Searching for the Phenotype of Female Metabolic Syndrome in Relation to Polycystic Ovary Syndrome and for the Genetic Background of Polycystic Ovary Syndrome

Doctoral dissertation

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ABSTRACT
Metabolic syndrome (MBS), i.e. the clustering of many type 2 diabetes and cardiovascular risk factors, is today in the scientific limelight because of the global epidemic of obesity and its accompanying burdens. However, little is known about the gynecological and endocrinological profile of women with MBS and of the role of polycystic ovary syndrome (PCOS) in MBS. PCOS is a heterogeneous clinical entity, characterized by signs and symptoms of hyperandrogenism and anovulatory disorders often associated with infertility, obesity and insulin resistance. The underlying pathogenesis has remained unknown.

The a priori hypothesis was that PCOS would be concentrated in women with MBS because of the overlapping of several long-term health disease risk factors. The first aim was to clarify this connection at the population level. The second goal of this study was to evaluate the genetic heterogeneity of PCOS with genetic association studies, namely the peroxisome proliferator-activated receptor-γ (PPARγ) in which polymorphism is associated with type 2 diabetes; microsomal epoxide hydrolase (EPHX) which has a role in female reproduction abnormalities; apolipoprotein E (apoE) as a genetic marker for dyslipidemia and atherosclerosis and tumor necrosis factor α (TNF-α) which has a role in insulin resistance and obesity.

Altogether 243 Caucasian women living in a defined area and aged 44 to 54 years were screened in two different ways. At the beginning of the study MBS was defined by a self-constructed model from family history of type 2 diabetes, BMI, WHR, hypertension, hypertriglyceridemia, low HDL-cholesterol, abnormal glucose metabolism, and hyperandrogenemia. Three criteria were necessary for MBS diagnosis. Later MBS was defined according to the criteria proposed by the National Cholesterol Education Program (NCEP). The control group consisted of 62 overweight women without central obesity or MBS and 53 healthy lean women. In the candidate gene association studies, the study population consisted of 58-112 PCOS women and 91 healthy controls.

The prevalence of MBS appeared to be 19%, increasing with age. PCOS defined as anovulatory periods, signs of hyperandrogenism and typical ovaries in ultrasound were found at a similar frequency (13%-15%) in women with MBS, simplex obesity and the lean controls. There were no differences between the groups regarding parity, infertility problems or obstetric outcome. However, with aging, oligomenorrhea appeared to be more common in the MBS women. The clinical features of hyperandrogenism and also the cutaneous markers of insulin resistance, such as androgenic alopecia and acanthosis nigricans were detected only in the MBS group. A markedly lower insulin sensitivity index, lower SHBG level and higher free androgen index (FAI) were detected in MBS women. Abdominal obesity and increased diastolic blood pressure were significantly associated with high FAI.

Our genetic studies support a role for PPARγ gene polymorphism in the pathogenesis of PCOS, with the presence of the Ala isoform being protective against the development of PCOS. This confirms findings that the action and signaling of insulin are important factors in the pathogenesis of PCOS. In addition, our study provides evidence to support the heterogeneity of the pathogenesis of PCOS, since the use of two intragenic single nucleotide polymorphisms in the gene encoding EPHX jointly in haplotype association analysis demonstrated that genetically determined low-activity haplotype C-G (Ile113-Arg139) was significantly associated with PCOS. This multipoint association may be of biological significance, since these genetic variations reduce enzyme activity, which in turn may have an impact on the reproductive system and contribute to miscarriage. On the other hand, it appeared that apoE does not play a major role in the development of dyslipidemia in the group with PCOS. In addition, polymorphism of the TNF-α gene (C857T) is unlikely to contribute to PCOS risk.

Finally, surprisingly few MBS women presented with symptoms classically associated with PCOS, which were as often detected among women without MBS. Although MBS and PCOS are clearly closely interwoven pathophysiologically, and their genetic background shares similarities with regard to insulin action and signaling, PCOS only accounts for a subgroup of a much wider problem, the metabolic syndrome.

National Library of Medicine Classification: WP 320, WD 200, QZ 50
Medical Subject Headings: cross-sectional studies; metabolic syndrome X; polycystic ovary syndrome; insulin resistance; obesity; sex hormones; hyperandrogenism; oligoamenorrhea; prospective studies; polyunsaturated chain reaction; polymorphism, peroxisomal proliferators; epoxide hydrolases; genetics, apolipoproteins E; tumor necrosis factor; Finland; female
"Liian pukun perhoseksi..."
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Mikkeli, November 2003

Seija Korkonen
ABBREVIATIONS

A   androstenedione
ACTH  adrenocorticotropic hormone
ApoE  apolipoprotein E
BMI  body mass index
Chol  cholesterol
CRP  C-reactive protein
CVD  cardiovascular disease
DHEA  dehydroepiandrosterone
DHEAS  dehydroepiandrosterone sulfate
E₁  estrone
E₂  estradiol
EPHX  epoxide hydrolase
FAI  free androgen index
FFA  free fatty acid
FSH  follicle stimulating hormone
GH  growth hormone
GLUT  glucose transporter protein
GnRH  gonadotropin releasing hormone
HDL  high density lipoprotein
HOMA  homeostasis model assessment
I/G  impaired fasting glycemia
IGT  impaired glucose tolerance
IRS  insulin receptor substrate
LDL  low-density lipoprotein
LH  luteinizing hormone
MBS  metabolic syndrome
NCEP  National Cholesterol Education Program
NGT  normal glucose tolerance
NO  nitric oxide
OGTT  oral glucose tolerance test
OMIM  Online Mendelian Inheritance in Man
PCO  polycystic ovaries
PCOS  polycystic ovary syndrome
PCR  polymerase chain reaction
PAI-1  plasminogen activating factor 1
PPARγ  peroxisome proliferator-activated receptor gamma
QUICKI  quantitative insulin sensitivity check index
SHBG  sex hormone-binding globulin
SNP  single nucleotide polymorphism
T  testosterone
TNF-α  tumor necrosis factor alpha
Trigly  triglyceride
WHO  World Health Organization
WHR  waist-hip ratio
LIST OF ORIGINAL PUBLICATIONS

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


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ORIGINAL PUBLICATIONS
1 INTRODUCTION

Studies in several populations have shown clustering of hyperinsulinemia, glucose intolerance, mild dyslipidemia, hypertension and obesity; this is commonly referred to as “metabolic syndrome” (MBS). This syndrome is of great importance because of its association with the subsequent development of type 2 diabetes and cardiovascular disease (CVD). It seems that the prevalence of MBS has increased, while sedentary lifestyle and poor physical activity are contributing factors to overweight and its accompanying burdens. Although the pathogenesis of MBS has multiple origins and is still very unclear, environmental and genetic factors contribute to both the development of overweight and the propensity to insulin resistance and other manifestations of the MBS. The recently published definitions of the World Health Organization (WHO) (Alberti and Zimmet 1998) and the National Cholesterol Education Program (NCEP) (NCEP, 2001) have clarified clinical and epidemiological research into MBS.

Polycystic ovary syndrome (PCOS) is a heterogenic and commonly diagnosed female endocrinopathy, particularly in younger women. The true incidence of the syndrome has not been established accurately due to the failure to agree on a single definition. However, anovulatory periods and hyperandrogenism are known to be typical features of PCOS. Although this symptom complex is not a new phenomenon, its underlying pathophysiology is uncertain. The recognition of a relationship between hyperandrogenism and insulin metabolism dates back to 1921, but new findings in the 1980s established the important components of insulin resistance and hyperinsulinemia in the syndrome (Burghen 1980; Barbieri 1983; Chang 1983). This information has sparked new interest in PCOS and the similarities to MBS have aroused concern about long-term health consequences of PCOS.

Although the connection of insulin resistance and PCOS has been shown beyond doubt, no studies have assessed this association the other way around; that is the frequency of gynecologic problems and endocrine profile in females with evident MBS and this was the first aim of this study. Although MBS and PCOS are two different overlapping entities, they are clearly closely interwoven pathophysiologically. The Finnish population is considered to be a genetic isolate, and therefore it is ideal for
genetic association studies (Peltonen et al. 1999). In PCOS, it has been possible to identify several attractive candidate genes such as the genes affecting steroid synthesis, insulin resistance and follicle maturation. The purpose of this study also was to clarify the pathogenesis of PCOS via association analysis to evaluate candidate genes modifying lipid metabolism, insulin resistance and signaling and protein stability.
2 REVIEW OF LITERATURE

2.1 Metabolic syndrome (MBS)

2.1.1 General aspects

The metabolic syndrome is a cluster of lipid and non-lipid cardiovascular disease risk factors (Zimmet 1999) considered as the first step towards type 2 diabetes. Alone, each component in the cluster carries a cardiovascular risk, but, in combination, they become more powerful (Kaplan 1989).

About 15 years ago, the concept of syndrome X was introduced by Reaven to explain already earlier described clustering of cardiovascular risk factors: this syndrome combined insulin resistance, dyslipidemia and hypertension (Reaven 1988). He proposed that insulin resistance was at the core of a syndrome characterized by a clustering of factors associated with increased cardiovascular disease (CVD) risk. Before that, three population studies – the Helsinki Policemen Study (Pyörälä 1972), Paris Prospective Study (Ducimetiere et al. 1980) and Busselton Study (Welborn and Wearne 1979) had demonstrated that high fasting or 2-h plasma insulin levels were independent predictors of coronary heart disease risk in non diabetic subjects. In the same line of thought, the notion put forward as early as 1947 by Vague suggested that regional distribution of adipose tissue seemed to be an important indicator of metabolic and cardiovascular alterations, has been confirmed in many prospective studies (Larsson et al. 1984; Lapidus et al. 1984; Folsom et al. 2000). The complex nature of the MBS poses considerable methodological challenges to researchers in the field and the syndrome has also been given several other names, including the deadly quartet (Kaplan 1989), the insulin resistance syndrome (Haffner et al. 1992), the civilization syndrome (Björntorp 1993), small-baby syndrome (Barker et al. 1993). A wide variety of ethnic groups have been found to have such clustering (Zinter 1999). Phenotypic expression varies in diverse ethnic groups (Young et al. 2002).

Due to the close linkage of these metabolic features with the abdominal body fat distribution pattern and its biological mechanism, the more collective term “metabolic
syndrome” was subsequently introduced. This name was chosen primarily because it was not considered established that insulin resistance was the root cause of all the components of the syndrome (Alberti and Zimmet 1998). The concept that insulin resistance would be a single factor behind this syndrome has sparked much controversy and recent studies of endothelial dysfunction and lipodystrophies suggest that insulin resistance may simply be a marker for these pathogenetic disorders.

To unify the field of research, the World Health Organization (WHO) (Alberti and Zimmet 1998) proposed in 1998 and later the National Education Program Expert Panel (NCEP, 2001) also presented a distinct definition for the MBS (Table 1). The latter is more suitable for clinical use. The working definition of WHO has not been accepted without criticism. Microalbuminuria, a marker of endothelial dysfunction, which predicts proteinuria, nephropathia and mortality to CVD (Stehouwer et al. 1992), was originally proposed by the WHO as a core component of the MBS. However, microalbuminuria in non diabetic individuals is uncommon, and NCEP did not include microalbuminuria in their definition. The appropriate measure of abdominal obesity and whether it should be included to the diagnosis are also in dispute. Reaven did not discuss obesity per se as a part of this constellation. WHO defined abdominal obesity as waist–hip ratio (WHR) or body mass index (BMI) and WHR may carry information about disease endpoints independently of waist girth or BMI in the Iowa Women’s Health Study (Folsom et al. 2000). On the other hand, waist circumference correlates well with visceral fat deposits as measured by computerized tomography (CT) (Pouliot et al. 1994). Most novel recommendation for non-diabetic individuals of American Association of Clinical Endocrinologists (Bloomgarden 2003) do not include obesity among the criteria, but have viewed obesity assessed by BMI and waist circumference as a physiological variable that increases insulin resistance. Furthermore, the euglycemic hyperinsulinemic clamp is not practical for epidemiologic research. The European Group for the Study of Insulin Resistance (EGIR) recommended the use of fasting insulin levels (upper quartile of the non-diabetic population) or impaired fasting glycemia (IFG) to estimate insulin resistance (Balkau and Charles 1999). The EGIR proposed also lower cut offs for hypertension (≥ 140/90 mmHg) that are in accordance with current WHO-International Society of Hypertension (WHO-ISH) and the Sixth
Joint National Committee recommendations (Joint National Committee 1997, WHO-ISH 1999).

**Table 1. Definitions for MBS of WHO and NCEP**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Presence of diabetes or impaired glucose tolerance and/or insulin resistance and, in</td>
<td>At least three of the following:</td>
</tr>
<tr>
<td>addition, presence at least two of the following:</td>
<td>- abdominal obesity (waist circumference &gt; 88 cm women, &gt; 102 cm men)</td>
</tr>
<tr>
<td>- abdominal obesity (WHR &gt; 0.85 women, &gt; 0.90 men, and/or BMI &gt; 30 kg/m²)</td>
<td>- Blood pressure systolic ≥ 130 mmHg</td>
</tr>
<tr>
<td>- Serum triglycerides ≥ 1.70 mmol/l</td>
<td>and/or diastolic ≥ 85 mmHg</td>
</tr>
<tr>
<td>- HDL-cholesterol (&lt; 1.0 mmol/l women; &lt; 0.9 mmol/l men)</td>
<td>- Serum triglycerides ≥ 150 mg/dL (1.70 mmol/l)</td>
</tr>
<tr>
<td>- Hypertension (≥ 140/90 mmHg or medication)</td>
<td>- HDL-cholesterol &lt; 50 mg/dl (1.30 mmol/l)</td>
</tr>
<tr>
<td>- Microalbuminuria</td>
<td>women and &lt; 40 mg/dl (1.0 mmol/l) men</td>
</tr>
<tr>
<td></td>
<td>- Fasting plasma glucose ≥6.1 mmol/l</td>
</tr>
</tbody>
</table>

In a population-based cohort of middle-aged Finnish men (Laaksonen et al. 2002) the WHO and NCEP definitions appeared to be valid, identifying those with an increased likelihood of developing diabetes during the follow-up.

Partly due to the lack of generally accepted criteria for the definition of the metabolic syndrome, the prevalence has varied markedly (Table 2).

In the report of Ford et al. (2002), Mexican-Americans had the highest age-adjusted prevalence (31.9 %) as compared to white (23.8 %) and to African-Americans (21.6 %). Asian populations are known for their strong susceptibility to suffer type 2 diabetes, especially when migrating to a "westernized" environment like the Indian population in London (Mather et al. 1998) or the Japanese immigrants in America (Fujiimoto et al. 2000).

In the Botnia study (Isomaa et al. 2001), the risk for coronary heart disease and stroke was increased threefold and cardiovascular and overall mortality was 1.8 fold in 35- to 70-year-old persons with a family history of type 2 diabetes who had the MBS defined by the WHO. However, cardiovascular disease and diabetes were present already at baseline in one third of the cohort.
Table 2. Prevalence of MBS with different diagnostic methods

<table>
<thead>
<tr>
<th>Study</th>
<th>Criteria of MBS</th>
<th>Country</th>
<th>Population</th>
<th>Prevalence of MBS %</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanhala et al. 1997</td>
<td>Self constructed</td>
<td>Finland</td>
<td>841 community-based study</td>
<td>f: 8 - 20 m: 17 - 30</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Rantala et al. 1999</td>
<td>Modified WHO</td>
<td>Finland</td>
<td>600 drug-treated hypertensive men</td>
<td>c: 8.8 h: 35</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Lee et al. 2000</td>
<td>Type 2 DM, hypertension, dyslipidemia</td>
<td>China</td>
<td>Hong Kong 767 subjects 43±14 y</td>
<td>10</td>
<td>Chinese</td>
</tr>
<tr>
<td>Iinomaa et al. 2001</td>
<td>WHO</td>
<td>Finland, Sweden</td>
<td>4483 35 70 y women and men</td>
<td>NGT: f 10/m 15 IFG/IGT: f 42/m 64 type 2 DM: f 78/m 84</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Abdel-Rahim et al. 2001</td>
<td>WHO</td>
<td>Israel</td>
<td>Palestinian West Bank community. 500 rural and 492 urban 30-65 y. men and women</td>
<td>1/</td>
<td>Palestinian</td>
</tr>
<tr>
<td>Ford et al. 2002</td>
<td>NCEP</td>
<td>USA</td>
<td>8814 men and women</td>
<td>22% (age-adjusted) f 23.4 / m 24 20-29 y: 6.7 60-69 y: 43.5 70 y+: 42.0</td>
<td>Mexican-Americans, African-Americans</td>
</tr>
<tr>
<td>Laaksonen et al. 2002</td>
<td>WHO / NCEP</td>
<td>Finland</td>
<td>1005 middle-aged men</td>
<td>Baseline / 4-y follow-up WHO: 22 - 24.9 NCEP: 11.4 - 13.7</td>
<td>Caucasian</td>
</tr>
</tbody>
</table>

C = controls; f = female; m = men; h = hypertensive men; NGT = normal glucose tolerance; IFG = impaired fasting glycaemia; IGT = impaired glucose tolerance.

Cardiovascular and overall mortality was increased in middle-aged men with the MBS during the 11-year follow-up in a population-based cohort in Eastern Finland (Lakka et al. 2002). This increased mortality was independent of other risk factors such as smoking, alcohol consumption, serum LDL cholesterol level and baseline CVD and diabetes. In this study, the prevalence of the MBS ranged from 8.8 % to 14.3 %, depending on the definition.

The prevalence of overweight and obesity has increased significantly in Finland and throughout the world. The prevalence of type 2 diabetes has increased in Finland (Reunanen 2002) and CVD is likely to follow this trend. The impact of obesity-related diseases on medical care and disability is a major concern. A recent experimental animal study (Roberts et al. 2001) concluded that diet therapy could potentially reverse the abnormalities seen in the MBS. Exercise could also positively affect many of the
abnormalities found in this syndrome (Shahid and Schneider 2000). Recent evidence from the Finnish Diabetes Prevention Study (Tuomilehto et al. 2001) and Diabetes Prevention Program (Knowler et al. 2002) suggest that even modest lifestyle interventions are effective in decreasing the risk for diabetes in glucose intolerant individuals.

2.1.2 Pathogenesis of MBS

The pathogenesis of MBS is poorly understood, but environmental factors combined with sedentary lifestyle and diet as well as still largely unknown genetic factors clearly interact to produce the disorder.

Neuroendocrine factors are hypothesized to have a role in the pathogenesis of MBS (Björntorp and Rosmond 2000). Maladaptation to stress, disturbances in the adrenal-pituitary axis and aberrations in glucocorticoid metabolism have been proposed to contribute to the development of abdominal obesity, insulin resistance, in fact the entire metabolic syndrome, but evidence for these abnormalities as the primary mechanism for the pathogenesis is still circumstantial. Further, intrauterine malnutrition may predispose to chronic diseases in adulthood, such as MBS and this may be mediated by altered sensitivity of the hypothalamic-pituitary axis (Law et al. 1992). Abnormalities of the sympathetic nervous system may cause obesity and impaired glucose tolerance in rodents and humans (Nonogaki 2000).

The insulin resistance that is found in MBS is linked with the action of insulin on glucose uptake, as well as its suppression of lipolysis in adipose tissue and its vasodilatative effect, but the insulin action on growth and mitogenesis does not appear to be diminished (Lebovitz 2001). Genetic abnormalities explain a small number of rare syndromes with insulin resistance (Lebovitz 2001). The molecular mechanisms of insulin action explain some disturbances, such as the ability of increased levels of free fatty acid (FFA) to mediate a decrease in insulin-mediated glucose transport (Dresner et al. 1999); exercise to mobilize GLUT-4 to the plasma membrane independent of insulin (Lund et al. 1995); and TNFα to increase serine phosphorylation of IRS-1 which has
been shown to inhibit insulin receptor tyrosine kinase reducing the insulin signal (Peraldi and Spiegelman 1998).

Adipose tissue seems to have an important role in the development of the MBS. It has been recognized that sensitivity to insulin is related to body fat content. Clausen et al. (1996) claimed that at BMI >30 kg/m², nearly all subjects had a low insulin sensitivity index, and on the other hand, upper-body fat deposition and insulin resistance are closely associated (Björntorp 1988). The association between insulin resistance and abdominal obesity seems to be evident. The abdominal fat depot comprises of subcutaneous fat, subcutaneous fat (anterior and posterior) and intra-abdominal fat. Subcutaneous fat is the most variable of the abdominal depots (1-20 kg). Intra-abdominal fat comprises omental (0.5-3 kg), mesenteric (0.5-2 kg) and retroperitoneal perirenal (0.5-2 kg) fat (Abate 2000). Omental and mesenteric fat drain mostly to portal vein. The liberation of FFAs from the visceral depot is thought to be the link between insulin resistance and visceral obesity (Björntorp 1990). The Portal Theory holds that increased release of FFA from visceral adipose depots leads to insulin resistance through effects on the liver, but it lacks supporting in vivo evidence (Manco et al. 2000; Shulman 2000). Recently, studies with selective catherization have shown increased release of FFA from subcutaneous abdominal fat (Martin and Jensen 1991; Guo et al. 1999). These and other studies suggest a non-portal mechanism in connection between subcutaneous trunkal adipose tissue of insulin resistance (Abate et al. 1995, 1996; Goodpaster 1997; Kelley et al. 2000).

In addition, a deficiency of adipose tissue is associated with insulin resistance (Ganda 2000). The insufficient adipose mass leads to TG storage in other tissues (Robbins et al. 1982). It seems that the correct number of functional adipocytes is required for correct energy homeostasis (Kinney 2002). Both excess adipose tissue in obesity and extreme loss of adipose tissue can lead to insulin resistance and the development of diabetes (Kinney 2002).

The currently prevalent line of research has proposed new hypotheses that may explain the established links between adiposity tissue and disease (Ravussin and Smith 2002). The first is ectopic fat storage and the second is an endocrine function of adipocytes. An excess fat weight is associated with increased release of FFA from the
adipose tissue. In obesity, the fat cells are already overloaded with TG and their storage capacity is overwhelmed i.e. they fail to carry out their role of protecting other tissues from the influx of dietary fatty acids (Frayn 2001). The increased plasma FFA concentration and triglyceridermia have acute adverse effects on insulin sensitivity but also lead with time to accumulation of TG in other tissues (Frayn 2001). This steatosis causes beta cell dysfunction in the pancreas and abnormal glucose metabolism in liver and muscle. In recent studies, a strong relationship between hepatic steatosis and insulin resistance has been documented (Pagano et al. 2002). With noninvasive magnetic resonance spectroscopy (MRS), one can quantify intracellular fat in muscle or in liver (Koistinen 2002). Hepatic steatosis does not occur only in subjects with generalized or regional obesity but also in non-obese subjects (Chitturi et al. 2002) and in patients with other adipose tissue disorders such as lipodystrophies (Pagano et al. 2002). In skeletal muscle, the elevated FFAs impair glucose uptake (Manco et al. 2000). Recent studies suggest that the underlying defect may be at the level of glucose transport (Kelley and Mandarino 2000).

Furthermore, the adipocyte is an important endocrine gland releasing numerous peptides into the circulation that affect metabolism (Ailhaud 2000) (Figure 1). The list of proteins and factors secreted by the adipocyte is growing rapidly.

Instead of there being a secretory effect of large fat cells on the regulation of insulin sensitivity, it is possible that fat cells are only a manifestation (end organ response) of some other unknown pathogenic factors (Ravussin and Smith 2002). These factors (hormones, neuropeptides) may lead independently to enlarged adipocytes, insulin resistance and other traits of the MBS. Large adipocytes from obese individuals express more TNF-α than control adipocytes and TNF-α may induce insulin resistance via several potential mechanisms (Moller 2000). The observations seem to indicate that TNF-α is a paracrine mediator of insulin resistance (Moller 2000).
Figure 1. Some proteins act as endocrine hormones influencing distant tissues and others act locally promoting or inhibiting adipocyte proliferation/differentiation. Both act to modulate insulin action (modified from Ravussin and Smith 2002). IGF-1= Insulin-like growth factor-1; IGFBP= Insulin-like growth factor binding protein; TNF-α= Tumor necrosis factor-α; TGFβ= Transforming growth factor-β; FGF=Fibroblast growth factor; EGF=Epidermal growth factor; PAI-1=Plasminogen activator inhibitor-1.

2.1.3 Clinical manifestations of MBS

2.1.3.1 Insulin resistance, hyperinsulinemia, and glucose intolerance

Impaired glucose tolerance or type 2 diabetes is an important component of the MBS (Zimmet 1999). Insulin resistance and hyperinsulinemia predict type 2 diabetes in several ethnic groups, even when adjusted for other components of the MBS (Haffner 1997). In addition, in cross-sectional studies, it has been shown that persons with a family history positive for NIDDM are more insulin resistant than those with a negative family history (Eriksson et al.1989), even when their glucose tolerance is normal (Gulli et al. 1992). In such people, insulin resistance may be genetically determined (Ishikawa et al. 1998; Vauhkonen et al. 1998; Bush and Hegele 2001).
The development of type 2 diabetes is characterized by a progressive deterioration of glucose tolerance from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) to frank diabetes (Alberti and Zimmet 1998). When pancreatic hypersecretion as a result of chronic insulin resistance fails to maintain glucose homeostasis, than diabetes develops. In type 2 diabetics, the prevalence of MBS is higher than in nondiabetics (table 2). However, type 2 diabetes is a heterogenous disease (Bush and Hegele 2001) and not all type 2 diabetic patients are insulin resistant. Beta-cell dysfunction is a characteristic abnormality found in type 2 diabetes. Genetic factors may further enhance the deterioration of insulin secretion that occurs in some patients with aging (Grundy 1998).

Most methods for assessing insulin sensitivity measure the effects on glucose metabolism. The euglycemic hyperinsulinemic clamp is the gold standard (DeFronzo et al. 1979). However, it is time-and labor-intensive, and not practical for clinical use. Fasting insulin correlate with insulin clamp in non diabetic individuals \( r = 0.66 \), in subjects with IGT \( r = 0.47 \) but less well in subjects with type 2 diabetes \( r = 0.48 \) (Laakso 1993). Changes in insulin sensitivity explain about 30 % of the variance in fasting insulin concentrations. The quantitative insulin sensitivity check index (QUICKI) (Katz et al. 2000) is based on fasting insulin and glucose levels and correlated to insulin sensitivity measured with clamp with a coefficient of 0.78. Another computational index is the homeostasis model assessment (HOMA) which has a 0.60 correlation coefficient with clamp. Matsuda and DeFronzo developed an index of whole-body insulin sensitivity from the data obtained from the oral glucose tolerance test (OGTT) and this index correlates strongly \( r = 0.73 \) with clamp (Matsuda and DeFronzo 1999).

2.1.3.2 Fat accumulation

Metabolic syndrome occurs most common in obese subjects, especially in those with the abdominal type of obesity, but in some cases it can be seen in those with normal body weight (Meigs et al. 1997; Yarnell et al. 1998).
The European Group for the Study of Insulin Resistance (EGIR) concluded on the basis of over 1100 direct measures of insulin-mediated glucose disposal that about 25% obese individuals could be classified as being insulin resistant. This estimate was similar whether BMI or abdominal girth was used as the index of obesity (Ferrannini et al. 1997).

The current guidelines on obesity define overweight as a BMI of 25 - 29.9 kg/m² and obesity as a BMI of > 30 kg/m² (WHO, 2000). Despite its crudeness, BMI provides a good index of overall adiposity at the population level. Finally, the prevalence of diseases associated with insulin resistance (diabetes and coronary heart disease) increases as BMIs increase from 20 to 27 kg/m², with still further increases at higher BMIs (Ruderman et al. 1998).

The methods used to calculate percent body fat are skinfold measures and bioelectrical impedance. The most accurate methods are underwater weighing and dual-energy X-ray absorptiometry (Ilaapala et al. 2002), although these methods are not practical for most epidemiological studies.

A central distribution of body fat is thought to be a more important factor for the development of MBS than the absolute degree of obesity. This type of fat distribution is associated with a high risk CVD. CT allows one to determine the visceral intra abdominal and subcutaneous abdominal area. Magnetic resonance imaging (MRI) provides similar results without exposing the individual to ionizing radiation. Waist circumference determines the extent of abdominal obesity (visceral and subcutaneous), and correlates with visceral fat in both women and men (Pouliot et al. 1994). The WHR describes the regional distribution of adipose tissue.

It seems that waist circumference as a simple measure provides more information about absolute amount of abdominal tissue whereas WHR explains the relative accumulation of abdominal fat (Figure ?).
Figure 2. Modified from Despres et al. 2001

2.1.3.3 Dyslipidemia

The prevalent lipoprotein phenotype of the MBS includes moderate elevation of plasma triglycerides and reduced high-density lipoprotein (HDL) cholesterol levels (NCEP Panel 2001). Low HDL cholesterol levels, especially HDL₂ levels are associated with reduced lipoprotein lipase activity, which on the other side it is connected with hormonal factors in women. Considerable evidence supports a link between dyslipidemia and all of the manifestations characteristic of MBS. Both elevated triglycerides and reduced HDL-cholesterol levels carry an increased risk for CVD (Hokanson and Austin 1996; Boden 2000). The qualitative changes of the LDL particles towards small-dense LDL are also typical of MBS (Austin et al. 1990).

Genetic variation in the apoE gene influences the expression of hyperlipidemia. The human apoE gene is polymorphic with three common alleles coding for three isoforms ε2, ε3 and ε4. Types III and IV hyperlipoproteinemia in type 2 diabetes and insulin resistance have been related to the apoE ε2 allele. In hyperinsulinemic insulin resistant diabetic patients with ε2 and ε3 alleles, plasma insulin and glucose levels were positively correlated with plasma triglyceride levels, but not in patients carrying the ε4 allele (Uusitupa et al. 1996). In population genetics, the ε3 allele is most common in all human groups, but the ε4 allele remains higher in some genetically isolated populations (Corbo and Scacchi 1999), suggesting that it confers some kind of advantageous
property. Nowadays ε4 as a “thrifty” allele is viewed as a susceptibility allele for CVD and Alzheimer’s disease.

2.1.3.4 Hypertension

The relationship between insulin resistance and blood pressure is the most controversial part of the MBS. According to recent domestic recommendation, the normal systolic blood pressure is \(< 130 \text{ mmHg}\) and diastolic \(< 85 \text{ mmHg}\) and elevated levels \(> 140 \text{ mmHg/} > 90 \text{ mmHg}\) (Current Care Guidelines 2002). Modan et al. (1985) suggested that insulin concentrations are associated with hypertension independently of glucose tolerance or obesity. Although hyperinsulinemia predicts the development of hypertension (Skanfor et al. 1991), no more than approximately 50\% of essential hypertensives are insulin resistant/ hyperinsulinemic (Zavaroni et al. 1992). In a Finnish study, hyperinsulinemia was present in 45\% of hypertensive men and in 25\% of hypertensive women (Vanhala et al.1998). Ferrannini et al. (1987) showed that insulin sensitivity is lower in non-obese hypertensive than in normotensive subjects.

2.1.4.5 Associated disorders

*Hemostatic dysfunction.* Increased production of thrombotic parameters such as fibrinogen and PAI-1 have recently been suggested to be part of the MBS (Imperatore et al. 1998; Sakkinen et al. 2000). Hyperinsulinemia has been shown to stimulate liver production of these parameters (Juhan-Vague et al. 1996; Festa et al. 1999).

*Endothelial dysfunction.* In the presence of insulin resistance peripheral vasodilatation is impaired. The vasodilatative effect of insulin on vascular smooth muscle is mediated by nitric oxide (NO) production (Scherrer et al. 1994).

By reducing NO production, insulin resistance promotes endothelial dysfunction and inflammation in the vessel wall since NO attenuates the binding of inflammatory cells such as monocytes and macrophages to the vascular wall. NO also inhibits the activity of inflammatory cytokines, such as TNF-α and the production of cytokines (Abate 2000).
Subclinical inflammation is suggested to be a part of the MBS. Inflammatory parameters such as high-sensitive C-reactive protein (CRP) correlated to insulin resistance in a population at risk for type 2 diabetes (Temelkova-Kurtschiev et al. 2007). It seems that CRP increases with the number of manifestations of MBS (Fröhlich et al. 2000). High sensitive CRP seems to be a stronger predictor of cardiovascular events than LDL cholesterol in a prospective 27939 women study (Ridker et al. 2002).

*Hyperuricemia.* Hyperuricemia appears to be a member of the cluster of abnormalities in MBS (Reaven 1994). An increased uric acid level is an independent risk factor for CVD (Brand et al. 1985; Niskanen et al. in press) and seems to be a marker of reduced insulin sensitivity, not simply attributable to the concomitant elevation of triglycerides (Vuorinen-Markkola and Yki-Järvinen 1994; Costa et al. 2002).

*Hyperleptinemia.* Plasma leptin levels correlate with measures of obesity, although for any level of obesity, the levels vary widely. Leptin is positively correlated with insulin sensitivity in subjects with MBS (Zimmet et al. 1999) and in healthy first-degree relatives of type 2 diabetes patients (Jansson et al. 2002). However, the role of leptin in pathogenesis of MBS needs clarification.

2.1.3.6 Steroid hormone aberrations

*Glucocorticoids*

The phenotype of MBS has similarities to the cushingoid form of obesity. In central obesity, total glucocorticoid turnover and production rates are increased, though circulating cortisol levels are normal or even low (Walker et al. 2000). There is evidence for altered peripheral enzymatic activity which can affect the clearance of cortisol and compensatory activation of the HPA axis and glucocorticoid production (Figure 3).

There is an increase in 5α-reduced metabolites suggesting an increase in 5α-reductase conversion of cortisol and this enhanced 5-α reduction has been correlated with central adiposity in three cross-sectional studies (Andrew et al. 1998; Fraser et al. 1999; Reynolds et al. 2001).
In addition, there is evidence for altered adipocyte 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) activity. Glucocorticoids can be produced locally and the enzyme 11βHSD1 converts active glucocorticoid from its inactive form. Hepatic conversion of cortisone to cortisol by 11βHSD1 is impaired in obesity, but increased in adipose tissue (Walker 2001). The elevated 11βHSD1 activity in fat presents a novel therapeutic possibility because 11βHSD1 inhibitors are predicted to lower intra-adipose cortisol concentrations and the degree of obesity. In addition, the thiazolidinedione (TZD) class of antidiabetic agents that are ligands for peroxysome proliferator-activated receptor (PPAR)γ markedly reduce adipocyte 11β HSD-1 mRNA and activity both in vivo and in vitro (Berger et al. 2001).

![Figure 3](image_url)

**Figure 3.** Pathways of cortisol metabolism. Enhanced cortisol clearance in impaired hepatic reactivation of cortisone to cortisol affect feedback activation of hypothalamic-pituitary axis. However, changes in 11βHSD1 are tissue specific. Modified Andrew et al. 2002.
Sex steroids

In women, the abundance of visceral fat is reported to be associated with declining levels of SHBG and higher androgen production rates and higher free testosterone and free estradiol (E₂) levels (Kirschner et al. 1990) but not with changes in total testosterone (Leenen et al. 1994). In addition, Armellini et al. (1994) reported a negative correlation between total serum testosterone and intraabdominal fat evaluated by CT in obese women, as in men. Women with lower body obesity have an increased amount of estrone (E₁) resulting from peripheral aromatization processes (Kirschner et al. 1990).

Visceral fat has a high density of androgen receptors and testosterone has the documented effect on lipid mobilization, causing as expected a decrease in visceral fat depots (Björntorp 1997). However, female-to male transsexuals treated with testosterone show an increase in visceral fat (Elbers et al. 1997), but only when oophorectomized. This observation suggests that estrogen would be protective. Estrogen treatment down regulates the density of the androgen receptor (Björntorp 1997). Progesterone seems to act through glucocorticoid receptors. There is growing evidence to suggest that the ovarian hormones have major effects on lipid and carbohydrate metabolism in animal studies (Campbell and Febbraio 2001). These data indicate that the impact of 17-β-estradiol will be mediated through overall metabolic flexibility of skeletal effects, including PPARγ and PPARα expression which are up-regulated by treatment with 17-β-estradiol. Conversely, progesterone negates both of these effects.

The increased peripheral clearance and the obesity-associated acceleration in overall adrenocortical function may lead to an increase in adrenal androgen production. In premenopausal women, the DHEA concentration correlates positively with trunk fat and negatively with leg fat accumulation whereas no such correlation is seen in men (Williams et al. 1993; Usiskin et al. 1990). In perimenopausal women, higher levels of DHEA and DHEAS may be indicative of increased CVD risk (Johannes et al. 1999).

Sex-Hormone Binding Globulin

Due to its high affinity for several potent sex steroid hormones, SHBG is the major regulator of the biological effects of these hormones (Selby 1990). Increased upper body adiposity is associated with decreased hepatic clearance of insulin, whereas it
shows a strong correlation with the concentration of SHBG in both men and women. SHBG levels are already subnormal in hyperinsulinemic obese children (Galloway et al. 2001). Moreover, a prospective population study has identified a low SHBG level as a risk factor for the development of type 2 diabetes in women (Lindstedt et al. 1991; Hasler et al. 1993) and in men (Stellato et al. 2000). Low serum levels of SHBG coincide frequently with components of the MBS (Pugeat et al. 1995; Teichner et al. 1997). Low plasma levels of SHBG are associated with coronary heart disease in postmenopausal women independently of insulin, obesity markers and dyslipidemia (Reinecke et al. 2002).

2.1.4 Genetics of MBS

Currently it is not known which of the components of MBS are primary and perhaps genetically determined and which are secondary. Each factor of MBS has its own heritability (Groop 2000). Insulin resistance clusters in families: 45% of first-degree relatives of patients with type 2 diabetes are insulin resistant, compared with 20% of people without a family history of diabetes (Groop 2000). Lehtovirta et al. (2000) reported in a twin study that the heritability for insulin resistance was lower than the heritability of insulin secretion.

The heritability of hypertension is about 40 - 50% (Ferranini et al. 1987). The heritability in the level of HDL-cholesterol is stronger than the heritability of triglycerides (Groop 2000). Microalbuminuria clusters in families and the heritability of albumin excretion is about 30% (Groop 2000). In obesity, heritability according to family studies is 30 - 50%, according to adoption studies 10 - 30% and according to twins studies 50 - 80%. About 40% of the variation in body fat can be attributed to genetic factors (Bouchard et al. 1993). It seems that genetic factors explain 60% of the variance in abdominal fat of postmenopausal women (Groop 2000). Also cortisol secretion is strongly genetically regulated: homozygotic twins show an almost identical diurnal HPA axis activity (Linkowski et al. 1993).

The results of classic twin analysis (Poulsen and Vaag 2001) suggested that in MBS there was a core complex of abnormalities that were linked, including insulin resistance,
dyslipidemia and obesity. Glucose intolerance and hypertension, were independent and only distantly associated with this complex. The study confirmed the concept of a multifactorial etiology of the components including genetic and nongenetic factors. In identifying major genetic loci influencing phenotypes of metabolic syndrome, Kiss eba et al. (2000) (OMIM 606532) performed a genomewide scan in 2209 subjects including 507 Caucasian families. Quantitative trait locus (QTL) on chromosome 3q27 was strongly linked to 6 traits (weight, waist circumference, leptin, insulin, insulin/glucose ratio, and hip circumference). This QTL exhibited a mild epistatic interaction with a second QTL (605572) on chromosome 17p12 that was strongly linked to plasma leptin levels. Francke et al. (2001) replicated locus on 3q27 for the MBS and diabetes. There are positional candidate genes which encode proteins known to influence glucose-insulin homeostasis: Glut2, at 3q26-q27; the catalytic α polypeptide of phosphoinositide 3-kinase at 3q26.39 and Glut4, at 17p13. There are also genes encoding for proteins thought to influence lipid homeostasis and energy balance: adiponectin gene apM1 at 3q27, gC1qR at 17p13.3 (the receptor protein known to bind to globular heads of the complement C1q) and PPAR-α at 17p12-p11.2.

The recent update of the human obesity gene map includes 222 reports of positive associations identifying 71 candidate genes, with over 300 genes, markers and chromosomal regions putative linked with BMI, body fat or other obesity phenotypes (Chagnon et al. 2003). The obesity gene map shows putative loci on all chromosomes except Y.

Some rare Mendelian disorders with obesity as a clinical feature have been identified in humans (Arner 2000) suggesting that single genes could cause obesity in humans. Furthermore, some other types of monogenic obesity in humans have been described (leptin, leptin receptor, pro-opiomelanocortin, prohormone convertase-1, melanocortin-4 receptor). These mutations are rare and they can only explain obesity in a few families. There is also evidence that several genes affect the differences in the response to overfeeding (Bray 1997; Blundell and Cooling 2000). A disturbance can be connected with regulation of food intake control and satiety, the effectiveness of energy consumption or with adipogenesis.
Using a genome wide scan approach in family studies, a number of chromosomes have been identified as possibly being involved in common forms of human obesity, these are chromosome 2 (Comuzzi et al. 1997, 2001), 10 (Hager et al. 1998, OMIM 603188), 11 (Norman et al. 1998, 601693) and 20 (Lembertas et al. 1997, 607025). Uncoupling protein2 (UCP2), located at 11q13 and widely expressed in adult human tissues, is upregulated in white fat in response to fat feeding, has been proposed as a possible key component of β-cell glucose sensing and this protein could represent a critical link between obesity, β-cell dysfunction and type 2 diabetes (Zhang et al. 2001).

The heritability of phenotype of MBS is no doubt multifactorial and those quantitative trait locus (QTL), polymorphisms and mutations of genes of insulin resistance, type 2 diabetes, glucocorticoid receptor, regulation of sympathetic nervous systems seem to influence the propensity to the phenotype of MBS.

Through the candidate gene approach, several genes have been identified which might be considered as minor obesity genes because polymorphism in these genes is associated with obesity in some but not all subjects. Most of the minor candidate genes control important functions in adipose tissue (Table 3).

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor subfamily of transcription factors. These are functional targets for a new class of insulin sensitizers, the thiazolidinediones. Located in chromosomal region 3p25, the PPARγ gene is mainly expressed in the adipose tissue where it promotes the differentiation of preadipocytes into adipocytes (Elbrecht 1996; Beamer et al. 1997). There are studies showing an association of polymorphisms in the PPARγ gene to the components of MBS. In vitro experiments have indicated that the less frequent alanine allele of PPARγ Pro12Ala, presenting with an allele frequency of approximately 15% among Caucasians, is associated with reduced transcriptional activity (Deeb et al. 1998). In a meta-analysis, this variant seemed to associate with a decreased risk of type 2 diabetes (Altshuler et al. 2000).
### Table 3. Genes involved in adipose biology and metabolism

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene product</th>
<th>Localization</th>
<th>OMIM</th>
<th>Disease phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEP</td>
<td>Leptin</td>
<td>7q31.3</td>
<td>164160</td>
<td>Obesity, hypogonadism</td>
</tr>
<tr>
<td>LEPR</td>
<td>Leptin receptor</td>
<td>1p31</td>
<td>601007</td>
<td>Obesity, hypogonadism</td>
</tr>
<tr>
<td>POMC</td>
<td>Proopiomelanocortin</td>
<td>2p23.3</td>
<td>176830</td>
<td>Hypoadrenalism</td>
</tr>
<tr>
<td>PC1 (PCSK1)</td>
<td>Prohormone convertase 1</td>
<td>5q15-q21</td>
<td>162150</td>
<td>Obesity, impaired prohormone processing</td>
</tr>
<tr>
<td>MC4R</td>
<td>Melanocortin 4 receptor</td>
<td>18q22</td>
<td>155541</td>
<td>Obesity, increased linear growth</td>
</tr>
<tr>
<td>RSTN</td>
<td>Resistin</td>
<td>19</td>
<td>605565</td>
<td>?</td>
</tr>
<tr>
<td>PPAR γ</td>
<td>Induces transcription of genes involved in insulin sensitivity, adipocyte differentiation and inflammation</td>
<td>3p25</td>
<td>601487.0001</td>
<td>Obesity</td>
</tr>
<tr>
<td>V&gt;M290</td>
<td></td>
<td></td>
<td>601487.0008</td>
<td>Severe insulin resistance and diabetes</td>
</tr>
<tr>
<td>P&gt;L467</td>
<td></td>
<td></td>
<td>601487.0007</td>
<td></td>
</tr>
<tr>
<td>P&gt;A 12</td>
<td></td>
<td></td>
<td>601487.0002</td>
<td></td>
</tr>
<tr>
<td>ADRB2</td>
<td>β-2-adrenergic receptor</td>
<td>5q32-q43</td>
<td>109690</td>
<td>Decreased lipolysis, obesity</td>
</tr>
<tr>
<td>ADRB3</td>
<td>β-3-adrenergic receptor</td>
<td>8p12-p11.2</td>
<td>109691</td>
<td>Obesity, Type 2</td>
</tr>
<tr>
<td>UCP 1</td>
<td>Uncoupling protein-1</td>
<td>4q31</td>
<td>113730</td>
<td>Decreased thermogenesis</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor - α</td>
<td>6p21.3</td>
<td>191160</td>
<td>Improve insulin sensitivity, impaired lipolysis, apoptosis</td>
</tr>
<tr>
<td>LDL-R</td>
<td>Low-density lipoprotein receptor</td>
<td>19p13.2</td>
<td>606945</td>
<td>Increased lipid uptake</td>
</tr>
<tr>
<td>HSI</td>
<td>Hormone-sensitive lipase</td>
<td>19q13.1-q13.2</td>
<td>151750</td>
<td>Impaired lipolysis</td>
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<tr>
<td>Ghrelin</td>
<td></td>
<td></td>
<td>605353</td>
<td>Obesity</td>
</tr>
<tr>
<td>AGRP</td>
<td>Agouti related protein</td>
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<td>602311</td>
<td>Obesity</td>
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<td>ApM1</td>
<td>Adiponectin</td>
<td>3q27</td>
<td>605441</td>
<td>Obesity</td>
</tr>
</tbody>
</table>

In an animal model, transgenic mice overexpressing adipocyte 11β-hydroxysteroid dehydrogenase type 1 developed visceral obesity with metabolic complications, i.e. a phenotype of MBS that was exacerbated by feeding the animals a high-fat diet (Masuzaki et al 2001).
2.2 Polycystic ovary syndrome (PCOS)

2.2.1 General aspects

Polycystic ovary syndrome (PCOS) is a heterogeneous clinical entity characterised by anovulatory disorders and signs of hyperandrogenism such as acne and hirsutism. The essential feature, the arrest of follicular development in the ovaries, leads to typical ovarian structure and abnormal steroid synthesis, relative hyperandrogenism, maintained estrogen secretion and a low level of progesterone. The women with PCOS typically present with irregular menstrual cycles as well as infertility problems associated with increased pregnancy loss (Franks 1995). There are also significant ethnic and racial variations in the clinical presentation of PCOS, especially concerning hyperandrogenism. The frequency of obesity, insulin resistance and incidence of diabetes mellitus associated with PCOS also varies (Hopkinson 1998; Wild 2002a).

Acanthosis nigricans – a cutaneous marker of insulin resistance - a gray-brown velvety discoloration of the skin-usually found in the neck, groin and axillary regions of the body has been reported especially in obese PCOS women (Dunaif 1997).

When compared to MBS, the diagnostic criteria of PCOS are even less well established. The evident association with insulin resistance aroused new interest in PCOS as representing possibly one manifestation of MBS (Burghen et al. 1980; Hopkinson 1998).

When Stein and Lewenthal first described the connection between bilateral polycystic ovaries, oligoamenorrhea, hirsutism and infertility often associated with obesity, the diagnosis was based on ovarian histology. The syndrome was seen as an ovarian end-organ disease and named polycystic ovarian disease (Stein and Lewenthal 1935, Stein 1964). The typical histological features for polycystic ovaries (PCO) include thickened fibrous capsule, increased number of small subcapsular follicle cysts and hyperplastic ovarian stroma (Goldzieher and Green 1962). Hormonal assays revealed that there were more complex endocrinological abnormalities associated with this situation and the term polycystic ovary syndrome was introduced (Yen 1980). The ultrasound criteria most often used for PCO have been described by Adams 1985: the
typical polycystic ovary was defined by the presence of 10 or more follicular cysts, measuring 2-8 mm in diameter and either arranged subcapsular or scattered throughout the stroma (Adams et al. 1985).

The major discrepancy concerning the diagnostic criteria for PCOS applies to the use of ultrasound. In Europe, the presence of PCO in ultrasonographic examination in conjunction with biochemical and/or clinical features of hyperandrogenism and anovulation have been generally required for the diagnosis of PCOS (Homburg 1996; Yen et al. 1999). At present, and especially in USA, ultrasound is considered as being as too unspecific since it can be confused by many other anovulatory conditions. In the National Institutes of Health (NIH) Conference on PCOS in 1990, no consistent definition was achieved, but the majority of the participants proposed that PCOS should be defined as 1) ovulatory dysfunction 2) evidence of hyperandrogenism, either clinical (hirsutism, acne or male pattern balding) or biological (elevated androgen levels) and 3) exclusion of related diseases (Cushing syndrome, late onset adrenal hyperplasia, virilizing ovarian and adrenal tumors, hyperprolactinemia, hypothyroidism) (Knochenhauer et al. 1998). US examination was not essential. It seems that medication can change the structure of the ovaries (Isojärvi et al. 1993).

There is little known about the prevalence of PCOS in the healthy population (Table 4). Depending on the criteria used, about 5 - 10% of the women of reproductive age are affected (Franks 1995; Knochenhauer et al. 1998). The pooled prevalence is higher, when ultrasound is included in the diagnosis, as high as 19% (Balen and Michelmore 2002). The isolated finding of polycystic ovaries in ultrasound seems to be quite a common finding in the healthy population, 10 - 33% according different studies (Botsis et al. 1995, Polson et al. 1988, Michelmore et al. 1999). In one sample of women of South Asian origin, as much as every second woman (52%) presented with PCO (Rodin et al. 1998) but without ovulatory disorders these women are not considered to have PCOS. The women with polycystic ovaries seem, however, to show often at least one feature of PCOS when compared to those with normal ovarian structure (Polson et al. 1988; Koivunen et al. 1999). The ovarian appearance does seem to correlate strongly with menstrual history. Most of the oligomenorrheic women (85%) as part of amenorrhoic women (26%), present also with PC-ovaries (Adams et al.
1986; Polson et al. 1988). The prevalence appears to be lower among older women (Kolvunen et al. 1999).

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Prevalence (%)</th>
<th>Diagnosis of PCOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al. 1996</td>
<td>34835, cohort in Iowa</td>
<td>1.4</td>
<td>Self-reported</td>
</tr>
<tr>
<td>Knochenhauer et al. 1998</td>
<td>36918 45 y, 74 white, 195 black, USA</td>
<td>4.0</td>
<td>NIH</td>
</tr>
<tr>
<td>Diamantis-Kandarakis et al. 1999</td>
<td>192 17-45 y, Greek, island of Lesbos</td>
<td>6.8</td>
<td>NIH</td>
</tr>
<tr>
<td>Asuncion et al. 2000</td>
<td>145 18-45 y, Madrid</td>
<td>6.5</td>
<td>NIH</td>
</tr>
</tbody>
</table>

### 2.2.2 Pathogenesis of PCOS

The underlying pathogenesis of PCOS is uncertain. There is a strong evidence of a peripubertal onset of PCOS (Apter et al. 1995; Yen 1999). At the onset of puberty, there is an increase in the pulse amplitude of luteinising hormone (LH), increasing androgen concentrations and often irregular menstrual cycles. Multicystic ovaries seen on ultrasound examination are a common and normal feature of puberty. Transitory insulin resistance and increased serum insulin concentrations especially in obese girls and reduced growth hormone (GH) secretion have been shown (Morales et al. 1996). If the cycles do not normalize to regular ovulatory periods within two years after menarche, abnormalities may persist into adulthood (Apter and Vihko 1990). It is not clear, whether the primary cause of PCOS is a hypothalamic defect or secondary to alterations in peripheral or local signaling (Taylor 1998).

#### 2.2.2.1 Abnormal gonadotropin secretion

Increased LH secretion in adult PCOS women was first reported by Yen et al. (Yen et al. 1970). Later studies have shown that typically there are increases of both LH-pulse frequency and amplitude and hyperreactivity to gonadotropin-releasing hormone (GnRH) stimulation. (Koskinen et al. 1996; Yen et al. 1999). Many studies have shown a difference between lean and obese PCOS subjects, hypersecretion of LH being
especially common in lean women (Anttila et al. 1991). The secretion of follicle
stimulation hormone (FSH) remains rather unchanged or low. The synthesis and
secretion of these two gonadotropins are dependent on the pattern of GnRH stimulus,
which seems to be altered by still unknown mechanisms (Taylor 1998).

2.2.2.2 Ovarial dysfunction in PCOS

According to the two-cell, two-gonadotropin-model of ovarian function, LH stimulates
theca-cells to secrete androgens, especially androstenedione (A), which normally is
converted to estrogen by the aromatase enzyme activated by FSH in granulosa cells
(Figure 4) (Rosenfield 1999). During normal ovulatory periods, when the dominant
follicle emerges, the secretion of E₂ predominates. Chronic stimulation of LH in PCOS
induces hypersecretion of androgens, however, which partly disturbs the selection and
maturation of the dominant follicle (Yen 1999).

One key step in androgen formation is the regulation at the levels of enzymes 17-
hydroxylase and 17,20-lyase, both which are mediated by the cytochrome P450c17
(Figure 4). Hyperactivity of this enzyme has been proposed to represent the primary
mechanism leading to ovarian hyperandrogenism occurring in the great majority of
PCOS patients. Expression and activation of P450c17 gene in ovary are regulated by a
number of hormones and growth factors including LH, ACTH, insulin and IGFs
(Magoffin 1989; John et al. 1986; Apter et al. 1994). Irrespective of the mechanism in
PCOS subjects, both the granulosa and thecal cells demonstrate a hyperresponse to the
gonadotropins (Rosenfield 1999).

Granulosa cells in the arrested follicles in PCOS are few in number and the
aromatase activity is low (Yen 1999). This results in an increased androgen to estrogen
ratio. There is data showing that many factors stimulating granulosa cells and aromatase
enzyme activation may be abnormal in PCOS. The paper by Norman et al. (2001) for
example claimed that the circulating level of activin is lower but that of follistatin, an
activin-binding protein, is higher in PCOS subjects than in controls (Norman et al.
2001). There also are theories concerning aromatase mutations as a basic defect in the
PCOS (Gabrilove 2002), resulting in an abnormal ratio of androgen to estrogen.
Figure 4. Ovarian main steroid biosynthesis according to the two-cell, two-gonadotropin model. LH stimulates androgen synthesis within theca-cells. FSH regulates estradiol synthesis from androgens in granulosa-cells. A key step in androgen synthesis is the regulation of 17-hydroxylase and 17,20-lyase enzymes, both of which are mediated by cytochrome P450c17. StAR= steroidogenic acute regulatory protein; 3β=Δ5-isomerase; 3β-hydroxysteroid dehydrogenase; 17β=17β-hydroxysteroid dehydrogenase; 5α-R= 5α-reductase. Modified from Rosenfield (1999).

2.2.2.3 Adrenal androgen secretion

Hyperandrogenism of adrenal origin often coexists with that of ovarian origin in many PCOS women (Martikainen et al.1996; McKenna et al. 1997). There is data showing that girls with premature adrenarche are at a high risk for developing a PCOS-like situation at puberty (Oppenheimer et al. 1995). The exact mechanism is not known, however. The analogous mechanism to the ovary has been suggested, i.e., increased androgen formation by cytochrome P450c17 enzymes (Ehrman et al. 1995a). These activities may be regulated differently in the adrenals than in ovaries, however. ACTH
affects both cortisol and adrenal androgen production, but only cortisol feeds back to
regulate its physiological level. Thus, even changes in cortisol metabolism could induce
excessive adrenal androgen production (Rodin et al. 1994).

2.2.2.4 Peripheral steroid metabolism

Only about 25% of the T in the blood of women arises directly from the ovaries, with
25% from the adrenals. The main part is produced from peripheral conversion of
precursors to T by enzymes such 5α-reductase in the skin and fat cells (McKenna et al.
1997). Peripheral conversion of circulating androgens to estrogens in adipose tissue is
also increased in PCOS, and explains the elevated estrone levels. The aecyclic production
of extraglandular estrogen further disturbs the gonadotropin secretion having a positive
feedback on LH and negative feedback on FSH secretion, respectively (Rosenfield
1999).

Although hyperandrogenism belongs to the criteria of PCOS, many individuals may
present with completely normal androgen levels (Taylor 1998). Hepatic synthesis of
SHBG is decreased by insulin and androgens, but increased by estrogen (Botwood et al.
1995). Due to the low SHBG level in PCOS subjects, it is common to detect elevated
levels of biologically active T (Rosenfield 1999).

2.2.2.5 Role of Insulin in PCOS pathogenesis

Although the finding of a relationship between hyperandrogenism and abnormal insulin
metabolism dates back to the beginning of the 20th century, it was not until the 1980’s
when insulin resistance was suspected as being a factor in the pathogenesis of PCOS
(Burghen 1980; Barbieri 1983; Chang 1983). Although insulin may influence steroid
metabolism in many ways to lead to hyperandrogenism (Table 5), the association with
the pathogenesis of PCOS is not clear. It is known that androgens may produce mild
insulin resistance. However, decreasing androgen levels by treatment with GnRH
agonists or antiandrogens does not restore normal insulin sensitivity, (Dunaif et al.
1990; Moghetti et al. 1996). On the other hand, insulin suppression obtained after
diazoxide administration has been shown to reduce T and to increase SHBG concentrations in obese and hyperandrogenic PCOS women without affecting body weight. According to most reports, insulin resistance can be shown in about half of PCOS subjects (Sozen and Ariici 2000). The comparison of the studies is especially hampered by the variable populations which have been evaluated, the different diagnostic criteria used as well as ethnic reasons.

### Table 5. Possible effects of insulin on steroid metabolism (modified from Poretsky et al. 1999)

<table>
<thead>
<tr>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>- direct stimulation of ovarian and adrenal androgen secretion, possibly through stimulatory effects on the 17α-hydroxylase/17-20-lyase and on 3β-hydroxysteroid dehydrogenase enzymes</td>
</tr>
<tr>
<td>- upregulates ovarian type-I IGF receptor with amplification of IGF-I, IGF-II and insulin actions in the ovary</td>
</tr>
<tr>
<td>- increases the LH ovary receptor number and probably sensitises LH secreting pituitary cells to GnRH stimulation</td>
</tr>
<tr>
<td>- promotes ovarian growth and cyst formation synergistically with LH/hCG</td>
</tr>
<tr>
<td>- inhibits IGFBP-1 production, both in the liver and in the ovary</td>
</tr>
<tr>
<td>- decreases hepatic synthesis of SHBG, with concomitant elevation of free androgen tissue availability</td>
</tr>
<tr>
<td>- stimulates or inhibits aromatase</td>
</tr>
</tbody>
</table>

At the ovarian level, insulin interacts both with its own and the IGF-1 receptor, which have been detected in human cell models throughout all ovarian compartments, e.g. granulosa, thecal and stromal tissues (Dunaif et al. 1992). IGF-I and IGF-II are well known effectors of ovarian function. In normal-weight PCOS women, IGF bioavailability seems to be increased by various mechanisms such as insulin-induced hepatic and ovary IGFBP-1 suppression and GH-induced hepatic IGF stimulation and so may be involved in the pathogenesis of the hyperandrogenism in the PCOS. On the contrary, in obese PCOS, IGF-1 bioavailability seems to be reduced in comparison to their normal-weight PCOS counterparts, although it may be relatively higher than in nonaffected women because of the combination of low GH and high insulin levels (Dunaif 1999).

Euglycemic glucose clamp studies have demonstrated a significant decrease in insulin-mediated glucose uptake in PCOS subjects (Dunaif et al. 1987; Morales et al.
1996; Morin-Papunen et al. 2000). However, many recent clamp studies have failed to confirm this finding in lean PCOS women (Ovesen et al. 1993; Holte et al. 1994; Morin–Papunen et al. 2000). Also some of the obese PCO women present with normal insulin sensitivity, suggesting that there is heterogeneity in insulin action in this syndrome.

Fasting hyperinsulinemia is often present in obese PCO women and this appears to be secondary to the increased basal insulin secretion rates (O’Meara et al. 1993). Insulin responses to oral glucose load seem to be increased both in lean and obese PCO subjects but acute insulin responses to an intravenous glucose load, first phase insulin secretion, are similar to weight–matched controls (Dunaif et al. 1987, 1996). Evidence for β cell dysfunction in PCO is provided by the studies of Erhmann et al. (1995b). These defects were greatly pronounced in PCO women who have a first degree relative with type 2 diabetes (Erhmann et al. 1995b). The early-phase secretion of C-peptide, which more accurately reflects pancreatic β cell secretory function, did not differ between PCOS and control subjects in a Finnish study by Morin-Papunen et al. (2000). Basal hepatic glucose production was significantly increased only in obese PCO women (Dunaif et al. 1987, 1992). The obese PCOS women have been shown to have lower hepatic extraction of insulin in comparison to the controls (Morin-Papunen et al. 2000).

Studies of insulin action in isolated PCO adipocytes show a decrease in the maximal rates of glucose uptake. This may be secondary to a significant decrease in the abundance of adipocyte GLUT4 glucose transporters (Pugeat et al. 2000). Insulin receptor function studies made by using cultured skin fibroblasts part of PCOS subjects show defects in the tyrosine kinase activity of the insulin receptor, thereby decreasing the signaling pathway (Dunaif et al. 1995).

2.2.2.6 Impact of obesity in PCOS

Obesity is encountered in 30 – 60 % of PCOS cases (Yen 1980; Franks 1995). Body fat in PCOS is generally located centrally or on the upper body (Gambari 2002). Obesity seems to modify the clinical picture of PCOS so much that many researchers divide women with PCOS into subgroups according to their weight. Obese PCOS women tend
to be more hyperandrogenic, hyperinsulinemic and have a more atherogenic lipoprotein pattern than their normal weight counterparts. Dysregulation of the gonadotropin function appears to be more dominant in lean PCOC subjects, this being detected for example as higher LH/FSH levels, whereas insulin resistance appears to be a rarity (Acien et al.1999).

Obesity itself represents a risk for hyperinsulinemia. Obesity alters many endocrinological parameters: e.g. concentrations of SHBG are typically decreased which evokes hyperandrogenism and hyperestrogenism (Peiris et al. 1987, Plymate et al. 1988). Alterations in the cortisol-cortisone metabolic pathways include to obesity (Gambineri 2002). Reduced GH secretion has been observed in obesity (Kopelman 1994). The role of leptin in this context is still under debate (Laughlin et al. 1997). Most endocrine disturbances disappear with weight reduction. Also metformin therapy seems to be beneficial in PCOS women, improving ovarian cyclicity and the fertility in some of these subjects (Velazquez et al. 1994; Nestler et al. 1998; Morin Papunen et al. 1998). However, the exact mechanism of action of metformin is not clear, although a decrease in abdominal obesity has been reported (Morin-Papunen et al. 2000).

2.2.3 Natural course of PCOS

There is a paucity of prospective long-term follow-up studies concerning PCOS. Recent information is mainly based on cross-sectional studies or rather small samples evaluated without unambiguous criteria for PCOS. It seems, however, that gynecological symptoms associated with PCOS can resolve with aging (Winters et al. 2000). Menstrual cycles become more regular and the signs of hyperandrogenism less evident. This may be associated with declining reserves of theca cells (Elting et al. 2000; Winters et al. 2000). However, because of the relative hyperestrogenism, there is thought to be an elevated risk for endometrial cancer (Dahlgren et al. 1991; Kaaks et al. 2002). Obesity itself is known to increase the risk. Theoretically there could also be an association with breast, ovarian and colon cancer, though this has not been confirmed (Anderson et al. 1997; Pierpoint et al. 1998).
**Risk for type 2 diabetes.** The prevalence of IGT and conversion to type 2 diabetes is 5-10 fold greater in PCOS subjects according to recent data (Ehrmann et al. 1999; Legro et al. 1999). Obesity and a family history of type 2 diabetes, i.e. first degree relatives, increase the risk (Legro et al. 1999). In a Swedish study by Dahlgren et al. (1992), the prevalence of diabetes appeared to be 15% among perimenopausal PCOS women (n = 32) wedge resected 20-30 years earlier but only 2.3% in a control group. There was a strong influence of heredity for metabolic diseases among PCOS women, with 85% reporting at least one of their parents as suffering from cardiovascular disease or type 2 diabetes or both (Dahlgren et al. 1992). A four times higher prevalence of diabetes was detected compared with the random population in another study in women who had 50 years previously undergone ovarian wedge resection (Cibula et al. 2000). Also women with type 2 as well as type 1 diabetes seem to present more often than expected with PCOS (Escobar-Morreale et al. 2000).

**Hypertension.** In many studies, blood pressure is comparable between PCOS women and controls (Zimmermann et al. 1992; Sampson et al. 1996). Dahlgren, however, reported four times more hypertension in wedge resected perimenopausal PCOS women than in controls (Dahlgren et al. 1992). Holte et al. (1996) found higher daytime and higher ambulatory mean arterial blood pressure in a group of 34 PCOS women as in weight-matched controls. In the study of Elting et al. (2001) a total of 346 PCOS patients were interviewed by telephone. A higher hypertension rate (χ2 5) was detected among PCOS, who were also more obese (Elting et al. 2001). In animal studies, hyperandrogenism may stimulate angiotensin II activity and this may lead to vasoconstriction and elevated blood pressure (Reckelhoff 2001).

**Dyslipidemia.** According to most studies, an abnormal lipid profile in PCOS is a common finding. Typically low HDL-cholesterol and apolipoprotein A1 and increased triglyceride and VLDL levels are found. Higher apolipoprotein B and LDL-cholesterol levels have been reported (Wild 2002a). Hyperinsulinemic PCOS women show an even more abnormal lipid profile than their normoinsulinemic counterparts (Mather et al. 2000). It seems that the degree of obesity has its own impact on the findings. A greater reduction of HDL-cholesterol together with higher increase of triglycerides and total cholesterol levels were observed in obese subjects with respect to the normal-weight
PCOS women (Talbott et al. 1998). In this cross-sectional study of Talbott et al., the difference of LDL-cholesterol and total cholesterol levels disappeared between PCOS women and weight and age-matched controls after the age of forty.

At present, there is no data on whether there is higher CVD morbidity or mortality among PCOS women compared with other populations (Pierpoint et al. 1998; Wild et al. 2000). It has been speculated that the relative hyperestrogenism or the elevated levels of endothelial growth factor could even be protective for the cardiovascular system (Pierpoint et al. 1998, Wild et al. 2000). On the other hand there is data showing more widespread atherosclerotic plaques on carotis ultrasound examination in PCOS women compared to age matched controls (Talbott et al. 2000).

2.2.4 Genetic background of PCOS

Several reports indicate that PCOS is a familial disorder (Cooper et al. 1968; Hague et al. 1988; Legro et al. 1998; Cotilla et al. 2001; Franks et al. 2001). Various features of the syndrome may be differentially inherited (Franks et al. 1997). There are studies suggestive of an autosomal dominant inheritance (Carey et al. 1993; Carey et al. 1994; Govind et al. 1999). Carey described an association to premature male-patterned baldness, and association with a change in the promoter region of the CYP17 gene, which modified the expression of the syndrome in some families but did not appear to be the primary genetic defect.

On the other hand, in the family study of Hague there was the suggestion that the Mendelian autosomal dominant mode could not explain the mode of inheritance of the syndrome (Hague et al. 1988) while in another study, an X-linked model was postulated (Givens 1988). As a result, the mode of inheritance remains unclear and more than one gene defect seems to participate in the pathogenesis of the syndrome, each contributing a small effect (Xita et al. 2002). The recent consensus is that PCOS is an oligogenic complex disorder.

The study of PCOS is also hampered by the lack of a universally accepted definition for PCOS.
Unlike many other diseases, the genetic studies of PCOS have focused on identifying and testing candidate genes (Urbanek et al. 1999). There are two main reasons for this approach. Firstly, localizing PCOS genes by classic systematic linkage study has been difficult because of the heterogeneity of the disease etiology, the uncertainty of the type of inheritance and the non-existent male phenotype. Secondly, it has been possible to name several attractive candidate genes such as the genes affecting metabolic or regulatory pathways of steroid synthesis, regulatory pathways of gonadotropin action, the insulin signaling pathway, pathways regulating body weight and follicle maturation. Those studies showing a positive association with PCOS are listed in Table 6.

<table>
<thead>
<tr>
<th>Study</th>
<th>Gene</th>
<th>Action</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gharani et al. 1997</td>
<td>CYP 11α</td>
<td>encodes P450 side-chain cleavage enzyme</td>
<td>15q24</td>
</tr>
<tr>
<td>Diamanti-Kandarakis et al. 2000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waterworth et al. 1997</td>
<td>INS VNTR</td>
<td>Regulation of insulin secretion</td>
<td>11p15.5</td>
</tr>
<tr>
<td>Bennett et al. 1997</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eaves et al. 1999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Michelmore et al. 2001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tucci et al. 2001</td>
<td>C/T SNP in exon 17 of INSR</td>
<td>Insulin receptor function</td>
<td>19p13.2</td>
</tr>
<tr>
<td>Siegel et al. 2002</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

INS VNTR = minisatellite of insulin gene - variable number of tandem repeats
INSR = insulin receptor

Gharani et al. (1997) demonstrated a significant allelic association with polymorphism of CYP11α, an enzyme involved in the catalysis of the cholesterol side-chain cleavage, in PCOS or hyperandrogenemia and they suggested a role for this variation in the etiology of the hyperandrogenism as a common characteristic of PCOS. This association has been confirmed by Diamanti-Kandarakis et al. (2000), but another study by San Millan et al. (2001) found no influence of this polymorphism on hyperandrogenism.

Defects in both insulin action and insulin secretion contribute to the predisposition in diabetes in PCOS. Coltita et al. (2001) studied 33 PCOS women and 48 nondiabetic first degree relatives and concluded that there was a heritable component to β-cell
dysfunction in families of PCOS women. Earlier Waterworth et al. (1997) found an association between PCOS and the allelic variation at the INS VNTR locus. The INS VNTR influences INS expression both in vivo and in vitro and the class III alleles of VNTR predispose to the alteration in insulin secretion. This association was later confirmed in the studies of Eaves et al. (1999) and Michelmore et al. (2001). Recently Tucci et al. (2001) claimed that a susceptibility gene for PCOS was located on chromosome 19p13.3 in the insulin receptor (INSR) gene region. Siegel et al. (2002) found an association between a C/T SNP at exon 17 of the INSR gene and PCOS. The frequency of T allele was significantly increased in lean PCOS women compared with lean controls.

Urbanek et al. (1999) tested a total of 37 candidate genes and the strongest evidence for linkage was with the follistatin gene in affected sib pairs. Follistatin neutralizes the biological activity of activin in vitro and in vivo. Overexpression of follistatin might be expected to arrest follicular development, increase ovarian androgen production, reduce levels of FSH and impair insulin release. However, later in a larger sample of families, the evidence for linkage between PCOS and follistatin was weak (Urbanek et al. 2000) and not seen at all in the study of Tucci et al. (Tucci et al. 2001).

The variant form of LH is thought to influence the genetic heterogeneity on reproductive functions, e.g. a genetic variant of LH that contains two missense point mutations in the LHβ gene frequency was found less frequently in obese Dutch and Finnish PCOS patients (Tapanainen et al. 1999). Also genetic variability in the gene encoding microsomal epoxide hydrolase (EPHX) could contribute to reproductive functions disorders (Wang et Ahl. 1998; Zusterzeel et al. 2001), though its association to PCOS has not yet been studied.

In animal studies of Burks et al. (2000) described was that deletion of insulin receptor substrate-2 (IRS-2), a component of the insulin/IGF-1 signalling cascade, caused female infertility. However, mice deficient for IRS-2 gene do not represent a good model of PCOS, although they have small, anovulatory ovaries with a reduced number of follicles, increased food intake and obesity, but unlike the human PCOS patients these mice have low levels of LH, prolactin, sex hormone and gonadotropin.
3 AIMS OF THE PRESENT STUDY

This study aimed to clarify the gynecological and endocrinological profile of women with metabolic syndrome in relation to polycystic ovary syndrome. The second goal of this study was to evaluate the genetic heterogeneity of PCOS with genetic association studies.

The specific aims of the present study were:

To assess the frequency of gynecologic problems in women with MBS.
To investigate the relationship of the MBS and obesity to PCOS.
To determine the sex hormone profile among premenopausal women having MBS.

Based on recent opinion that PCOS is an oligogenic complex disorder, the aim was to clarify the pathogenesis to PCOS with genetic association studies.

- to examine the link between insulin resistance and PCOS by determining the frequency of the PPARγ polymorphism in women with PCOS.
- to determine whether genetic variability in the gene encoding for microsomal epoxide hydrolase (EPHX) could contribute to individual differences in the susceptibility to the development of PCOS.
- to investigate the possible role of apoE in the dyslipidemia often seen in PCOS.
- to clarify whether TNF-α polymorphism is associated with PCOS.
4 SUBJECTS AND METHODS

4.1 Subjects

4.1.1 Subjects in population-based studies (Studies I and II)

Study I.

Two-phase screening for metabolic syndrome

Study participants (n = 204) were recruited from a sample of Caucasian women born in the years 1942, 1947, 1952, 1957 and 1962 in a defined area in eastern Finland (Figure 5). The sample members were drawn from updated census register and invited by a letter to the two-phase screening. Altogether 543 of the eligible 730 women participated in the initial screening during the years 1993 and 1994 carried out by Vanhula et al. (1997). In the first phase, the clinical markers of insulin resistance (type 2 diabetes in first-degree relative, obesity, central obesity, hypertension) were investigated. Those who presented with at least one of the markers, were invited to laboratory tests (269 women).

The MBS was considered to be present if the study participant met at least three of the following eight criteria: 1) first-degree relative with type 2 diabetes, 2) obesity as defined by a BMI ≥ 30kg/m², 3) abdominal adiposity as defined by a WHR ≥ 0.88, 4) hypertension as defined by systolic blood pressure ≥ 160mmHg or diastolic blood pressure ≥ 95 mm Hg or drug treatment for hypertension, 5) fasting serum triglycerides ≥ 1.70 mmol/l, 6) fasting HDL-cholesterol <1.20 mmol/l, 7) abnormal glucose metabolism according to the WHO criteria and 8) hyperinsulinemia (fasting insulin > 13 nmol/l).

MBS, according to these less strict criteria, was found in 106 (19.5 %) women and this group formed group MBS in study I. Of these 106 women, 34 (8 %) met the stricter criteria for MRS (simultaneous presence of dyslipidemia and either hyperinsulinemia or abnormal glucose tolerance).
Updated census register  
\( n = 736 \), of whom 24 were ineligible

Did not participate  
\( n = 169 \)

Participated  
\( n = 543^* \)

1-phase screening -  
\( n = 274 \)

1-phase screening +  
\( n = 269 \)

Lean Controls  
BMI < 27 kg/m\(^2\)  
none risk factor  
\( n = 53 \)

Obese controls  
BMI > 27 kg/m\(^2\)  
WHR < 0.88  
\( n = 62 \)

MBS +  
\( n = 106 \)

MBS -  
\( n = 163 \)

n = 53

n = 50#

n = 59

n = 58#

n = 92

n = 84#

Clinical examination, questionnaire

1 alt.test

* born in 1942 \( n = 76 \); in 1947 \( n = 155 \); in 1952 \( n = 124 \); in 1957 \( n = 121 \); in 1962 \( n = 67 \)

# Stratification according to the NCEP criteria is shown in Figure 6

**Figure 5.** Flow diagram on the formation of the study population (Study I).

**Obese control group** was formed from these participants who were invited to the laboratory tests. Exclusion criteria were abdominal obesity (WHR \( \geq 0.88 \)) and MBS. The inclusion criteria was a BMI over 27 kg/m\(^2\). None of these women had more than one of the diagnostic criteria for MBS. 62 women fulfilled these criteria.

**Lean control group** was chosen from the original screening population in alphabetical order having a BMI less than 27 kg/m\(^2\) and none of the risk factors mentioned earlier.
This whole study population were invited to repeat laboratory tests and a 2-hour oral glucose tolerance test during the years 1996-1997.

Although only 3 criteria were required for including women in the group with metabolic syndrome, this group differed significantly from the lean group according to all 8 screening characteristics and also from the obese group except for HDL-cholesterol. Peri- or postmenopausal status were classified as amenorrhea for more than six months and increased FSH level (> 20 IU/L).

**Study II**

In study II, MBS was considered with the concept of National Cholesterol Education Program (NCEP 2001) from the whole study population (n = 192) from those who participated in repeated laboratory tests and underwent an oral glucose tolerance test (Figure 6). MBS was defined as the presence of at least three NCEP criteria: waist circumference > 88 cm, blood pressure ≥ 130 mmHg and/or ≥ 85 mmHg, fasting serum triglycerides ≥ 1.70 mmol/l, fasting HDL-cholesterol < 1.30 mmol/l, and fasting glucose > 6.1 mmol/l. Altogether 85 women fulfilled the criteria and of these 63 were premenopausal and they were included in the study group. Twenty five women were classified as peri-or postmenopausal on the basis of history of amenorrhea for more than six months and increased FSH level (>20 IU/L) and they were excluded.

Of the 63 premenopausal MBS women, 26 (41.3 %) women fulfilled three, 26 (41.3 %) had four, 9 (14.3 %) exhibited five and two (3.2 %) had all six criteria. Fifty-three of the women (84.1 %) presented with waist circumference > 88 cm, 56 (88.9 %) with systolic blood pressure ≥ 130 mmHg, 46 (73.0 %) with diastolic blood pressure ≥ 85 mmHg, 30 (47.6 %) with triglyceride ≥ 1.7 mmol/l, 43 (68.3 %) with HDL-cholesterol < 1.3 mmol/l and 14 (22.2 %) with fasting glucose ≥ 6.1 mmol/l.
Figure 6. Flow diagram on the formation of the study population for Study II.

The control group consisted of 88 age-matched women from the original population. The controls could present with one (n = 30, 34.1 %) or two (n = 30, 34.1 %) NCEP criteria of MBS but not three or more. Of the 88 controls, 28 (31.8 %) women had none of the NCEP criteria.

4.1.2 Subjects in genetic association studies (Studies III – VI)

Study groups consisted of 135 (Study III), 112 (Study IV), 58 (Study V), and 87 (Study VI) PCOS women and 91 (Study V) – 115 (Studies III, IV, VI) controls.

PCOS patients were recruited from population-based study population (n = 27, studies III - VI), from the endocrinology/infertility clinic Kuopio University Hospital and Mikkeli Central Hospital (n = 31 - 85, Studies III - VI) and from the endocrinology/infertility clinic Oulu University Hospital (n = 30, Study III).

Information was collected retrospectively. Blood sample for lipids and DNA was collected after written contact. 91 (Study V) - 115 (studies III, IV, VI) controls were nonhirsute, fertile Caucasian Finnish women with regular cycles and normal ovaries who delivered at the Kuopio University Hospital between January 1999 and December 1999.

Reference population (n = 649) (Study III) including both men and women, the latter of unknown PCOS status, were derived from the same geographical (eastern Finland) area (Pihlajamäki et al. 2000).
**Diagnostic criteria for PCOS**

In studies I and II, PCOS was diagnosed so that in addition to the polycystic ovary ultrasound finding and anovulation, one or more of the following features were present: clinical marker of hyperandrogenism, anamnestic fact about infertility or earlier diagnosed PCOS (patient record). PCOS were found in 11 (13.1 %) of MBS group, in 9 (15.3 %) of the simplex obese group and in 7 (13.2 %) of the lean control group women.

In studies III-VI, the diagnosis of PCOS was based on an observation of anovulation and polycystic ovaries in ultrasound (≥ 8 (Oulu) – 10 small (<10 mm) subcapsular follicles in either ovary and increased stroma) and at least one of the following symptoms: clinical manifestations of hyperandrogenism such as hirsutism scored according to Ferriman and Gallwey, infertility, laboratory testing revealing androgen excess and an elevated LH/FSH ratio and exclusion of other reasons of anovulation and hyperandrogenism such as hypothyroesosis, hyperprolactinemia, hypercortisolism and late onset of congenital adrenal hyperplasia.

The design and size of the studies are summarized below in Table 7.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Size</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Cross-sectional population-based study</td>
<td>92 women with MBS 59 simplex obese controls 53 lean controls</td>
<td>Lipid/glucose profile Gynecological history/findings</td>
</tr>
<tr>
<td>II</td>
<td>Cross-sectional population-based study</td>
<td>63 premenopausal MBS women with NCEP 88 controls</td>
<td>Androgenic sex profile</td>
</tr>
<tr>
<td>III</td>
<td>Retrospective case-control</td>
<td>133 PCOS women 115 healthy controls</td>
<td>Pro12Ala polymorphism of PPARγ</td>
</tr>
<tr>
<td>IV</td>
<td>Retrospective case-control</td>
<td>112 PCOS women 115 healthy controls</td>
<td>2 SNP of EPHX</td>
</tr>
<tr>
<td>V</td>
<td>Retrospective case-control</td>
<td>58 PCOS women 91 healthy controls</td>
<td>ApoE allele and genotype frequencies</td>
</tr>
<tr>
<td>VI</td>
<td>Retrospective case-control</td>
<td>87 PCOS women 115 healthy controls</td>
<td>C-850T polymorphism of TNFα</td>
</tr>
</tbody>
</table>
4.2 Methods

4.2.1 Patient History

A structured questionnaire soliciting the social and medical history was distributed to all participants in studies I and II. Information about gynecologic history, smoking, use of alcohol, chronic diseases, and medication was obtained. Subjects who were smoking daily were recorded as smokers. Patient records were reviewed with respect to diseases and operations.

4.2.2 Clinical examination

All 204 subjects from the population sampling frame were examined and personally interviewed with the questionnaire by the same gynecologist (SK) in 1996 and 1997.

The blood pressure was taken twice at two-min intervals with the subject seated after she had rested for 15 minutes. Systolic and diastolic blood pressure were measured with a calibrated manometer from the right arm and recorded to the nearest 2 mmHg. Blood pressures were defined as the points of the appearance and disappearance of Korotkoff sounds, respectively (level V). The result of the latter measure was recorded.

For anthropometric measurements, subjects were examined wearing light clothes. BMI was calculated as Weight (kg)/Height (m²). Waist and hip circumferences were measured to the nearest centimeter with a tape measure while the subject was standing. The waist was measured at the smallest girth midway between the lowest rib margin and the iliac crest, and the hip circumference was measured at the level of the greater trochanter to calculate the waist/hip ratio.

The clinical features of acanthosis nigricans and androgenic alopecia were assessed.

Hirsutism was classified according to the Ferriman-Gallwey score (Ferriman and Gallwey 1961).

Endometrial evaluation was carried out by taking an endometrial sample with a special brush (Ori endometrial brush; Medical AB, Malmö, Sweden). Whenever
possible, both histologic and cytologic evaluation were performed by a pathologist in the Kuopio University Hospital Department of Pathology.

4.2.3 Vaginal ultrasonography

Transvaginal ultrasonography of the ovaries and uterus was performed with 5.0-MHz Aloka Echo Camera SSD-500, Aloka Company, Tokyo, Japan. The form and dimensions of the uterus and the thickness of the endometrium were assessed. The morphologic features and dimensions of ovaries were determined. Ovaries were considered as polycystic if there were ≥ 10 small (< 10 mm) subcapsular follicles in either ovary.

4.2.4 Laboratory assays

Study population of studies I and II, visited the laboratory three times: the first visit was after an overnight fast in the early follicular phase (days 3 - 7) or on the next day after examination when there was amenorrhea. Blood samples for lipids and FSH, LH, E2, E1, T, A, DHEAS, SHBG, prolactin (prol), leptin, THS and first cortisol were collected. Postmenopausal hormone replacement therapy, if used (4/16, 6/17 and 6/9 of MBS, obese and lean women, respectively) was stopped 1 - 2 month before blood tests. The 2-hour oral glucose tolerance test was performed with 75 g glucose (Nutricia Nederland BV, Zoetermeer, The Netherlands) according to the WHO recommendations. The second cortisol level was measured after a low dose (1 mg) overnight dexamethasone. Samples for assay of P were collected three weeks later.

Serum samples, except lipids were frozen at ~20°C until analyzed. Details of the assays used are given in Table 8. All assays were performed according to the instructions of the reagent manufacturers.

The free androgen index (FAI) was calculated of the quotient at testosterone (nmol/l)/ SHBG (nmol/l) x 1000. The unbound testosterone concentration in serum was based on the formula testosterone x 10 x (2.28 - 1.38 x log (SHBG (nmol/l)/ 10)) according to Anderson et al. (1974).
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method</th>
<th>Reference range</th>
<th>C.V. of intrassay variation (%)</th>
<th>C.V. of interassay variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>ILMA</td>
<td>F: 3-12 U/L postm. &gt;25</td>
<td>3.5</td>
<td>5.3</td>
</tr>
<tr>
<td>LH</td>
<td>MEIA</td>
<td>F: 4-12 U/L postm. &gt;10</td>
<td>4.1</td>
<td>5.8</td>
</tr>
<tr>
<td>F₁</td>
<td>RIA</td>
<td>F: 0.09-0.35 nmol/L</td>
<td>4.8</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Postm. &lt; 0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E₁</td>
<td>RIA</td>
<td>E: 60-300 pmol/L</td>
<td>4.3</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L: 80-900 pmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Postm. &lt; 2x0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>RIA</td>
<td>0.8-3.5 nmol/L</td>
<td>5.0</td>
<td>6.4</td>
</tr>
<tr>
<td>SHBG</td>
<td>IRMA</td>
<td>30-90 nmol/L</td>
<td>3.2</td>
<td>1.58-8.33</td>
</tr>
<tr>
<td>A</td>
<td>RIA</td>
<td>3-10 nmol/L</td>
<td>5.2</td>
<td>8.0</td>
</tr>
<tr>
<td>DHEAS</td>
<td>RIA</td>
<td>1.8-12 µmol/L</td>
<td>6.6</td>
<td>7.5</td>
</tr>
<tr>
<td>Prog</td>
<td>RIA</td>
<td>L: 7-68 nmol/L</td>
<td>4.3</td>
<td>5.0</td>
</tr>
<tr>
<td>Cortisol</td>
<td>RIA</td>
<td>180-682 mmol/L</td>
<td>3.0</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>121-390</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prol</td>
<td>ILMA</td>
<td>40-470 mU/L</td>
<td>7.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Leptin</td>
<td>RIA</td>
<td></td>
<td>5.0</td>
<td>6.0</td>
</tr>
<tr>
<td>TSH</td>
<td>TR FIA</td>
<td>0.4-5.0 mU/L</td>
<td>2.5</td>
<td>2.6</td>
</tr>
<tr>
<td>B-Gluc</td>
<td>EA</td>
<td>3.3-5.0 mmol/L</td>
<td>0.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Insulin</td>
<td>RIA</td>
<td>5-20 mU/L</td>
<td>5.2 (&lt;20)</td>
<td>5.7 (13.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.6 (&gt;20)</td>
<td>6.3 (107.4)</td>
<td></td>
</tr>
<tr>
<td>Chol</td>
<td>FA</td>
<td>3.6-6 mmol/L</td>
<td>1.5 (5.1 mmol/L)</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.8 (7.2 mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-c</td>
<td>EA</td>
<td>1.07-2.25 mmol/L</td>
<td>2.6 (0.82 mmol/L)</td>
<td>4.1 (0.84)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3 (1.62 mmol/L)</td>
<td>3.8 (1.69)</td>
<td></td>
</tr>
<tr>
<td>Trigly</td>
<td>EA</td>
<td>0.6-2.2 mmol/L</td>
<td>0.8 (2.52 mmol/L)</td>
<td>1.6 (2.45)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5 (1.26 mmol/L)</td>
<td>5.2 (1.25)</td>
<td></td>
</tr>
</tbody>
</table>
Insulin resistance was also estimated by a recently validated quantitative insulin sensitivity check index (QUICKI) based on fasting insulin and glucose concentrations \((\log(\text{insulin}) + \log(\text{glucose}))^{-1}\) (Katz et al. 2000).

**Polymerase chain reaction (PCR) analysis**

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood lymphocytes using a standard phenol-chloroform extraction method. PCR and single strand conformation polymorphism (SSCP) was used in study III and PCR and restriction fragment length polymorphism analysis (RFLP) in studies IV-VI. Primers and restriction enzymes used in analyses are described in Table 9.

<table>
<thead>
<tr>
<th>Study</th>
<th>Restriction enzyme</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>III PPARγ</td>
<td></td>
<td>5'-GACAAATATCACTGTAATTACAGC-3' and 5'-CCCAATAGGGTAATGGAAG-3'</td>
</tr>
</tbody>
</table>
| IV EPRIH |  | 1) 5'-GGG GTG CAT TGT TAT TTT CCT CC-3' and 5'-CAG CTT TAG TCT TGA AGT GAC GGT-3',
 | 1) Ty113His in exon 3 | Thh111 I |
 | 2) His139Arg in exon 4 | Rsal |
 | 2) 5'-TTCT GCC AGA GCC TGA CCG TGC-3' and 5'-ATG GAA CCT CTA GCA GCC CCC TAC C-3' |
| V ApoE | Hha I | 5'–GCACGGCTGTCCAAGGAGCTGCAGGC-3' and 5'-GGCGCTCGCGGATGCGCTGAG-3' |
| VI TNFα | Hinc II | TP203FM (mismatch) and TPO306R |

The PCR conditions are described in detail in each original article.

**4.3 Statistical analysis**

The statistical analyses described in Table 10 in studies I - VI were performed using SPSS for windows (SPSS, Chicago, IL) version 8.0 (study I), version 9.0 (III -VI) and version 10.1 (study II). Data are shown as the mean ± SEM or simple percentages. Normal distribution of the variables was tested with Kolmogorov-Smirnov test. Triglyceride, glucose, insulin concentrations, testosterone, SHBG, free testosterone and
FAI were corrected for skewing using log transformation, but are presented using untransformed values (studies I, II). Sample size and power determinations were performed using nQuery Advisor 4.0 (study VI) and 3.0 (study IV) software (Statistical Solutions, Sangus, Ma, USA). Hardy-Weinberg equilibrium was assessed using Associate program, version 2.31 (studies IV - VI) and in study III by using Genepop web version 3.1c (http://wbiomed.curtin.edu.au/genepop/). The an expectation-maximization algorithm to obtain maximum-likelihood estimates was assessed using Arlequin ver 2000 software in study IV as well as pairwise linkage disequilibrium (LD) analyses.

<table>
<thead>
<tr>
<th>Table 10. Statistical analyses used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
</tr>
<tr>
<td>1-way analysis of variance</td>
</tr>
<tr>
<td>Scheffe / Tambane</td>
</tr>
<tr>
<td>$\chi^2$-test/Fisher</td>
</tr>
</tbody>
</table>
5 RESULTS

5.1 Population-based studies (Studies I and II)

MBS prevalence increased with age so that until perimenopause it was highest (Table 11), whereas the incidence of PCOS decreased with age.

<table>
<thead>
<tr>
<th>Age</th>
<th>34 y (%)</th>
<th>39 y (%)</th>
<th>44 y (%)</th>
<th>49 y (%)</th>
<th>54 y (%)</th>
<th>Together</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population (n)</td>
<td>67</td>
<td>121</td>
<td>124</td>
<td>155</td>
<td>76</td>
<td>543</td>
</tr>
<tr>
<td>Overall MBS</td>
<td>8 (11.9)</td>
<td>11 (9.0 )</td>
<td>15 (12.1)</td>
<td>33 (21.3)</td>
<td>18 (23.7)</td>
<td>85</td>
</tr>
<tr>
<td>MBS with PCOS</td>
<td>6 (9.0)</td>
<td>3 (2.5)</td>
<td>3 (2.4)</td>
<td>1 (0.6)</td>
<td>1 (1.3)</td>
<td>14</td>
</tr>
<tr>
<td>MBS without PCOS</td>
<td>2 (3.0)</td>
<td>8 (6.6)</td>
<td>12 (9.7)</td>
<td>32 (20.6)</td>
<td>17 (22.4)</td>
<td>71</td>
</tr>
<tr>
<td>PCOS without MBS</td>
<td>5 (7.5)</td>
<td>6 (5.0)</td>
<td>2 (1.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>13</td>
</tr>
</tbody>
</table>

5.1.1 Study I

With two-phase screening, we could identify the target female population, i.e., they were more abdominally obese, had higher blood pressure, dyslipidemia, higher plasma insulin levels and abnormal glucose tolerance was more common than in simplex obese and the lean control group; these women could be considered to have metabolic syndrome (Table I/study I). Summarized clinical and endocrine characteristics of the study population divided among menopausal status are shown in Tables 12 and 13.
Table 12. Summarized clinical and endocrine characteristics of the study groups in premenopausal women

<table>
<thead>
<tr>
<th></th>
<th>MBS group (n = 68)</th>
<th>Obese group (n = 41)</th>
<th>Lean group (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.3 ± 0.67</td>
<td>43.9 ± 0.9</td>
<td>41.3 ± 0.9</td>
</tr>
<tr>
<td>Body mass index kg/m²</td>
<td>31.± 0.1/6*</td>
<td>28.6 ± 0.4/#</td>
<td>22.5 ± 0.5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>99.9 ± 1.5*</td>
<td>87.9 ± 1.1/6#</td>
<td>74.3 ± 0.8</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.92 ± 0.005*</td>
<td>0.82 ± 0.007/#</td>
<td>0.78 ± 0.006</td>
</tr>
<tr>
<td>NIDDM in first degree relative</td>
<td>29 (44%)*</td>
<td>13 (32%)#</td>
<td>1 (2.4%)#</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>142.6 ± 2.0**</td>
<td>131.9 ± 2.2</td>
<td>123.3 ± 2.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>87.5 ± 1.2*</td>
<td>82.6 ± 1.3#/</td>
<td>74.5 ± 1.2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.5 ± 0.11*</td>
<td>5.3 ± 0.15</td>
<td>4.9 ± 0.12</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.73 ± 0.09**</td>
<td>1.17 ± 0.10</td>
<td>0.88 ± 0.08</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.28 ± 0.04*</td>
<td>1.36 ± 0.05</td>
<td>1.43 ± 0.05</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.6 ± 0.09*</td>
<td>5.3 ± 0.07/#</td>
<td>5.0 ± 0.07*</td>
</tr>
<tr>
<td>2 h glucose (mmol/l)</td>
<td>6.1 ± 0.23*</td>
<td>5.5 ± 0.21/#</td>
<td>4.8 ± 0.17*</td>
</tr>
<tr>
<td>Fasting plasma insulin (mU/l)</td>
<td>11.9 ± 0.9*</td>
<td>9.1 ± 0.6/#</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>2 h insulin (mU/l)</td>
<td>57.8 ± 6.7*</td>
<td>35.8 ± 3.4</td>
<td>26.3 ± 1.8</td>
</tr>
<tr>
<td>FSH</td>
<td>13.0 ± 2.0</td>
<td>15.6 ± 3.0</td>
<td>8.6 ± 1.0</td>
</tr>
<tr>
<td>LH</td>
<td>10.2 ± 1.9</td>
<td>11.2 ± 2.5</td>
<td>6.1 ± 0.9</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>0.82 ± 0.07</td>
<td>0.74 ± 0.06</td>
<td>0.78 ± 0.07</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>37.8 ± 3.1*</td>
<td>46.8 ± 3.0/#</td>
<td>59.3 ± 4.0*</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.38 ± 0.06</td>
<td>1.32 ± 0.07</td>
<td>1.35 ± 0.08</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>20.90 ± 1.14*</td>
<td>17.29 ± 0.93</td>
<td>16.46 ± 1.07*</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>7.4 ± 0.3</td>
<td>6.7 ± 0.3</td>
<td>6.9 ± 0.4</td>
</tr>
<tr>
<td>DHEAS</td>
<td>4.8 ± 0.3*</td>
<td>4.1 ± 0.3</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>FAI</td>
<td>52.0 ± 6.1*</td>
<td>30.6 ± 2.1</td>
<td>26.9 ± 2.3*</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.18± 0.02</td>
<td>0.17 ± 0.02</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>Estrone</td>
<td>246.2 ± 12.6*</td>
<td>209.4 ± 10.3</td>
<td>204.9 ± 11.9*</td>
</tr>
<tr>
<td>Progesterone (mmol/l)</td>
<td>12.6 ± 1.6*</td>
<td>14.6 ± 2.3</td>
<td>21.5± 2.9</td>
</tr>
</tbody>
</table>

*significant difference between MBS group and lean group
•between MBS group and obese group
#between obese and lean group. Values are expressed as mean ±SEM
Table 13. Summary of the clinical and endocrine characteristics of the study groups in peri- and postmenopausal women

<table>
<thead>
<tr>
<th></th>
<th>MBS group (n = 16)</th>
<th>Obese group (n = 17)</th>
<th>Lean group (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.5 ± 0.53</td>
<td>52.2 ± 0.6</td>
<td>53.0 ± 0.7</td>
</tr>
<tr>
<td>Body mass index kg/m²</td>
<td>30.9 ± 1.5        *</td>
<td>29.2 ± 0.7</td>
<td>22.5 ± 0.9</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.2 ± 3.4        *</td>
<td>89.2 ± 1.7</td>
<td>75.7 ± 2.7</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.89 ± 0.01       *</td>
<td>0.83 ± 0.01</td>
<td>0.79 ± 0.02</td>
</tr>
<tr>
<td>NIDDM in first degree</td>
<td>10 (59 %) *</td>
<td>5 (31 %)</td>
<td>1 (10 %)</td>
</tr>
<tr>
<td>relative Systolic blood pressure (mmHg)</td>
<td>150.0 ± 4.3</td>
<td>143.2 ± 5.2</td>
<td>135.6 ± 5.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>90.2 ± 2.4</td>
<td>86.4 ± 2.4</td>
<td>85.2 ± 1.6</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.6 ± 0.19       *</td>
<td>6.29 ± 0.19</td>
<td>6.1 ± 0.52</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.74 ± 0.29</td>
<td>1.48 ± 0.18</td>
<td>1.01 ± 0.09</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.37 ± 0.07</td>
<td>1.29 ± 0.05</td>
<td>1.84 ± 0.19</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.4 ± 0.13</td>
<td>5.2 ± 0.10</td>
<td>5.13 ± 0.2</td>
</tr>
<tr>
<td>2 h glucose (mmol/l)</td>
<td>6.7 ± 0.33       **</td>
<td>5.2 ± 0.39</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>Fasting plasma insulin (mU/l)</td>
<td>11.8 ± 1.4       *</td>
<td>9.4 ± 0.9</td>
<td>6.1 ± 0.9</td>
</tr>
<tr>
<td>2 h insulin (mU/l)</td>
<td>70.3 ± 9.7        **</td>
<td>36.4 ± 6.8</td>
<td>28.1 ± 6.5</td>
</tr>
<tr>
<td>FSH</td>
<td>45.9 ± 6.7</td>
<td>47.1 ± 6.7</td>
<td>50.3 ± 8.0</td>
</tr>
<tr>
<td>LH</td>
<td>31.7 ± 3.9</td>
<td>375 ± 3.8</td>
<td>41.7 ± 7.0</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>0.92 ± 0.2</td>
<td>0.76 ± 0.06</td>
<td>0.88 ± 1.0</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>42.6 ± 4.8</td>
<td>46.1 ± 4.7</td>
<td>70.5 ± 5.6</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>3.3 ± 0.2</td>
<td>1.2 ± 0.08</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>18.7 ± 7.1</td>
<td>16.1 ± 1.7</td>
<td>9.9 ± 1.4</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>5.0 ± 0.6</td>
<td>5.12 ± 0.5</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>DHEAS</td>
<td>2.9 ± 0.2</td>
<td>3.3 ± 0.4</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>FAI</td>
<td>32.0 ± 3.7       *</td>
<td>30.5 ± 4.7</td>
<td>12.9 ± 2.1</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.26 ± 0.1</td>
<td>0.12 ± 0.04</td>
<td>0.08 ± 0.05</td>
</tr>
<tr>
<td>Estrone</td>
<td>298.0 ± 64.4</td>
<td>198.3 ± 26.0</td>
<td>157.5 ± 25.6</td>
</tr>
<tr>
<td>Progesterone (mmol/l)</td>
<td>1.2 ± 0.6</td>
<td>0.6 ± 0.4</td>
<td>0.2 ± 0.2</td>
</tr>
</tbody>
</table>

*a*significant difference between MBS group and lean group;  
*between MBS group and obese group  
#between obese and lean group. Values are expressed as mean ±SEM

Women with MRS have an increased free androgen index (FAI) as compared to simplex obese and lean control groups. SHBG levels were significantly decreased in MBS and obese groups compared to lean control group (Table I/Study I).

There were no differences in the mean age at menarche between the groups (Table II/Study I). The occurrence of irregular cycles in their late adolescence also did not differ. In adulthood, however oligomenorrhea was a more common disorder in the
group with MBS compared to others (p = 0.03). When MBS was defined by using more stringent criteria, oligomenorrhea seemed to be more common. Furthermore, hypermenorrhea was a more common complaint in these subjects (p = 0.001).

Most of the women in each group were living in a stable sexual partnership and there were no differences in parity and infertility problems. With respect to frequency of miscarriages there were no significant differences between the groups.

Evaluation of the patient’s records showed that the frequencies of previous endometrial hyperplasia were similar in the groups (Table III / Study I). In addition, there were no differences in the frequency of hysterectomy.

Transvaginal ultrasonographic evaluation showed that frequency of myomas was comparable in all groups. Polycystic like ovaries were not found more commonly in the MBS women than in the other groups (Table IV/Study I).

Cutaneous markers of insulin resistance, such as alopecia areata and acanthosis nigricans, were detected only in the group with MBS. Hirsutism and acne tended to be more common in the MBS and obese groups than among lean women (Table IV/Study I).

The number of peri- and postmenopausal women was comparable among the 3 groups. The onset of climacteric symptoms occurred at a similar age (mean 47 y, Table II /Study I). Only some of the postmenopausal women in MBS group (7/19) and in the obese group (7/17) used HRT for climacteric symptoms, whereas the use of hormones tended to be more common among lean subjects (7/10).

Endometrial samples with a cytobrush appeared to be representative in 122/131 (93.2 %) of cases, and histological evaluation was possible in 104/122 (85.2 %) of the samples. In about every third case in the MBS (32 %) and obese (29 %) groups, the histological finding of the endometrium did not correspond to the cycle, whereas this kind of discordance was a rare finding among lean controls (14 %). Hyperplasia was not detected in any of the 122 representative endometrial samples evaluated. In one case, there were atypical cells (PAP smear class II) and in two individuals a decidual reaction due to an intrauterine device was present.

The frequency of current smokers was highest in the lean control group (40.0 %) compared to both the MBS and simple obese groups (22 % and 19 %, p <0.05,
respectively). Light-to-moderate consumption of alcohol (1 - 50 portion/month) was reported by 55 (65.5%) in the MBS group, 43 (74.1%) in the obese group and 47 (94.0%) in the lean group. High alcohol consumption (> 51 - 100 portions/month) was found only in the MBS group (5%).

5.1.2 Study II

In this study MBS was considered with NCEP criteria. Only premenopausal women were included. Table 1/study II shows that premenopausal MBS women were more obese, dyslipidemic, hypertensive and insulin resistant than controls. Although only three criteria were demanded, the groups differed significantly in all of the examined criteria. Controls were handled as an intact group and not divided to simplex obese and lean as in study I.

MBS women presented with a marked decrease in insulin sensitivity index and this was associated inversely with waist circumference ($r = -0.65$) (Figure 7).

![Figure 7. The mean insulin sensitivity index (\( (\log (\text{insulin}) + \log (\text{glucose})^{1/3} \)) in MBS and control groups according to the tertiles of each group’s waist circumference. P <0.002 between top vs. mid tertile; p <0.001 between top vs. low tertile and between mid vs. low tertile within the MBS group. P = 0.01 for low vs. top tertile in controls.](image-url)
There were no statistically significant differences between the gonadotropin levels in the MBS and control group. Estrone levels were significantly higher in the MBS group than in controls.

Total testosterone, androstenedione and estradiol levels did not differ between the groups. Mean DHEAS level was significantly higher in MBS group than in controls. The mean SHBG level was significantly lower, whereas free testosterone levels and the FAI were significantly higher among MBS women than in the control group.

FAI correlated significantly with waist circumference ($r = 0.26$), but this correlation disappeared when controlled for insulin sensitivity index. When analyzed in tertiles, MBS women had higher FAIs, although the increase was not strictly linear in women with waist circumference exceeding 95 cm (Figure 8).

![Figure 8](image)

**Figure 8.** Free androgen index (FAI) in MBS and control groups according to the tertiles of each group’s waist circumference.

Table 3 of study II shows that waist circumference $> 88$ cm, insulin $> 11.4$ mU/L, fasting glucose $\geq 6.1$ mmol/l, diastolic blood pressure $\geq 85$ mmHg, and triglyceride $\geq 1.7$ mmol/L were associated with the risk of hyperandrogenism, whereas in multiple logistic regression analyses only waist $> 88$ cm and diastolic blood pressure $\geq 85$ mmHg were significantly associated with the risk of hyperandrogenism.
5.2 Genetic association studies (Studies III – VI)

Genotype and allele distributions are presented in each of the original studies: table 2 / study III; table 2 / study IV; table 2 / study V and table 2 /study VI. Table 3 /study IV shows estimated haplotype frequency distributions of EPHX gene exon 3 and 4 polymorphisms in chromosomes from women in the PCOS and control group.

Study III. Pro124Ala polymorphism of PPARγ (located 3p25). There was a statistically significant different allele distribution between PCOS groups and in the controls. The frequency of the Ala isoform was reduced in PCOS women (12.6 % v. 19.1. %) (p = 0.045), at an OR of 0.609 (95 % CI:0.374-0.991). The frequency in controls corresponded well to the population frequency (16.0 %).

The genotype distribution was of borderline significance in the PCOS group and in the controls (P=0.051). Using the control group as a reference, pooled Pro/Ala and Ala/Ala were associated with protection against PCOS at a borderline level of significance (p = 0.055) and an OR of 0.581 (95 % CI:0.333-1.014). The mean BMIs of genotypes did not differ in the PCOS group.

PPARγ genotypes were found to be in Hardy-Weinberg equilibrium in both the study and control groups.

Study IV. Two single nucleotide polymorphisms of EPHX (located in 1q42.1). In association of EPHX SNP at exon 3 (T >C; Tyr113His) and at exon 4 (A >G; His139Arg) there were no significant differences in genotypes and alleles. However, the G allele of the exon 4 (Arg139) was slightly over-represented in the PCOS group (20%) compared with the control group (14 %) without achieving statistical significance. To further explore this finding with haplotype estimation analysis, we could demonstrate that the low activity haplotype C-G (His113-Arg139) was significantly over-represented in the PCOS group (p = 0.026; OR 2.28 (95 % CI: 1.1-4.8). Pairwise LD analysis between exon 3 and 4 loci revealed no LD for the pairs of loci in the PCOS group or in the controls.

Study V. ApoE allele and genotype (located 19q13.2). The frequency of the apo e4 allele was similar in PCOS women and in controls (17.2 % v. 18.7 %), which corresponded well with the population frequency. None of the apoE genotypes was
significantly over-represented. When PCOS women were divided into subgroups according to the number of ε4 alleles, there was still no difference either within the PCOS women or between PCOS and controls. An equilibrium state between apoE alleles and genotypes was established in both PCOS and control groups.

**Study VI. C850T polymorphism of TNF-α (located in 6p21.3).** A similar C and T allele distribution for the C-850T polymorphism was observed in the PCOS women and in controls, with the frequency of the variant T allele being 8.6 % in the PCOS women compared to 9.6 % in the control group (p = 0.862). Accordingly, the profile of genotype frequencies was similar in the groups. The OR for PCOS associated with the pooled TT and CT genotypes was 0.88 (95% CI 0.41-1.91). Power analysis based on data from the genotypes frequencies showed that one would need to examine more than 5495 patients and controls to have an 80 % chance of finding a difference between TNF-α frequencies at a significance level of p<0.05.
6 DISCUSSION

The diagnostic criteria for MBS are primarily intended for screening people at an increased risk of type 2 diabetes and cardiovascular disease and aims at primary prevention of these endpoints. In general, high-risk people are elderly, but insulin resistance and obesity now occur increasingly in younger people. Prevention is desirable, since the features of the MBS can be present for up to ten years before detection of the glycemic disorders.

PCOS as diagnostic entity is a common denominator for chronic anovulatory and hyperandrogenemic states of reproductive-age women after exclusion of specific diseases of the pituitary, ovary and adrenal glands (Kelly et al. 2000). Overlapping in the phenotypes of MBS and PCOS women has raised the question of their interrelationship.

In two population-based studies, we wanted to clarify the relation between these two entities. The hypothesis was that disorders that were similar to PCOS would be concentrated especially in women with MBS, because emerging evidence suggests that insulin resistance may have a primary etiologic role in PCOS. This specific question has not been previously evaluated. Although without doubt there are problems with the changing criteria of MBS as also with PCOS, the results were surprising, i.e., the common view that the typical phenotype of PCOS overlaps with the phenotype of MBS, was not confirmed, i.e. PCOS did not concentrate among MBS women instead being found also among females with no signs of MHS. Based on this finding we were justified to classify PCOS as its own entity and thus secondly we wanted to clarify the pathogenesis of PCOS with different genetic association studies.

6.1 Population-based studies

Studies I and II were cross-sectional population-based studies where the subjects were screened from the target population aged 34 to 54 years in a defined area in eastern Finland. Criteria for MBS in study I were self-constructed as described earlier by Vanhala et al. 1997, since previously there were no internationally accepted criteria for
MBS. In this study about one fifth of the women (19 %) fulfilled the criteria for metabolic syndrome. This is somewhat less than in USA (Table 2. Ford et al. 2002) using NCEP criteria in women. In Finland the prevalence of MBS in middle-aged men was 27.7-24.9 % with WHO criteria and 11.4-13.7 % with NCEP criteria (Table 2). If we used more the stringent criteria, the prevalence in our women would be 8 %.

These studies have advantages over many others studies since they were population based, representing different age groups, examined by one gynecologist. Although the clamp technique was not used for measuring insulin resistance, we did detect a population with reduced insulin sensibility. Hyperinsulinemia as a surrogate for insulin resistance has been considered to be valid for epidemiological studies (Laakso 1993) and QUICKI, which we used has been suggested to even improve the estimation of insulin resistance (Katz et al. 2000). Although only 3 criteria were required, the group with MBS differed from the lean control group in all eight characteristics and also from the group with simple obesity.

The study population was studied comprehensively, thus, we were able to rule out other clinical diseases.

In study II we had to change the criteria to NCEP criteria and analyzed only premenopausal women. One advantage with this change was that NCEP criteria are internationally accepted. A positive finding was that the hormonal profiles of the subjects were comparable despite the changing diagnostic criteria.

6.1.1 Phenotype of MBS women has similarities to phenotype of PCOS women without being the same entity

The working hypothesis was that symptoms especially associated with PCOS would be more common among MBS women than in controls, i.e., obese subjects without MBS and lean healthy women. Although anovulatory periods and mild hyperandrogenism seen in blood tests were more common features among MBS women, actually no other features for PCOS were detected. We could not find any difference between the MBS group and the control groups with respect to menarche and irregular menstrual periods before the age of 20 years. This phenomenon differs from the onset of PCOS, which often occurs at menarche, and is characterized by a failure to establish a regular pattern
of menses. Furthermore there were no differences regarding fertility or obstetric outcome among the study groups, even though MBS women appeared to be more obese already at a young age. Even the prevalence of polycystic ovaries on ultrasound examination was about the same in all female groups. Clinical hyperandrogenism presenting as acne and hirsutism were also not more prevalent.

This study was cross-sectional but it demonstrated that MBS women had quite normal ovarian function until the time when they gained body weight and developed increased insulin resistance but only a part of these women revealed gynecological disturbances. Oligomenorrhea with anovulatory periods was associated with central obesity, since no difference between women with peripheral obesity and lean controls was found. Similar findings regarding the association between upper body obesity and anovulation have been reported (Moran et al. 1999; Bray 1997). Even when stricter criteria for MBS were used, i.e., the distinct group presenting both with abnormal glucose metabolism and dyslipidemia, over 70% of the women with MBS did not show the classical PCOS phenotype, only oligomenorrhea (46%). Oligomenorrhea has been associated cross-sectionally with insulin resistance and type 2 diabetes among Pima Indian women (Roumain et al. 1998), although the increased risk was not statistically significant after adjustment for obesity. In the Nurses health prospective study (Solomon et al. 2001) women with long or highly irregular menstrual cycles at age 18 to 22 years had a significantly increased the risk of developing type 2 diabetes that was not completely explained by obesity. Further, within PCOS cases, the ultrasound appearance of polycystic ovaries did not appear to further intensify the cardiovascular disease risk profile in these women (Loucks et al. 2000). In a longitudinal study, Tapvenen et al. (2003) showed that self-reported symptoms of oligomenorrhea and/or hirsutism could detect women with typical endocrine features of PCOS and signs of decreased insulin sensitivity.

The finding that oligomenorrhea is the major gynecologic disturbance in MBS women provides support for the concept that oligomenorrhea is the only important gynecologic symptom which associates with insulin resistance and subsequent risk factors for health in later life. The ovarian ultrasound findings are important, especially in fertility treatment since women with polycystic ovaries exhibit a risk of
hyperstimulation. In older women and in women approaching menopause, ovarian findings do not have any major clinical significance. According to this study, the ultrasound findings did not help us to identify women with obvious MBS. One factor influencing the findings is also the age distribution of the women examined, most being over 35 years old. There is data showing that polycystic ovaries are more rare in older women (Koivunen et al. 1999). PCOS women also are thought to obtain more regular cycles with increasing age (Elting et al. 2000; Winters et al. 2000). On the other hand, a PCO finding as such hardly is not sufficient to consider that an individual has an increased risk for CVD.

6.1.2 Hyperandrogenism in MBS women

Although the total testosterone concentration was not increased and the free testosterone level only marginally, the elevated FAI seemed to be an essential component of female MBS. Further, adrenal DHEAS levels were higher in MBS group than in controls. The finding that the clinical features of hyperandrogenism (hirsutism and acne) were not more common in MBS women than in controls, indicates that hyperandrogenism in MBS women is generally mild. However, the cutaneous markers of hyperinsulinemia (alopecia areata and acanthosis nigricans) were also only rarely detected in the MBS group. Acanthosis nigricans occurs most commonly in association with hyperinsulinemia (Torley et al. 2002).

There is no data on sex hormone profile of MBS women fulfilling NCEP or WHO criteria. A number of studies have assessed androgen levels in women with central obesity. However, different measures for localization of fat have posed problems. In our study, women having the same waist circumference or insulin sensitivity index were more hyperandrogenic in the MBS group than in the control group. These results may explain why previous investigators have reported contradictory results concerning the effect of obesity on the sex hormone profile. When waist circumference reached 94 cm, the association to FAI no longer appeared to be linear.

Several mechanisms associated with the actions of insulin can explain the altered steroid metabolism favouring hyperandrogenism in MBS. Insulin, as such, acts both on
the ovaries and adrenals to increase androgen secretion (Willis and Franks 1995; Moghetti et al. 1996). Studies on hyperandrogenic women with PCOS have shown that even in a generalized insulin resistant state with accompanying hyperinsulinemia, the ovaries continue to be sensitive to insulin. On the other hand, there is no conclusive evidence to show that ovarian androgen production is clearly elevated in women with MBS. If hyperinsulinemia were responsible for the hyperandrogenemia, one would expect that levels of androgens should increase with increasing insulin levels.

Insulin has also a direct inhibitory effect on SHBG synthesis and secretion in cultured hepatocytes (Botwood et al. 1995) and this can influence the expression of androgens. There is evidence that insulin may be the humoral mediator of the weight-dependent changes in SHBG concentrations, resulting in higher levels of free testosterone and estradiol. The mean SHBG level in MBS women was significantly lower than in controls in this study. The decreased SHBG levels in turn is known to predict type 2 diabetes in women (Haffner 1993). MBS is commonly the first step towards type 2 diabetes and results of this study demonstrating low SHBG levels in MBS women support this concept. The serum SHBG level has been claimed to be rather effective as a single marker for detecting women with PCOS (Escobar-Morreale et al. 2001; Taponen et al. 2003). Our finding suggests that low SHBG is also a marker of overall insulin resistance. This finding is in line with that of Reinecke et al. (2002) who found that postmenopausal women with coronary heart disease had lower SHBG levels. No associations of coronary heart disease with history of clinical symptoms indicating hyperandrogenism (e.g., oligomenorrhea, infertility, or hirsutism) were found in this study.

There is evidence, that obese women have altered glucocorticoid metabolism and disturbed feedback in hypothamus-pituitary axis. In our study there were no significant differences between the groups in cortisol levels after administration of one mg dexamethasone, but it is possible, that this test is not sufficiently sensitive at identifying mild dysfunction in the hypothamus-pituitary axis (Björntorp and Rosmond 2000).

The study of MBS in women requires follow-up research to clarify the association between hormonal and metabolic abnormalities. This study did not answer the question of whether hyperinsulinism or hyperandrogenism is the initiating event. However, this
study does show, that the elevated FAI seems to be an essential component of female MBS. Correspondingly, women with a history of preeclampsia, studied 17 years after their first pregnancy, had mild hyperandrogenism, elevated fasting insulin levels, and elevated blood pressure (Laivuori et al. 1996). Therefore, in gynecological practice, it is important to be aware of the markers of MBS, the cluster of insulin resistance, elevated blood pressure, elevated plasma glucose, a prothrombotic state and atherogenic dyslipidemia. Although central obesity was not included in the original definition of the MBS, later studies have emphasized its key role. Obesity is an increasing problem throughout of world, so it is clear that also the incidence of MBS will increase.

6.1.3 Effect of aging

This cross-sectional study is not able to demonstrate what happens when a women ages. However, the prevalence of MBS seems to be higher in older groups, whereas clinical PCOS cases become less frequent. Especially in women, changes reminiscent of MBS become common with advancing age. In menopause, the most dramatic change is the decrease in the estradiol plasma concentrations. DHEA and DHEAS decline steadily, but A and T decline just before or at the menopause (Longcope 1998). Aromatization of DHEA, A and T to estrone and estradiol increase with aging (Longcope 1998). The redistribution of fat is also age-related. The visceral fat depots increase, this could be related to an increase in androgenic activity in postmenopausal subjects. The activity of the growth-hormone-IGF-I-axis decreases, while cortisol levels remain constant (Casson et al. 1997). There are also unfavourable changes in lipid metabolism. Ferrannini et al. (1996) claimed that in healthy Europeans, age per se is not a significant cause of insulin resistance but other factors which could be considered as age-related changes in body composition do have effects on insulin action.

There are only limited data of what actually happens to PCOS women with aging. It seems that the symptoms of PCOS decrease and menstrual periods become more regular. In the cross-sectional study of Winters et al. (2000), the hyperandrogenism partly resolved before menopause. Similarly in the 6-18 y follow up study of 38 PCOS women, hyperinsulinemia and insulin resistance tended to worsen without any
deterioration of the hyperandrogenism (Pasquali et al. 1999). Long-term estrogen-
progestagen treatment countered this tendency to insulin resistance, probably because it
improved the pattern of body fat distribution, by reducing abdominal fat depots. In fact
there is no clear evidence confirming that PCOS women have a significantly increased
mortality from CVD among PCOS women (Pierpoint et al. 1998). It has been even
speculated that the relatively high and stable estrogen levels could protect the blood
vessels and support endothelial function.

6.2 Genetic association studies

There is evidence that PCOS is clustered in families and probably is inherited as a
complex trait. However, studies in families have revealed that even within individual
PCOS pedigrees, the clinical phenotype is very heterogenous in females and in males. It
is unlikely that one major gene is responsible for PCOS. Many factors, which
participate in the pathogenesis of this syndrome need to be evaluated. Clearly, relating
genotype to phenotype in an appropriate manner will be one of the future challenges in
diseases that result from varying susceptibility to a wide range or environmental factors
and which are mediated by many different genes. Family studies have highlighted the
importance of genetic factors in polycystic ovary syndrome but the precise mode of
inheritance and the molecular basis for the disorder remain uncertain. Overall we found
an association between PCOS and the PPARγ and EPHX genes, but we did not find
associations with the ApoE and TNF-α genes.

6.2.1 Subjects and methods of studies III - VI

One limitation of our studies is their relatively small sample size, which theoretically
increases the likelihood of a type II error. However, based on power analysis of studies
with negative association, it is unlikely that our findings are false negatives. In addition,
alleles and genotypes of our genetic studies were found to be in Hardy-Weinberg
equilibrium in both the PCOS and control groups. Population frequencies derived from
the same geographical area were used for comparison to increase the power in studies
III and V.
6.2.2 Association with PPARγ reflects insulin action in PCOS

We found that a functional variant, the Ala allele, which caused reduced transcriptional activity of the PPARγ gene, appeared to exert a protective effect against PCOS. This is support for the hypothesis that in Caucasians there is an overlap between PCOS and type 2 diabetes.

PPARγ is a member of the nuclear hormone receptor subfamily of transcription factors. The common Pro12Ala polymorphism has been interesting in the relation to diabetes and obesity. The Ala allele has been associated with a lower BMI in a sample taken from Japanese and Finnish populations (Deeb et al. 1998), and lowered risk for diabetes, indicated a 15% risk reduction for diabetes associated with the Ala allele in Caucasians in the meta-analysis (allele frequency 15%) (Altshuler et al. 2000). In some studies on Caucasian, there was no association with diabetes (Beamer et al. 1998; Mancini et al. 1999). There are inconsistent associations across populations: Female Canadian Oji-Cree Indians with Ala allele had a higher risk to diabetes than the non-carriers. Associations to obesity are also inconsistent. In some studies Ala allele has been associated with obesity, but not invariably (Busch and Hegele 2001). In our study, there was no difference in genotype frequencies and BMI.

The finding of Hara et al. (2002) that Caucasian PCOS women who had the Ala allele, although still substantially insulin resistant, were less insulin resistant than those with two Pro alleles, supports our results, although we did not analyze the data separately for PCOS women with varying degrees of insulin resistance.

The Ala allele of the PPARγ gene may be offer some protection against the development of PCOS when gene-environment interactions are taken into account. In other words, these results imply that the Pro allele increases the susceptibility for PCOS without being essential for its appearance. The Pro allele has been associated with a 1.25 fold risk of diabetes in Caucasians. Our finding on the association between Pro12Ala PPARγ polymorphism and the protection against PCOS might be of biological significance if one wishes to evaluate the risk for those who have attenuated insulin resistance to convert to full-blown type 2 diabetes.
6.2.3 Positive association between PCOS and EPHX gene

We found that in the EPHX gene, the G allele of the exon 4 SNP was more frequently present in PCOS women than in controls. Further, according to the haplotype estimation analysis we found that the haplotype C-G (His113-Arg139) was significantly associated with PCOS.

EPHX is located in 1q42.1 and it encodes for catalyzes the phase 1 hydrolysis of epoxides, playing a role in detoxification processes and in the metabolism of endogenous and exogenous compounds (Hartsfield et al. 1998). The genetic variability of this gene in the development of PCOS or in relation obesity, diabetes or dyslipidemia is an unexplored area. Polymorphism in the EPHX gene may have a role in the female reproductive system and modify the susceptibility to spontaneous abortion (Wang et al. 1998) and ovarian cancer (Lancaster et al. 1996). There is one study reporting an association of this gene polymorphism to preeclampsia risk (Zusterzeel et al. 2001).

Two single nucleotide polymorphisms (SNPs) have been described in the coding region of the EPHX1 gene that produce two protein variants (Hassett et al. 1994). These polymorphisms are thought to be linked to protein stability.

In women who have suffered spontaneous abortions, the low-activity variant in exon 3 and the high-activity wild-type genotype in exon 4 are more frequent than in controls, indicating that the two variants have opposite associations with spontaneous abortion (Wang et al. 1998). In preeclampsia, the high activity genotype in exon 3 enhances the risk of disease (Zusterzeel et al. 2001). Our finding that the low activity haplotype C-G (His113-Arg 139) was significantly associated with PCOS, may have a clear biological significance, since these both overrepresented genetic variations together result in an effect in the same direction, both reducing enzyme activity. This seems to be a functional variant and our result is support for the hypothesis that C-G in the EPHX gene is a disease-associated haplotype. The haplotype estimation analysis used in this study is based on empirical data drawn from the literature currently supporting the feasibility of this analysis for detecting an association (Xu et al. 2002). The association between genotypes and PCOS may reflect enzymatic mechanisms involved in steroidogenesis, although the exact mechanism remains speculative at this point.
Another interesting finding was that SNP allele frequencies of the EPHX gene vary considerably across human ethnic groups and populations. The frequencies in our groups were similar to those found in some other white populations, whereas in the Netherlands, the population frequencies have been found to be similar to those of Chinese subjects. There are no studies indicating that polymorphism in the EPHX gene could play any role in MBS, and thus it rather would be a characteristic related to PCOS.

6.2.4 Negative associations between ApoE and TNF-α and PCOS

In our cohort of PCOS women, the apoE ε4 allele was not more frequent than in healthy controls or in the general population from the same geographical area. ApoE polymorphism is one of the genetic determinants of serum cholesterol values. An association between apoE isoforms and plasma lipid levels has been reported in several populations, and the presence of ε4/4 and ε3/4 alleles is associated with increased cholesterol and LDH-cholesterol levels and increased risk for CVD (Miettinen 1991; Ilveskoski et al. 1999). This association to dyslipidemia could also be of biological significance in PCOS. However, we did not detect any difference in the frequency of the apoE ε4 alleles between PCOS women and healthy fertile women, which was close to the rate in the general population in our area. Thus, there does not seem to be a major role for the apoE genotype in the dyslipidemia associated with PCOS. This finding is similar to those reported from a cohort of German PCOS patients (von Eckardstein et al. 1996).

Further, we could not demonstrate any association between C-850T polymorphism located in the promoter region of the TNF-α gene and PCOS. TNF-α is a cytokine which is overexpressed in adipose and muscle tissues of obese animals and humans. TNF-α can induce insulin resistance by inhibition of tyrosine phosphorylation of the insulin receptor β chain and insulin receptor substrate –1. There is a positive correlation between the level of TNF-α mRNA in fat tissue and the level of hyperinsulinemia. In animal studies neutralizing of TNF-α leads to improved insulin sensitivity but this result has not been duplicated in humans. There are two polymorphisms (G308A and G238A)
found but the results are conflicting with regard to any possible association to human obesity and insulin resistance. Serum TNF-α has been shown to be increased in normal weight women with PCOS, suggesting that factors other than obesity are the cause of elevated serum TNF-α in normal-weight PCOS women (Gonzalez et al. 1999). Serum TNF-α levels are also mildly increased in hyperandrogenic patients (Escobar-Morreale et al. 2001) and carriers of the −308A polymorphism presented increased androgens and 17-hydroxyprogesterone levels compared to controls. However, Millner and colleagues (1999) failed to show an association between PCOS and the −308 polymorphism. Their result of the TNF-α promoter polymorphism are in accordance with our finding. Thus, it seems that TNF-α does not play a significant role in the pathogenesis of PCOS.

6.3 Clinical perspectives based on the findings of the study

Overall, the results of this study suggest that irrespective of the overlapping clinical findings, MBS and PCOS are not different facets of a single clinical entity. However, should PCOS be present in a woman with MBS, it will worsen her metabolic disturbances.

Based on the current literature, a number of risk factors for CVD appear to cluster in women with PCOS. However, at present there is no data to show that all women with PCOS invariably develop cardiovascular complications later in life (Wild 2002b).

The actual morbidity is probably associated with other known risk factors for CVD unrelated to PCOS per se. In other words, the long-term effects of PCOS without concomitant cardiovascular risks remain unknown. Therefore, it is crucial to dissect the genetic background behind PCOS to be able to identify those women at risk. Similarities in the pathogenesis with different components of MBS or type 2 diabetes in addition to the PPARγ polymorphism found here indicate that there is a subgroup of PCOS women who are more susceptible abnormal glucose tolerance and type 2 diabetes, or dyslipidemia or hypertension than their counterparts and also later in their life, cardiovascular complications (Figure 9). Nonetheless, obesity increases rapidly and worsens both of syndromes. This will require an intervention such as lifestyle counseling at every stage of the life span of these women.
**Figure 9.** Genetic background affect long-term health risks as well as lifestyle.
7 CONCLUSIONS

A cross-sectional sample of Finnish women aged 34 – 54 years was studied to identify a group of MBS using either self constructed or NCEP criteria. About one fifth of the women (19.5 %) fulfilled the criteria for MBS. Defined in either way, MBS women were more overall overweight and abdominally obese, dyslipidemic, hypertensive and insulin resistant than the controls. The MBS women appeared to have mild hyperandrogenemia as assessed by free testosterone and the FAI. Furthermore, a low SHBG level was an essential feature in MBS women.

PCO ovaries and PCOS were not associated unambiguously with MBS. PCOS was found in 13 %, 15%, and 13 % in women with MBS, simplex obese and lean women, respectively. Problems associated with PCOS such as infertility, miscarriages, and endometrium hyperplasia were not more common in MBS women when compared to controls.

The clinical cutaneous markers of hyperandrogenemia and insulin resistance such as acanthosis nigricans and alopecia areata were found only in MBS women. Oligoamenorrhea and hypermenorrhea were more common in MBS women than in controls, but these disturbances appeared mainly in adulthood. Insulin sensitivity decreased linearly with waist circumference in MBS women, whereas FAI did not increase linearly with waist circumference. In multiple logistic regression analysis, only waist circumference and diastolic blood pressure were significantly associated with hyperandrogenism.

Our genetic studies support a role for PPARγ gene polymorphism in the pathogenesis of PCOS, with the presence of the Ala isoform being protective against the development of PCOS. In addition, we found that a functional haplotype of the EPHX gene was associated with PCOS, the low activity haplotype C G (Hie 113 Arg139) being significantly associated with PCOS. Further, the results of this study reveal that apoE does not play a major role in the development of dyslipidemia in the group of women with PCOS and that polymorphism of the TNF-α gene is unlikely to contribute to the PCOS risk.
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