjusted for smoking status and physical activity. Smoking generates systemic inflammation, and thus proinflammatory cytokines might be elevated (457). Regular exercise more than three times a week has an anti-inflammatory effect. During exercise IL-6 is produced by muscle fibers, and it inhibits the production of proinflammatory cytokines, especially tumor necrosis factor (TNF) alpha (356).

This study showed that insulin sensitivity, measured by the QUICKI index, and its correlations with adiponectin and inflammatory markers corroborate existing evidence that a low adiponectin level and low-grade inflammation are associated with insulin resistance.

### **6.5. Hypoadiponectinemia and pro-inflammation in the metabolic syndrome**

This study shows that proinflammatory cytokines and adiponectin are associated with the MetS. Cytokine levels were higher and adiponectin levels lower in subjects who had the MetS according to both definitions (IDF, NCEP) compared to subjects who had the MetS according to only one definition. The only-IDF definition group of the MetS had higher levels of hs-CRP and IL-1Ra compared to the group with only-NCEP definition, probably due to abdominal obesity as the central criterion for the syndrome. A similar stronger relationship with the IDF compared to the NCEP definition was found in a Chinese population with hs-CRP (458). Subjects with the MetS according to only the NCEP criteria had quite normal levels of adiponectin and inflammatory markers, which may indicate that these subjects are not at such a high risk for CVD and/or diabetes. On the other hand the levels of triglycerides were higher and HDL-cholesterol tended to be lower in this NCEP-only population. In a recent study a similar risk for CVD and diabetes was observed in subjects having the MetS according to the IDF, NCEP and WHO definitions (56).

The current study also confirmed that levels of adiponectin, hs-CRP and IL-1 Ra were similarly and linearly correlated with the number of components of the MetS according to the IDF and NCEP definitions in both sexes. Similar results have been reported previously with respect to the levels of hs-CRP (254,459) and adiponectin (220,460,461) in different populations. Despite the fact that hs-CRP and adiponectin have been found to be highly correlated with the components of the MetS, it is not yet recommended that they could be added to the definition criteria (53). Hs-CRP values are different in different ethnic groups, and adiponectin is different between the sexes and also more difficult to measure than the other accepted values. In the Finnish Diabetes Prevention Study hs-CRP was the best immunological predictor for the progression from impaired glucose tolerance to overt type 2 diabetes (357). Our findings show that according to any of the definitions of the MetS, conventional cardiovascular risk factors, insulin resistance measured by QUICKI, low adiponectin and high levels proinflammatory markers cluster in the same individuals.

The prevalence of the MetS was 38 % in men and 34% in women according to the IDF definition, and 34% and 27%, respectively, according to the NCEP definition. Our results agree with previous results from Finland (220). On the other hand, the level of agreement between both definitions was better in women. In large part both definitions of the MetS identified the same high-risk individuals, but the IDF definition more reliably identified individuals with more unfavourable proinflammatory parameters than did the NCEP definition.

In this study it was possible to compare the correlations of IL-1 Ra with hs-CRP and adiponectin in the same study population. The new finding was that IL-1Ra showed a similar correlation to those of hs-CRP and adiponectin with the MetS.



It can be concluded that decreased levels of adiponectin and increased levels of hs-CRP and IL-1 Ra possibly reflect the same phenomenon, and correlate linearly with the number of components of the MetS according to both the IDF and NCEP definitions. The levels of inflammatory markers (hs-CRP and IL-1 Ra) are higher among patients with the MetS defined by only the IDF criteria compared to those defined by only the NCEP criteria.

#### **6.6. Gender differences in hs-CRP, IL-1 Ra and adiponectin levels in the metabolic syndrome**

This study showed that the levels of proinflammatory markers, hs-CRP and IL-1 Ra, were significantly higher among women with the MetS compared to men with the MetS, independently of the definition used (NCEP, IDF). In contrast, no gender difference in these markers between men and women was observed in subjects without the MetS. If it is taken into account that obesity and the MetS are strongly related, our results are in line with earlier studies showing a stronger association between obesity and hs-CRP in women than in men. This could be related to the higher percentage of body fat in women (446,462). A recent finding in Finland shows that in women the waist had increased by 2.7 cm over the past 10 years and by only 1.0 cm in men. Waist circumference increased more than BMI among women, especially unemployed women (463). The increasing waistlines may be involved in increasing T2DM numbers among women, because girls are also "intrinsically" more insulin-resistant than boys. It was found that girls had 15-25% higher concentrations of cord insulin compared with boys, independent of many confounders (464). Is this genetic or epigenetic and what is the role of hormones? Although the answers are unknown, these questions have given rise to the "sex insulin hypothesis" (465). Insulin-resistant diabetes is common in Turner syndrome (monosomy X) (466) and in Klinefelter syndrome/variants (polysomy X) (467). Hormones complicate matters further; women are more insulin resistant during their prepubertal and postmenopausal years, whereas men seem to be more insulin-resistant during their reproductive years (468).

Hypoadiponectinemia is an early marker of this process and reflects the interaction of the adiponectin gene and environmental factors related to obesity, insulin resistance and the MetS (214). Decreased synthesis of adiponectin has been assumed to lead to dysregulation of the mechanisms that inhibit the production of pro-inflammatory cytokines (448). Recent data show that the reduction in mortality rates among diabetic patients has been limited to men (427). The low-grade inflammation in women with the MetS shown in our study could be one explanation why prediabetic women tend to have a more atherogenic risk profile than men years before the diagnosis of diabetes (469). This may also explain, at least in part, why the MetS is a stronger predictor of cardiovascular disease in women (470) than in men.

It is possible to conclude that women with the MetS had higher levels of hs-CRP and IL-1 Ra compared to men. These observed gender-differences in cytokines with long lasting inflammatory process may explain why type 2 diabetes is associated with relatively higher cardiovascular mortality in women than in men.

### **6.7. Implications for clinical practice and research**

Despite recent criticism of the diagnosis of the MetS, there is no question that risk factors for CVD cluster together. So far, no common clearly accepted etiology for the various components have been found. The question is whether this ever-increasing cluster of features conceals a distinctive clinical entity and whether it helps to identify people who clearly are at increased risk for CVD and T2DM.

However, the identification of subjects, as early as possible, with a high risk for CVD and T2DM is of great importance. Tools for identification in clinical practice are easily available and they are not expensive. We have a rather well-tested lifestyle modification, which should be started as early as possible among patients with the MetS. The prevention of childhood obesity epidemics now is of great importance for the near future. In the search for a common etiology of the MetS, T2DM and CVD ("common soil hypothesis") the new findings about the active role of adipose tissue via adipocytokines and chronic inflammation are emerging as at the core of this problem. These studies showed in a population-based level that decreased levels of adiponectin and increased levels of markers of chronic inflammation (hs-CRP and IL-1 Ra) correlate linearly with the number of components defined by the IDF or the NCEP criteria. What is the direction of causality? There is experimental evidence that inflammatory mediators alone can trigger insulin resistance in cells, experimental models and humans in the absence of other triggering factors, such as obesity (57). This suggests that inflammation is proximal to metabolic deterioration and may lead to diabetes. This question cannot, however, be answered in this study design, but the prevention of at least a relative change in BMI to higher levels during growth is desirable. The results tend support the view that when an obese child reduces his or her relative weight to become a non-obese adult, this may protect against the MetS and its complications (417).

Diabetes as a potentially preventable complication of the MetS is relatively more dangerous for women than for men. The recent data show that the reduction in mortality rates among diabetic patients has been limited to men. Significantly higher levels of cytokines among women with MetS compared to men found in this study could be one explanation why prediabetic women have a more atherogenic risk profile than men years before the diagnosis of diabetes. In contrast, no gender difference in the cytokines between men and women were observed in subjects without the MetS. This long-lasting inflammatory stress may in part explain why type 2

diabetes is associated with relatively higher cardiovascular mortality in women and thus lifestyle modifications should be started very early among women with the MetS. This large, partly unexplained excess risk for CVD in women with type 2 diabetes needs further prospective research.

For everyday clinical purposes the measurement of IL-1 Ra and adiponectin are not at present recommended despite the fact that they are good markers for the MetS. Maybe the use of hs-CRP as CHD risk estimate is the most useful among subjects at intermediate risk (471, 472). A better understanding of the genes involved in the interplay between the environment and the human body in terms of increased waist size and cytokines will help to identify the right candidates for preventive therapies, maybe the use of IL-1 Ra for prevention of T2DM is possible. Adiponectin replacement therapy or the enhancing adiponectin action through modulation of expression and/or function of adiponectin receptors may be a novel and promising therapeutic strategy for the Mets and T2DM in the future (214).

## 7. SUMMARY

This study was done to evaluate the associations and meanings of adiponectin and low-grade inflammation (measured by hs-CRP and IL-1 Ra) with the MetS in a community-based age-cohorts. Five different whole age groups with the mean age of 46 years living in the same geographical area were included. These cross-sectional studies were done during 1997-1998 and 923 of 1 294 (71.3%) individuals participated. The prevalence of the MetS according to the IDF definition was in men 38% and in women 34%. The corresponding figures according to the NCEP definition were 34% and 27%.

The study showed an association between the relative change in BMI from childhood to adulthood and the levels of adiponectin and markers of a low-grade inflammation. This suggests that low levels of adiponectin and elevated levels of IL-1 Ra and hs-CRP could be indicators of relative weight gain. The association was particularly strong among women. IL-1 Ra had the strongest correlations with relative BMI change. Inflammation associated with obesity could explain this observation by inhibiting adiponectin expression. These results support the hypothesis that adiponectin may be the link between overnutrition, weight gain, insulin resistance and cardiovascular disease.

Insulin sensitivity correlated significantly with adiponectin and IL-1 Ra levels, independently of confounding factors. Insulin sensitivity did not correlate with hs-CRP after adjustment for age, gender, BMI, smoking status and physical activity. The correlations of adiponectin and CRP with insulin sensitivity were similar in both sexes. The strongest correlation was observed between insulin sensitivity and IL-1 Ra, and this was observed especially in women.

Levels of adiponectin, hs-CRP and IL- 1 Ra were similarly and linearly correlated with number of the components of the MetS according to the IDF and NCEP definitions in both sexes. These cytokines and adiponectin are likely to be central components of the MetS. Cytokine levels were higher and adiponectin levels lower in subjects who had the MetS on both definitions (IDF, NCEP) compared to subjects who had the MetS on only one definition. Both definitions of the MetS largely identified the same high risk individuals.

The levels of proinflammatory markers, hs-CRP and IL-1 Ra, were significantly higher among women with the MetS compared to men with the MetS, independently of the definition used (NCEP, IDF). No gender difference in these markers was observed in subjects without the MetS. The relatively greater decrease in adiponectin level in women with the MetS could induce more pro-inflammation in women than in men. The low-grade inflammation in women with the MetS could explain why prediabetic women tend to have a more atherogenic risk profile than men years before the diagnosis of diabetes.

In conclusion, the MetS is associated with hypoadiponectinemia and low-grade inflammation. An association in this study was found between relative change in BMI between childhood and adulthood, insulin sensitivity, the number of components of the MetS and especially in female gender.

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## 9. ORIGINAL PUBLICATIONS I-IV

I Saltevo J, Vanhala M, Kautiainen H, Laakso M. Levels of Adiponectin, C-reactive Protein and Interleukin-1 Receptor Antagonist are associated with the relative change in body mass index between childhood and adulthood. *Diabetes and Vascular Disease Research*. 2007;4:328-31

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III Saltevo J, Vanhala M, Kautiainen H, Kumpusalo E, Laakso M. Association of C-reactive Protein, Interleukin-1 Receptor Antagonist and Adiponectin with the Metabolic Syndrome. *The Mediators of Inflammation*. 2007; Article ID 93573:1-8

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## Kuopio University Publications D. Medical Sciences

- D 411. Skommer, Joanna.** Novel approaches to induce apoptosis in human follicular lymphoma cell lines - precinical assessment.  
2007. 80 p. Acad. Diss.
- D 412. Kempainen, Kaarina.** Early maternal sensitivity: continuity and related risk factors.  
2007. 80 p. Acad. Diss.
- D 413. Sahlman, Janne.** Chondrodysplasias Caused by Defects in the Col2a1 Gene.  
2007. 86 p. Acad. Diss.
- D 414. Pitkänen, Leena.** Retinal pigment epithelium as a barrier in drug permeation and as a target of non-viral gene delivery.  
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- D 415. Suhonen, Kirsi.** Prognostic Role of Cell Adhesion Factors and Angiogenesis in Epithelial Ovarian Cancer.  
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- D 416. Sillanpää, Sari.** Prognostic significance of cell-matrix interactions in epithelial ovarian cancer.  
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- D 417. Hartikainen, Jaana.** Genetic predisposition to breast and ovarian cancer in Eastern Finnish population.  
2007. 188 p. Acad. Diss.
- D 418. Udd, Marianne.** The treatment and risk factors of peptic ulcer bleeding.  
2007. 88 p. Acad. Diss.
- D 419. Qu, Chengjuan.** Articular cartilage proteoglycan biosynthesis and sulfation.  
2007. 78 p. Acad. Diss.
- D 420. Stark, Harri.** Inflammatory airway responses caused by *Aspergillus fumigatus* and PVC challenges.  
2007. 102 p. Acad. Diss.
- D 421. Hintikka, Ulla.** Changes in adolescents' cognitive and psychosocial functioning and self-image during psychiatric inpatient treatment.  
2007. 103 p. Acad. Diss.
- D 422. Putkonen, Anu.** Mental disorders and violent crime: epidemiological study on factors associated with severe violent offending.  
2007. 88 p. Acad. Diss.
- D 423. Karinen, Hannele.** Genetics and family aspects of coeliac disease.  
2008. 110 p. Acad. Diss.
- D 424. Sutinen, Päivi.** Pathophysiological effects of vibration with inner ear as a model organ.  
2008. 94 p. Acad. Diss.
- D 425. Koskela, Tuomas-Heikki.** Terveyspalveluiden pitkäaikaisen suurkäyttäjän ennustekijät.  
2008. 253 p. Acad. Diss.
- D 426. Sutela, Anna.** Add-on stereotactic core needle breast biopsy: diagnosis of non-palpable breast lesions detected on mammography or galactography.  
2008. 127 p. Acad. Diss.
- D 427. Saarelainen, Soili.** Immune Response to Lipocalin Allergens: IgE and T-cell Cross-Reactivity.  
2008. 127 p. Acad. Diss.