The 6th FESCC Continuous Postgraduate Course in Clinical Chemistry

Under the Auspices of IFCC

NEW TRENDS IN CLASSIFICATION, MONITORING AND MANAGEMENT OF METABOLIC SYNDROME

Handbook

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Editorial

The Sixth FESCC Continuous Postgraduate Course in Clinical Chemistry:
New Trends in Classification, Monitoring and Management of Metabolic Syndrome

The Croatian Society of Medical Biochemists and Slovenian Association for Clinical Chemistry, together with the Forum of the European Societies of Clinical Chemistry (FESCC) - IFCC in Europe have organized the sixth in a series of postgraduate weekend courses under the auspices of IFCC. The Course entitled "New Trends in Classification, Monitoring and Management of Metabolic Syndrome" promotes continuous postgraduate education of professionals in clinical chemistry and laboratory medicine, and ensures the laboratory knowledge harmonization, this time on metabolic syndrome in particular.

The initial description and several definition of metabolic syndrome using different sets of criteria reflect contrasting views on pathogenic mechanisms. The use of these definitions of metabolic syndrome in diverse populations has resulted in different prevalence rates, inconsistencies and confusion in research and clinical practice. Therefore in 2005 the International Diabetes Federation (IDF) has proposed a new word wide definition of metabolic syndrome. In this specialized FESCC Course, renowned experts from European countries try to cover the clinical and laboratory aspects of metabolic syndrome. This Handbook contains the material prepared by these experts especially for this Course. The integrated knowledge of the authors, experts in different fields, is intended to provide the reader with optimal information.

The Handbook contents are divided into three chapters according to the Course program. The chapter BASIC CONCEPTS covers topics such as Pathophysiology of Metabolic Syndrome (MS), Prediabetes and MS, Genetic Susceptibility to the MS. In the chapter RISK ASSESMENT, Dislipidemia, Coronary Disease, Insulin Resistance andMS, Pro-inflamatory and thrombotic factors are described. The last chapter is dedicated to DIAGNOSTIC EXACTANESS OF BIOCHEMICAL MARKERS where Evidence Based Laboratory Medicine, Hypertension and Metabolic Syndrome, Approach to the treatment of MS are presented.

We do hope that the Course program as well as this Course Handbook meets the intended goals by presenting the state-of-the-art, contributing to harmonization of the new trends in diagnosis, monitoring and management of metabolic syndrome.

Zagreb, November 2006 *Elizabeta Topić*

1. PATHOPHYSIOLOGY OF METABOLIC SYNDROME

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1.1 Introduction

The metabolic syndrome is a constellation of interrelated abnormalities (namely obesity, dyslipidaemia, hyperglycaemia, and hypertension) that increase the risk for cardiovascular disease and type 2 diabetes. This is a common metabolic disorder which increases in prevalence as the population becomes more obese. The disorder is defined in various ways. Diagnostic criteria for the metabolic syndrome have been establish by the World Health Organisation (WHO) in 1998, by the National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III), in 2001, and more recently by the International Diabetes Federation (IDF), in 2005. The metabolic syndrome was introduced as a diagnostic category to identify the individuals that satisfy arbitrary chosen criteria to initiate lifestyle changes, and drug treatment when needed, with the goal of decreasing risk of cardiovascular disease and type 2 diabetes mellitus.

Table 1.1. *Definitions of the metabolic syndrome. By: a) World Health Organisation, b) National Cholesterol Education Program's Adult Treatment Panel III, c) International Diabetes Federation (1, 2, 3)*

a) World Health Organisation, 1998

b) National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III)**,** 2001

c) International Diabetes Federation, 2005

1.2 Pathogenesis of metabolic syndrome

1.2.1 Insulin resistance

The most accepted hypothesis to describe the pathophysiology of the metabolic syndrome is insulin resistance. That is why the metabolic syndrome is also known as the insulin resistance syndrome. Insulin resistance has been defined as a defect in insulin action that results in hyperinsulinaemia, necessary to maintain euglycaemia. Concept of insulin resistance provides a conceptual framework with which to place a substantial number of apparently unrelated biological events into a pathophysiological construct.

A major contributor to the development of insulin resistance is an overabundance of circulating fatty acids, released from an expanded adipose tissue mass. FFA reduce insulin sensitivity in muscle by inhibiting insulin-mediated glucose uptake. Increased level of circulating glucose increases pancreatic insulin secretion resulting in hyperinsulinemia. In the liver, FFA increase the production of glucose, triglycerides and secretion of very low density lipoproteins (VLDL). The consequence is the reduction in glucose transformation to glycogen and increased lipid accumulation in triglyceride (TG). Insulin is an important antilipolytic hormone. In the case of insulin resistance, the increased amount of lipolysis of stored triacylglycerol molecules in adipose tissue produces more fatty acids, which could further inhibit the antilipolytic effect of insulin, creating additional lipolysis.

1.2.2 Obesity and increased waist circumference

The WHO and ATP III definitions of metabolic syndrome both include abdominal obesity, but it is a necessary requirement in the IDF definition (Table 1.). That reflects the IDF position - though the pathogenesis of the metabolic syndrome and its components is complex, abdominal obesity is a key causative factor. Despite the importance of obesity in the model, we should remember that patients of normal weight can also be insulin resistant. Those are called metabolically obese, normal-weight individuals, typically having increased amount of visceral adipose tissue. According to some theories, with increases in visceral adipose tissue, a higher rate of flux of adipose tissue-derived free fatty acids to the liver through the splanchnic circulation would be expected, while increases in abdominal subcutaneous fat could release lipolysis products into the systemic circulation and avoid more direct effects on hepatic metabolism.

1.2.3 Dyslipidaemia

In general, with increases in free fatty acid flux to the liver, increased production of very low-density lipoproteins (VLDL) occurs. Under physiological conditions, insulin inhibits the secretion of VLDL into the systemic circulation. In the setting of insulin resistance, increased flux of free fatty acids to the liver increases hepatic triglyceride synthesis. Thus, hypertriglyceridaemia is an excellent reflection of the insulin resistant condition and is one of the important criteria for diagnosis of the metabolic syndrome.

The other major lipoprotein disturbance in the metabolic syndrome is a reduction in HDL cholesterol. This reduction is a consequence of changes in HDL composition and metabolism. In the presence of hypertriglyceridaemia, a decrease in the cholesterol content of HDL results from decreases in the cholesteryl ester content of the lipoprotein core with variable increases in triglyceride. In addition to HDL, the composition of LDL is also modified in a similar way. In fact, with fasting serum triglycerides > 2.0 mmol/L, almost all patients have a

predominance of small dense LDL. This change in LDL composition is attributable to relative depletion of unesterified and esterified cholesterol, and phospholipids, with either no change or an increase in LDL triglyceride. In some studies, this alteration in LDL composition is an independent risk factor for cardiovascular disease. However, more often this association is not independent, but related to the concomitant changes in other lipoproteins and other risk factors.

1.2.4 Glucose intolerance

The defects of insulin action in glucose metabolism include failure to suppress gluconeogenesis in the liver, and to mediate glucose uptake in insulin sensitive tissues (i.e. muscle and adipose tissue). To compensate for defects in insulin action, insulin secretion must be increased to sustain euglycaemia. If this compensation fails, defects in insulin secretion predominate and hyperglycaemia occurs.

Although free fatty acids can stimulate insulin secretion, prolonged exposure to excessive concentrations of FFA results in falls in insulin secretion. The mechanism for this alteration has been attributed to lipotoxicity.

1.2.5 Hypertension

The relation between insulin resistance and hypertension is well established. Several different mechanisms are proposed. First, insulin is a vasodilator when given intravenously to people of normal weight, with secondary effects on sodium reabsorption in the kidney. In the setting of insulin resistance, the vasodilatory effect of insulin can be lost, but the renal effect on sodium reabsorption preserved. Fatty acids themselves can mediate relative vasoconstriction. Hyperinsulinaemia may result in increased sympathetic nervous system (SNS) activity and contribute to the development of hypertension.

1.2.6 Other manifestations

Insulin resistance is accompanied by many other alterations that are not included in the diagnostic criteria for the metabolic syndrome. Increases in apo B and C-III, uric acid, prothrombotic factors (fibrinogen, plasminogen activator inhibitor 1), serum viscosity, asymmetric dimethylarginine, homocysteine, white blood cell count, pro-inflammatory cytokines, the presence of microalbuminuria, non-alcoholic fatty liver disease, obstructive sleep apnoea, and polycystic ovarian disease are all associated with insulin resistance.

Recommended literature:

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2. GENETIC SUCCEPTIBILITY TO METABOLIC SYNDROME

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2.1 Introduction

The first recognition of clustering of hypertension, hyperglycemia and gout came already in twenties of 20th century . In 1988 Reaven identified Syndrome X originating from insulin resistance (IR). In 2004 The National Cholesterol Education Program's Adult Treatment Panel III (ATPIII) defined the metabolic syndrome with alternative names: IR syndrome, Reaven syndrome, characterized by following components:

- insulin resistance
- abdominal obesity
- atherogenic dyslipidemia and cardiovascular disease
- hypertension
- proinfammatory state
- prothrombotic state

Diagnostic criteria for MS were prepared by ATPIII by International Diabetes Federation (IDF). Upon ATPIII agreement, patients having at least 3 of 5 characteristics can be diagnosed as having the metabolic syndrome: abdominal obesity, elevated triglycerides, decreased HDL-cholesterol, increased blood pressure or increased fasting plasma glucose. IDF declares criteria fairly consistent with ATPIII. Slight differences include central obesity as the major feature and fulfilled two of four other manifestations. The borderline glucose concentration is lower in IDP criteria, with strong recommendation for OGTT when exceeded.

Three possible etiologies for the metabolic syndrome were postulated: Obesity was found to be responsible for excess release of free fatty acids, cytokines and other proinflammatory products which are implicated in the development of IR, hypertension and dyslipidemia.

IR as the second possible cause of metabolic syndrome rises a question whether it is possible to dissociate between obesity and IR. Indeed, IR exists to various degrees in all particular classes of body mass index, suggesting an independent inheritable contribution of it to at least some extent. Some populations (South Asians) with mild overweight display IR and this is said to be primary IR. From this point of view IR can be classified as a separate etiological factor for metabolic syndrome. Hyperinsulinemia as a consequence of IR is capable to increase VLDL secretion from the liver and cause hypertension. IR of muscle can cause hyperglycaemia, exaggerated with gluconeogenesis in insulin-resistant liver.

The third etiology is thought to include independent factors: immunologic, vascular, hepatic, which are influenced by specific genetic and environmental factors.

2.2 Risk factors for metabolic syndrome

The following factors increase chances of having metabolic syndrome:

- *Age*. The prevalence of metabolic syndrome increases with age, affecting less than 10 percent of people in their 20s and 40 percent of people in their 60s. However, one study shows that about one in eight schoolchildren have three or more components of metabolic syndrome.
- *Race*. Hispanics and Asians seem to be at greater risk for metabolic syndrome than other races are.
- *Progressive weight gain*. Metabolic syndrome is present in about 5% of people with normal body weight, 22% of those who are overweight and 60% of those considered obese. Adults who continue to gain 5 or more pounds per year raise their risk of developing metabolic syndrome by up to 45%.
- *Obesity*. A body mass index (BMI) - a measure of your percentage of body fat based on height and weight - greater than 25 increases your risk of metabolic syndrome. So does abdominal obesity - having an apple shape rather than a pear shape.
- *History of diabetes*. You're more likely to have metabolic syndrome if you have a family history of type 2 diabetes or a history of diabetes during pregnancy (gestational diabetes).
- *Other diseases*. A diagnosis of high blood pressure, cardiovascular disease or polycystic ovary syndrome - a similar type of metabolic problem that affects a woman's hormones and reproductive system - also increases the risk of metabolic syndrome.
- *Low physical activity*. A sustainable exercise program, fore example 30 minutes 5 days a week is reasonable to start, providing there is no medical contraindication. (If you have any special concerns in this regard, check with your doctor first.) There is a beneficial effect of exercise on blood pressure, cholesterol levels, and insulin sensitivity, regardless of whether weight loss is achieved or not. Thus, exercise in itself is a helpful tool in treating metabolic syndrome.
- *Diet.* A detailed discussion of diet therapies, pros and cons of various diets etc. is beyond the scope of this article. However, there is now a trend toward the use of a Mediterranean diet -- one that is rich in “good” fats (olive oil) and contains a reasonable amount of carbohydrates and proteins (such as from fish and chicken). Mediterranean diet: A diet traditionally followed in Greece, Crete, southern France, and parts of Italy that emphasizes fruits and vegetables, nuts, grains, olive oil (as opposed to butter) and grilled or steamed chicken and seafood (as opposed to red meat). Plus a glass or two of red wine.
- *Lifestyle*: sedentary work, smoking, eating an excessively high carbohydrate diet, and consuming an alcohol-free diet.
- *Genetic factors*

Diet and exercise are still the preferred primary treatment of metabolic syndrome.

2.3 Genetic risk factors for metabolic syndrome

Genetic factors could influence each individual component of the syndrome, and the syndrome itself. A family history that includes obesity, type 2 diabetes and/or insulin resistance greatly increases the chance that an individual will develop the metabolic syndrome. However there are some genetic loci, which are in linkage disequilibrium with metabolic syndrome.

2.3.1 Genetics of metabolic syndrome

Kissebah et al. (2000) performed a genomewide scan by use of a 10-cM map in 2,209 individuals distributed over 507 nuclear Caucasian families and for the first identifying major genetic loci influencing the metabolic syndrome phenotypes. They showed a quantitative trait locus (QTL) on chromosome 3q27 strongly linked to 6 traits: weight, waist circumference, leptin, insulin, insulin/glucose ratio, and hip circumference (lod scores ranging from 2.4 to 3.5). A second QTL was found on chromosome 17p12 and was strongly linked to plasma leptin levels (lod = 5.0). Several candidate genes are located in both regions (Table 2.1.).

McCarthy and coworkers (2003) studied 207 SNPs in 110 candidate genes among coronary artery disease patients, a population enriched for metabolic abnormalities. The number of abnormalities (0 to 5) was determined in 214 male and 91 female patients, and the association with each polymorphism was evaluated. Polymorphisms in 8 genes were associated with metabolic syndrome in the whole population (P values ranging from 0.047 to 0.008): LDLR, GBE1, IL1R1, TGFB1, IL6, COL5A2, SELE) and LIPC. Variants in 7 additional genes showed significant gene interaction by gender. Separate analyses in men and women revealed a strong association with a silent polymorphism in the gene encoding low density lipoprotein receptor-related protein-associated protein-1 (LRPAP1) among females (P = 0.0003), but not males (P = 0.292). Several other genes showed association only in females; only 1 gene, PRCP, was significantly associated in men alone (P = 0.039).

Based on results of both genome wide studies, the genetic association studies with metabolic syndrome of genes listed in Table 2.1. are highly recommended.

Table 2.1. *Candidate genes for genetic association studies with metabolic syndrome*

In addition, Robitaille study in 2004 found among 632 men increased frequency of the val162 allele of the leu162-to-val polymorphism in the PPARA gene, among those with abdominal obesity, hypertriglyceridemia, high plasma apoB and low HDL plasma levels, which are components of the metabolic syndrome. The frequency of the V162 allele was approximately 10% in their group.

Animal models for metabolic syndrome studies

For studies of metabolic syndrome two of animal models were developed: a transgenic mice with 11-beta-hydroxysteroid dehydrogenase type 1 overexpressing and the Neil1 knockout mice.

The transgenic mice overexpressing 11-beta-hydroxysteroid dehydrogenase type 1 selectively in adipose tissue was to an extent similar to that found in adipose tissue from obese humans. These mice had increased adipose levels of corticosterone and developed visceral obesity that was exaggerated by a high-fat diet. The mice also exhibited pronounced insulin-resistant diabetes, hyperlipidemia, and, surprisingly, hyperphagia despite hyperleptinemia. Increased adipocyte 11-beta-hydroxysteroid type 1 activity may be a common molecular etiology for visceral obesity and the metabolic syndrome.

Vartanian et al. (2006) found that Neil1 knockout mice were born at expected mendelian ratios and the phenotype of Neil1 -/- pups was normal through the first 4 to 6 months of life. At about 7 months, however, male Neil1 -/- mice developed severe obesity, and female Neil1 -/- mice were modestly overweight. Mutant mice also showed dyslipidemia, fatty liver disease, and a tendency to develop hyperinsulinemia, similar to metabolic syndrome in humans. Histologic studies showed significant kidney vacuolization, and mitochondrial DNA from Neil1 -/- mice showed increased levels of steady-state DNA damage and deletions, compared to wildtype controls.

2.3.2 Genetics of individual components of metabolic syndrome

Genetic factors could influence each of components in metabolic syndrome individualy. In following chapters the genetic factors in obesity and insulin resistance as the main causes of metabolic syndrome, will be rewieved in short.

2.3.2.1 Genetics of obesity

The state of nutrition is best described by body mass index (BMI) which is, with some exceptions, in good correlation with the amount of total body fat. According to the BMI, the following categories of excessive body mass or nutrition are postulated:

BMI 25-30 kg/m2	overweight
BMI 30-40 kg/m2	obesity
BMI 40-50 kg/m2	morbid obesity
BMI >50 kg/m2	extreme obesity

Pathophysiology of obesity
Each individual has genetically determined the weight set-point and hence body weight is tightly regulated by an energetic homeostatic mechanism. Adipocytes secrete leptin and β-cells secrete insulin, both in proportion to body-fat content. The two hormones enter the brain.

They bind to their central receptors on the hypothalamic neurons exerting effects to reduce body weight. Hypothalamic nevrons express peptides and their receptors which could be categorized as orexigenic: neuropeptide Y, agouti-related protein (AgRP), melanin-concentrating hormone (MCH), orexins A and B; or anorexigenic: melanocortins (i.e. melanocyte-stimulating hormone, α-MSH) and cocaine and amphetamine related transcript (CART). In leptin or insulin abundance anorexigenic pathways prevail: increase of energy expenditure, increase of thermogenesis, diminished food intake. Particularly leptin-melanocortin anorexigenic signalling pathway appears to be very conserved among species and mutations in genes encoding for components of this pathway: leptin, leptin receptor, pro-opiomelanocortin (POMC), prohormone-convertase 1 (PC1), and melanocortin 4 receptor (Mc4R), cause rare forms of morbid monogenic obesity and lead to some naturally occuring murine models of obesity (ob, db, Ay and mg;). On the contrary, knockouts in genes for orexigenic pathways in mice fail to produce lean fenotypes, which demonstrates extremely powerful mutual effects of anabolism and weight gain system components.

Leptin and insulin mediate long-term body-mass regulation. They are active also in short-term signals that effect single meal to be initiated and terminated. In addition, there are some other shortly acting hormones/factors which accompany food intake: ghrelin, motilin, neuromedin U, neurotensin, cholecystokinin, peptide YY_{3-36} (PYY; 72) and glucagon-like peptide-1, all secreted by the gastrointestinal tract, and vagal afferent signalling .

Genetics of obesity

Today's high incidence of obesity could be explained by a »thrifty genotype« hypothesis: over periods of time the alleles were selected which favored weight gain and fat storage in order to provide enough nutrients for times of food deprivation. In today's times of food availability and decreased physical activity such genotypes cause obesity.

Besides monogenic forms of obesity there are at least 20 rare syndromes with obvious genetic basis which appears to be more complex as it predisposes more dysfunctions (mental retardation, multiple signs of hypothalamic disorder).

The common human obesity is thought to be oligogenic state and its expression is modulated by multiple modifier genes and by environmental factors: food intake, physical activity, and smoking. Genetic basis in the pathophysiology of obesity is estimated to be 40-80%. At least 204 putative gene loci associated with obesity have been identified, and those which have been confirmed by multiple studies are presented in Table 2.2.

Table 2.2. *A list of genes associated with obesity confirmed by 5 or more studies:*

2.3.2.2 Genetics of insulin resistance and diabetes mellitus type 2

Insulin resistance (IR) is defined as less than normal response to insulin, which leads to hyperinsulinemia for euglycaema to be maintained. The hyperinsulinemia causes disinhibition of gluconeogenesis, impaired uptake of glucose by muscle and disinhibition of lipolysis in adipose tissue. Clinical markers of IR are visceral obesity, acantosis nigricans, acne, hirsutism, hepatic steatosis.

Etiology of IR includes genetic factors resulting in syndromic forms of IR, and environmental factors: food intake, poor physical activity, aging, smoking or administration of drugs – thiazide diuretics, beta adrenergic antagonists, glucocorticoids, which can cause or contribute to IR. The most important factor is obesity which is usually of combined polygenetic and environmental origin. Abdominal adipose tissue is a source of free fatty acids and various hormones (adipokines) being implicated in the development of IR.

Genetic defects in insulin signalling
A minority of insulin resistant cases is characterized by a single genetic or acquired trait. Anti-insulin autoantibodies have been found in diabetes mellitus type 1. On the other hand, more than 60 mutations have been identified in the insulin receptor gene. Among them type A IR is associated with heterozygous mutation state which underlies decreased Tyr phosphorylation of the β-subunit after insulin binding. In Rabson-Mendenhall syndrome and leprechaunism (Donohue syndrome) insulin receptor gene mutations are presumed to impair insulin binding to the receptor. IR may be due to abnormal production of anti-insulin-receptor antibodies (type B IR). PPAR-γ mutations which are not associated with lipodystrophy are also reported to cause IR.

Genetics of DM2
Affected genes in monogenic forms of diabetes, insulin resistance and lipodystrophy represent an excellent base for the search of susceptibility genes for polygenic multifactorial DM2, although the latter can be distinguished from monogenic Mendelian diseases. Namely, on certain genetic backgrounds, with particular gene interaction – epistasis and with certain envinronment influence the same genes could contribute to DM2.

Maturity–onset diabetes of the young (MODY) is a monogenic from of diabetes and exists in 6 forms due to 6 affected MODY genes. From them, *HNF4A, TCF1* (or *HNF1A*) and *GCK* genes which encode for two transcriptional factors and glucokinase in the β-cells, respectively, were reliably proved to be involved in DM2.

From the genes responsible for monogenic form of insulin resistance, the gene for insulin with class III variable number tandem repeat alleles and the *PPARG* gene have been associated with multifactorial DM2 as well. Additionally, the *AGPAT2* (acylglycerolphosphate-acyltransferase) gene underlying Berardinelli-Seip congenital lipodystrophy and *Mt-tRNA Leu(UUR)* mitochondrial gene otherwise associated with

maternally inherited diabetes and deafness (MIDD) syndrome have been shown to be implicated in DM2 .

Search for susceptibility genes among the genes dealing with insulin secretion and action gave positive results for already mentioned *PPARG* gene with another polymorphism P12A (different than the one implicated in monogenic IR) and *KCNJ11* gene, encoding for protein contained in K^+_{ATP} channels. Arising but not yet definite evidence for implication in DM2 concerns *IRS-1*, *GLUT2* and *PGC1A* (co-activator of PPAR-γ) genes.

The only proved DM2 susceptibility gene found through genome wide-scan methods is *CPN10* which encodes for calpain-10 from the familiy of calcium-activated neutral proteases, expressed in β-cells. This gene was identified after strong linkage disequilibrium found with DM2, which was followed by positional cloning.

When studying the importance of particular susceptibility genes one must bear in mind that their effect is modest. The listed susceptibility genes does contribute to the basis for future diagnosis, prognosis and therapy, but for now, the mutual influences of various gene loci and interactions of genes with dietary and other lifestyle factors remain to be exactly determined and quantitated.

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3. HOW IS THE METABOLIC SYNDROME RELATED TO THE DYSLIPIDEMIA?

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3.1 Some historical aspects on the metabolic syndrome

The contemporary definition of the metabolic syndrome refers to a cluster of metabolic abnormalities related to a state of insulin resistance which is often associated with a high-risk overweight/obesity phenotype. Because such cluster increases the risk of coronary heart disease (CHD) and type 2 diabetes, numerous consensus groups have attempted to provide recommendations to identify in clinical practice patients with these atherogenic/diabetogenic metabolic abnormalities. Although there has been an exponential proliferation of scientific papers and conferences on the metabolic syndrome, the concept of a cluster of abnormalities such as obesity, diabetes, dyslipidaemia and hypertension is not new and several physicians/investigators have contributed to the development of such concept through astute clinical observations or epidemiological/metabolic studies.

The observation that obesity, dyslipidemia, diabetes and hypertension occur simultaneously in many people was first made by Crepaldi in 1967. In the late 1970s this clustering of conditions was termed the metabolic syndrome by German researchers. Since then the syndrome is described under a number of guises as " Insulin resistance syndrome, Syndrome X, Plurimetabolic syndrome and the metabolic syndrome" The syndrome is a multi-component disease brought on by combination of lifestyle and environmental factors, with some populations exhibiting a genetic susceptibility for its development. The original terminology-the metabolic syndrome-remains the most appropriate, as it is well established and best describes the conditions that comprise it. The escalating prevalence of the syndrome has important health implications. Each component of the metabolic syndrome is an established cardiovascular disease risk factor, and the presence of multiple components confers greater risk than the sum of the risk associated with the individual ones. The NCEP ATP III criteria were used to estimate the prevalence of the metabolic syndrome. The incidence of individuals exhibiting the syndrome is rising dramatically, it is currently estimated that 47 million US residents have the metabolic syndrome increasing their risk for coronary heart disease and stroke threefold. The prevalence of the syndrome increased with age from 7 % at the age of 20-29 year old to 44 % in 60-69. However alarmingly, the prevalence is increasing amongst children and adolescents very fast.

3.2 Defining the metabolic syndrome: an urgent need for an universal definition

In order to reduce the confusion in the medical community, universal agreement on the definition and clinical tools to assess the metabolic syndrome would be very helpful and efforts for additional international consensus activities have been made. Recent meetings have contribute to emphasize the notion that even if insulin resistance is indeed at the core of the metabolic syndrome, abdominal obesity is by far the most prevalent form of the metabolic syndrome. Therefore, to dissociate abdominal obesity/insulin resistance would be for the time

being of little help. We should rather work on cut-off values proposed for the various clinical tools used to optimally discriminate for the presence of the metabolic syndrome in several population of the world.

3.3 The concept of the metabolic syndrome

It is now well accepted that obesity represents a heterogeneous condition from a metabolic standpoint. A preferential deposition of adipose tissue in the abdominal cavity has been associated with a cluster of atherogenic and diabetogenic metabolic complications characterizing the metabolic syndrome. For instance, many studies have reported that excess visceral adipose tissue accumulation is associated with elevated plasma triglyceride concentrations, marked reductions in plasma HDL-cholesterol levels and an increased proportion of small, dense LDL particles, despite normal LDLcholesterol. Furthermore, there is also solid evidence to suggest that among obese patients, the most severe disturbances in indices of plasma glucose-insulin homeostasis resulting from an insulin resistant state are observed in patients with a high accumulation of visceral adipose tissue. Finally, abdominal obesity has been associated with hypertension and with a pro-inflammatory and thrombotic state. It is important to keep in mind that presumably normal weight individuals may nevertheless be characterized by an excess of visceral adipose tissue and therefore at increased risk of metabolic complications. Thus, under those circumstances, such normal weight subjects characterized by an excess of visceral adipose tissue may also show the features of the metabolic syndrome.The concept of the metabolic syndrome viewed as precursor to the development of both type 2 diabetes and cardiovascular disease has progressively emerged with a formal recognition by the World Health Organization (WHO) in 1998 and the National Cholesterol Education Program Adult Treatment Panel III in 2001 (NCEP ATP III), which have recently proposed a formal definition of the metabolic syndrome.

Table 3.1. *Definitions of the metabolic syndrome according to World Health Organisation (WHO) and National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria.*

The respective criteria proposed to define the metabolic syndrome are listed in Table 3.1. Both definitions include type 2 diabetes and impaired fasting glycaemia, as well as hypertriglyceridaemia and a low HDL-cholesterol concentration as component traits.The WHO definition included also the presence of impaired glucose tolerance determined with a glucose load test. There are also a few differences between these two definitions. The WHO criteria consider both central obesity (defined by the waist-to-hip ratio) and overall obesity (defined by the BMI) while the NCEP criteria consider only central obesity (defined by the waist circumference).Furthermore, blood pressure thresholds differ between the two criteria with a higher value in the WHO definition. In addition, elevated microalbuminuria is a component trait in the WHO definition while it is not considered for NCEP ATP III. These differences in the definition and proposed criteria for the definition of the metabolic syndrome show that some uncertainty persists in this area.

3.4 The "hypertriglyceridaemic waist" phenotype: review of evidence

The metabolic syndrome increases the risk of cardiovascular disease and type 2 diabetes. The simultaneous measurement and interpretation of waist circumference and triglyceride level, "the hypertriglyceridaemic waist", may be a simple tool to identify individuals at high risk.

The National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) has recognised the metabolic syndrome as a cluster of abnormalities increasing the risk of both cardiovascular disease (CVD) and type 2 diabetes. The NCEP-ATP III guidelines have also underlined the central role of abdominal obesity in the development of this syndrome. Nowadays, it is generally accepted that abdominal obesity is associated with numerous metabolic complications increasing the risk of type 2 diabetes and CVD .Results of the prospective Québec Cardiovascular Study have revealed that the presence of some features of metabolic syndrome found in viscerally obese men was predictive of a substantially increased risk of coronary heart disease (CHD). For instance, it has been shown in this study that men with the simultaneous presence of fasting hyperinsulinaemia, elevated apolipoprotein B levels, and an increased proportion of small LDL particles (a cluster that we have referred to as the atherogenic metabolic triad) were characterised by a 20-fold increase in the risk of developing CHD over the 5year follow-up period of the study, compared with men without this cluster of non-traditional risk markers.

In addition, the risk of CVD associated with the atherogenic metabolic triad remained significant even after adjustment for traditional risk factors such as LDL-cholesterol, triglyceride and HDL-cholesterol levels.Thus, waist circumference and fasting triglyceride levels were tested for their ability to identify high-risk men who might be carriers of the atherogenic metabolic triad (hyperinsulinaemia, elevated apolipoprotein B, and small LDL particles). In this regard, sensitivity and specificity analyses conducted in a sample of adult men (aged between 28 and 63 years) showed that a cut-off point of 90 cm for waist circumference combined with a cut-off point of 2.0 mmol/l for triglyceride levels provided the best indication to identify men with these features of the metabolic syndrome. For instance, 84% of men with the hypertriglyceridaemic waist phenotype (waist circumference ≥90 cm and fasting triglyceride levels ≥2.0 mmol/l) were carriers of the atherogenic metabolic triad. Additional analyses also underlined the clinical importance of the hypertriglyceridaemic waist phenotype in the assessment of risk of coronary artery disease (CAD) and type 2 diabetes.

Indeed, in a sample of 287 men who underwent coronary angiographic procedures for symptoms of CAD, it was found that men with both elevated waist circumference (≥ 90 cm)

and triglyceride levels (≥ 2.0 mmol/l) were characterised by a 3.6-fold increase in the risk of CAD compared with men without the hypertriglyceridaemic waist phenotype.4 Moreover, the prevalent odds ratio of being affected by diabetes was also markedly increased (12-fold increase) in men with simultaneous elevations in waist circumference and triglyceride levels. On the other hand, it has been suggested that the hypertriglyceridaemic waist phenotype (waist circumference ≥ 90 cm and fasting triglyceride levels ≥ 2.0 mmol/l) had a greater impact on CAD risk than the presence and/or absence of impaired fasting glucose.

3.5 The metabolic syndrome: role and importance of the lipid components

Individuals with the metabolic syndrome, particulary those with abdominal obesity, exhibit a highly atherogenic lipid profile which may account for their high risk of cardiovascular disease and premature death. The metabolic syndrome is characterised by the co-occurrence of obesity (especially central obesity), dyslipidaemia, hyperglycaemia, and hypertension.

The presence of the metabolic syndrome is of relevance to public health since it has been linked to an increased risk of both cardiovascular disease (CVD) and type 2 diabetes. In particular, recent evidence shows that the presence of metabolic syndrome is associated with an increased risk of coronary heart disease (CHD), myocardial infarction, and stroke in both sexes. This substantially higher risk of CV morbidity and mortality associated with the presence of metabolic syndrome appears independent of other significant, potentially confounding factors such as smoking, plasma LDL cholesterol levels or alcohol consumption. Dyslipidaemia is an integral part of the metabolic syndrome since both definitions include hypertriglyceridaemia (defined as serum triglycerides >= 150 mg/dl) and a low HDL cholesterol concentration (defined as HDL-C < 40 mg/dl for men and <50 mg/dl for women by NCEP ATP III, or HDL-C < 35 mg/dl for men and < 40 mg/dl for women by WHO) as component traits. Individuals with the metabolic syndrome, particularly those with abdominal obesity, exhibit a highly atherogenic lipid profile which may account for their high risk of CVD. Central fat accumulation and presence of insulin-resistance have both been associated with a cluster of dyslipidaemic features, i.e., elevated plasma triglyceride level, an increase in very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL), presence of small dense LDL particles, and a decrease in HDL-cholesterol. These abnormalities of lipoprotein metabolism are more likely to occur together than separately and constitute key component traits of the metabolic syndrome.

3.5.1 Hypertriglyceridaemia

Beyond the LDL-cholesterol level, the presence of elevated serum triglycerides substantially increases the risk of CVD. Recent prospective studies indicate that elevated triglycerides are an independent risk factor in CHD. Hypertriglyceridaemia is associated with several atherogenic factors including increased concentrations of triglyceride-rich lipoproteins and the atherogenic lipoprotein phenotype consisting of small dense LDL particles, and low high-density lipoprotein (HDL) cholesterol. Factors contributing to hypertriglyceridaemia in the general population include obesity, overweight, physical inactivity, excess alcohol intake, high-carbohydrate diet, type 2 diabetes, and some other diseases (e.g. chronic renal failure, nephrotic syndrome), certain drugs (e.g. corticosteroids, estrogens, retinoids, higher doses of adrenergic blocking agents), and genetic disorders (familial combined hyperlipidaemia, familial hypertriglyceridaemia, and familialdysbetalipoproteinemia).In daily practice, elevated serum triglycerides are predominantly observed in persons with metabolic syndrome. Many

previous studies indicate that hypertriglyceridaemia is strongly associated with all components of metabolic syndrome.

Patients with metabolic syndrome who have hypertriglyceridaemia most often exhibit elevated level of triglyceride-rich lipoproteins which are considered atherogenic. The latter are partially degraded VLDL, commonly called "remnant lipoproteins". In clinical practice, VLDL cholesterol is the most readily available measure of atherogenic remnant lipoproteins. Thus, VLDL cholesterol can be a target of cholesterol-lowering therapy. Recent guidelines identify the sum of LDL+IDL+VLDL cholesterol (termed "non-HDL cholesterol" [total cholesterol minus HDL cholesterol]) as a secondary target of therapy in persons with hypertriglyceridaemia.

3.5.2 Low HDL-cholesterol

Low levels of HDL-cholesterol are associated with increased risk of coronary artery disease (CAD). This relationship was observed irrespective of age, blood pressure level, obesity, total cholesterol or LDL-cholesterol levels. The term "isolated low HDL" has been used to describe the situation where total cholesterol or LDL-cholesterol are considered normal but HDL-cholesterol is low. Long-term follow-up of subjects with low HDL-C has demonstrated that their risk of developing CAD is similar to the risk for subjects with elevated total cholesterol or LDL-cholesterol. Low HDL-cholesterol is the strongest predictor of subsequent CV events in patients with angiographically proven CAD and levels of total cholesterol within the normal range.According to current guidelines, the presence of low HDL-cholesterol should be considered a major CV risk factor, which modifies the goal for LDL-lowering therapy and is used as a risk factor to estimate the 10-year risk for CHD.A low HDL-cholesterol level has several causes, some of which are associated with insulin resistance, i.e. elevated triglycerides, overweight and obesity, physical inactivity, and type 2 diabetes. The combination of a low HDL-C with elevated plasma triglyceride level has therefore been considered an insulin-resistant state. It should be noted that certain drugs also reduce the level of HDL-C (e.g. beta-blockers, anabolic steroids, progestational agents). Nevertheless, low HDL-cholesterol is an important component trait of metabolic syndrome and deserves close clinical attention and management since these patients are at high risk of CVD.

3.5.3 Total cholesterol/HDL-cholesterol ratio

The total cholesterol/HDL-cholesterol ratio is a well known predictor of CHD risk. We have recently shown that men characterised by the hypertriglyceridaemic waist phenotype had a substantially elevated total cholesterol/HDL-cholesterol ratio compared with those without this phenotype. In this study, only 3% of men with waist circumference < 90 cm and triglyceride levels < 2.0 mmol/l had a total cholesterol/HDLcholesterol ratio of 6 or higher. However, almost 50% of subjects characterised by the hypertriglyceridaemic waist phenotype had a ratio above 6. Similar conclusions were reached in other study populations.

3.6 Lipid Risk factors

There are different risk factors for identifying individuals at risk of developing the metabolic syndrom, it is important to consider the patients case history and to conduct a physical examination as visceral obesity. Risk assessment include a list of biological parameters wherein lipids play an important role especially Tg and HDL-particles. The traditional factors associated with the syndrome are obesity, insulin resistance, hyperglycemia, dyslipaemia,

hypertension and microalbuminia. Is the increase of the FFA flux due to excess of calories and sedentary lifestyle in favour of insulin resistance predominantly in muscles or stimulate the increased lipid flux trough all its metabolic pathways the oxidative stress, stimulating inflammation and disrupting insulin signalling.

Multivariate analysis revealed that dyslipidemia, insulin resistance and hypertension were significantly independent risk factors of coronary heart disease with dyslipidemia conferring the greatest risk. LDL levels are associated with increased CVD risk however the LDL particle distribution is more important and should be discussed in the metabolic syndrome. There are also data available where we can show the linear relationship between circulating LDL-ox and the metabolic syndrome. However the HDL-C is inversely related to CVD risk, and long-term follow-up of patients provided the protective effect of HDL-C, determining CVD outcomes. The low HDL-C levels in the metabolic syndrome is a missing link to CV-events. Special attentions should be given to low LDL versus low HDL.

3.7 Experts opinion

The metabolic syndrome: is it an important medical issue in your opinion (and specialty)? Why?
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For many years, it has been recognised that several risk factors for cardiovascular disease cluster together. These factors are low HDL-cholesterol and high triglyceride concentrations, overweight, hyperglycaemia, and high blood pressure.

The metabolic syndrome is now accepted as the term to define this condition. The key concept is that these risk factors combine to increase cardiovascular disease, even when they are just slightly abnormal. This is why the definition of the metabolic syndrome sets cut-off points for these risk factors that include a large portion of the population.

For example, in the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) definition, the cut-off point for high triglycerides is > 150 mg/dL (approximately 1.7 mmol/L), which is about the average for the population over age 50 years. The cut-off point for low HDL-cholesterol is < 40 mg/dL (1 mmol/L) for men and < 50 mg/dL (1.3 mmol/L) for women, which includes about 35% of the population. Thus, the definition of metabolic syndrome recognises that these lipids continuously increase risk throughout their ranges in the population. Even mild abnormalities in triglyceride and HDL levels, when coexisting with other risk factors of the metabolic syndrome, become an important indicator of high risk.

It is very important to recognise that high LDL or high total cholesterol are not components of the metabolic syndrome. In fact, LDL is often below average in patients with the metabolic syndrome. Thus, physicians must be aware that patients can still have a high risk of cardiovascular disease even if they have low LDL or total cholesterol. In essence, one can conceptualise the metabolic syndrome as a "non-LDL" type of risk, and just as important as LDL to recognise and to treat.

The incidence of the metabolic syndrome has been increasing throughout Europe and North America, in parallel with an increase in overweight, obesity, and diabetes. Since LDL has

been emphasised in treatment guidelines quite effectively, it is now time to turn the attention of clinicians to the metabolic syndrome. If this epidemic of overweight and diabetes is not stopped,cardiovascular disease will increase, and we will lose the progress we have made in the past 20 years.

The metabolic syndrome requires a multi-factorial approach to treatment, since all of its components combine to increase the risk of cardiovascular disease. Firstly, diet and exercise will improve all components by lowering triglycerides, glucose and blood pressure, and raising HDL. In fact, much of the metabolic syndrome can be attributed to "over nutrition". Secondly, pharmacological therapy for improving the components of the metabolic syndrome should be individualized in each patient.

3.8 Treatment of dyslipidemia in metabolic syndrome

Treatment of the dyslipidemia of metabolic syndrome should involve no pharmacologic interventions, including weight loss, exercise, and a low-fat diet. Reducing LDL-C levels with use of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors ("statins") is also appropriate for patients with metabolic syndrome. The ATP III guidelines recommend that LDL-C be the primary target of lipid-lowering therapy when a patient's triglyceride level is below 500 mg/dL (5.65 mmol/L).

Metabolic syndrome can be considered a coronary artery disease (CAD) equivalent. Thus, it is appropriate to have target LDL-C levels that are below 100 mg/dL (2.59 mmol/L). Achieving this goal usually requires addition of a cholesterol-lowering agent, such as a statin. However, for many patients, statin therapy does not correct abnormalities of triglyceride and HDL-C concentrations.

Modifying triglyceride and HDL-C levels with drug therapy improves cardiovascular risk beyond the benefits achieved with statins alone. The Coronary Drug Project and the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VAHIT) used drug interventions (niacin and gemfibrozil) designed to modify triglyceride and HDL-C levels, and these interventions were associated with a reduction in cardiovascular events in both studies. Caution should be exercised when using both a fibric acid derivative and a statin, because the risk of myositis is increased with combination therapy. In addition, creatine kinase levels should be monitored if symptoms such as myalgia develop, especially in the setting of combination therapy.

3.9 Conclusion

Metabolic syndrome represents a clustering of cardiovascular risk factors linked through their association with insulin resistance. Since insulin resistance is an independent risk factor for cardiovascular disease, its presence can lead to macro vascular complications long before other features of metabolic syndrome are evident.

Challenges remaining in the identification of high-risk persons include the introduction of clinical markers of insulin resistance, integration of post challenge glucose and lipid concentrations, and better definition of the role of inflammatory, prothrombotic, and genetic factors. Improved understanding of the risk factors for metabolic syndrome is required, and

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4. CORONARY DISEASE AND METABOLIC SYNDROME

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Data from the Framingham Offspring Study indicate that the risk of coronary heart disease (CHD) in men and women, who were followed for 16 years, was directly related to the number of coronary heart disease risk factors (high cholesterol, low HDL-cholesterol, high body mass index, high systolic blood pressure, high triglyceride levels, and high blood glucose). Each of these risk factors also is associated with obesity (Wilson et al. 1999). Data from the Framingham Offspring Study also demonstrate that small changes in body weight are associated with significant changes in the sum of CHD risk factors. A gain in weight of 2.25 kg or more over 16 years significantly increased the sum of risk factors for CHD by 20% in men and 37% in women. Conversely, a reduction in weight by 2.25 kg or more significantly decreased the risk factor sum by 48% in men and 40% in women.

The metabolic syndrome is also known as the insulin resistance syndrome, dysmetabolic syndrome, and syndrome X. There is no precise definition of this syndrome, but it represents a specific body phenotype in conjunction with a group of metabolic abnormalities that are risk factors for coronary heart disease (CHD). Characteristics of this syndrome include abdominal obesity, insulin-resistant glucose metabolism (hyperinsulinemia, high fasting plasma glucose concentrations, impaired glucose tolerance), dyslipidemia (hypertriglyceridemia, low serum HDL-cholesterol concentration), and hypertension. Recently, additional metabolic abnormalities associated with abdominal obesity that are also risk factors for coronary heart disease have been identified, such as increased serum concentrations of apolipoprotein B, small, dense low-density-lipoprotein (LDL) particles, increased C-reactive protein, increased plasminogen activator inhibitor 1 (PAI-1), and impaired fibrinolysis (Lemieux et al. 1999, 2001, Landin et al. 1990). Obesity itself is not a requirement for the metabolic syndrome, and metabolically obese, normal-weight persons, presumably with increased abdominal fat mass, have been identified (Lemieux et al. 2000).

Approximately 22% (47 million) of the US adult population have the metabolic syndrome, as defined by the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (ATP III) (Ruderman et al. 1998). This diagnosis was made by having 3 or more of the following: 1) abdominal obesity (waist circumference > 102 cm for men and > 88 cm for women), 2) hypertriglyceridemia (≥ 1.69 mmol/L), 3) low HDL cholesterol (< 1.04 mmol/L in men; < 1.29 mmol/L in women), 4) high blood pressure (≥ 130/86 mm Hg), and 5) high fasting glucose (6.1 mmol/L).

Recently, the metabolic syndrome was formally recognized as a distinct medical condition, and the ICD-9-CM code 277.7 for Dysmetabolic Syndrome X was approved by the Centers for Disease Control. This syndrome denotes the presence of a constellation of metabolic abnormalities, such as those listed in this figure, but does not require that a predetermined number of components be present.

In 2001, the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP Treatment Panel III, or ATP III) released updated guidelines for cholesterol

testing and management that included a definition and treatment recommendations for the metabolic syndrome. According to ATP III, the metabolic syndrome consists of a constellation of risk factors that place patients at risk for both the development of type 2 diabetes and atherosclerotic disease. The hallmarks of the syndrome are: abdominal obesity, atherogenic dyslipidemia – characterized by elevated triglycerides, small LDL particles, and low HDL, elevated blood pressure, insulin resistance with or without glucose intolerance, a prothrombotic state and a proinflammatory state.

These "lipid and non-lipid risk factors of metabolic origin" not only increase the risk of type 2 diabetes, but also enhance the risk for coronary heart disease "at any given cholesterol level" (Expert Panel, 2001).

Although it has been widely assumed that the metabolic syndrome is associated with an increased risk of cardiovascular disease, relatively little research has been done on the prevalence of cardiovascular morbidity and mortality in patients with the syndrome. Following the introduction of the WHO definition, Isomaa et al (2001) assessed cardiovascular morbidity and mortality in a cohort of subjects (N = 3,928; age, 35 to 70 years) being followed in a longitudinal study in Finland and Sweden (the Botnia study). Median follow-up was 6.9 years. Subjects meeting the WHO definition of metabolic syndrome were significantly more likely to have a history of coronary heart disease, myocardial infarction, and stroke than those without the syndrome. The presence of metabolic syndrome was associated with significantly increased risk of coronary heart disease (relative risk, 2.96, $P < 0.001$), myocardial infarction (RR 2.63, $P < 0.001$), and stroke (RR 2.27, $P < 0.001$). Overall, the prevalence of coronary heart disease, MI, and stroke were approximately 3-fold higher in the group with metabolic syndrome.

In an epidemiologic study of female nurses (The Nurses Health Study; age, 35-55 y) after 2.2 million person-years of follow-up, the relative risk of cardiovascular disease was significantly elevated prior to diagnosis of diabetes. During 20 years of follow-up, 110,227 women remained free of diabetes and 5894 were diagnosed with type 2 diabetes. 1556 new cases of myocardial infarction, 1405 strokes, 815 cases of fatal coronary heart disease, and 300 fatal strokes were documented. Among the nurses who developed diabetes, the age-adjusted relative risk of myocardial infarctions or stroke was 2.82 for the period before diagnosis and 3.71 for the period after diagnosis compared with women who did not develop diabetes during the same period. The relative risk of a myocardial infarction in subjects with a diagnosis of diabetes at baseline was 5.02. These results suggest that aggressive management of cardiovascular risk is warranted in individuals at increased risk for type 2 diabetes. This study provides strong evidence for adopting a strategy for diabetes prevention rather than just a policy screening frequently for type 2 diabetes in high-risk subjects. The latter strategy could not prevent cases of CVD that develop prior to the onset of clinical diabetes (Hu et al. 2002).

In a prospective cohort study among female registered nurses in the U.S., 44,702 women (age, 40-65 y) who were free of prior coronary heart disease, stroke, or cancer, provided waist and hip circumferences. After an 8-year follow-up, after adjusting for BMI, age (continuous), age2, smoking, parental history of myocardial infarction, alcohol consumption, physical activity, menopausal status, hormone replacement therapy, aspirin intake, saturated fat, and antioxidant score, waist circumference significantly correlated to an increased risk in coronary heart disease ($P < 0.001$ for trend). Waist circumference and waist-to-hip ratio (WHR) were independently strongly associated with increased risk also among women with a BMI $\geq$25.

After adjusting for reported hypertension, diabetes, and high cholesterol, a waist circumference of ≥30" or a WHR of ≥0.76 was associated with a 2-fold higher risk of coronary heart disease. (Rexrode et al. 1998).

Abdominal fat distribution increases the risk for coronary heart disease (CHD) among lean, overweight, and obese persons. The risk of CHD begins to increase at a normal BMI, which is 23 kg/m2 for men and 22 kg/m2 for women [Stamler et al, 1986]. Data from both the Iowa Women's Health Study [Folsom et al. 2000] and the Nurses' Health Study [Rexrode et al. 1998] found that women in the lowest BMI but highest waist-to-hip circumference ratio tertiles (a measure of abdominal adiposity) had a greater risk of fatal and nonfatal myocardial infarctions than women in the highest BMI but lowest waist-to-hip circumference ratio tertiles.

An increase in weight since young adulthood (18–20 years of age) in men and women is associated with increased risk of developing type 2 diabetes. A weight gain of 10 kg, which is the average amount of weight gained by US adults from 20 to 50 years of age, is associated with a two- to threefold increase in the risk of diabetes. Weight gain during adulthood is also associated with an increased risk of coronary heart disease, hypertension, and cholelithiasis compared with those who maintain their weight after 18 to 20 years of age (Willet et al. 1999).

It is estimated that obesity accounts for 6% of the total healthcare expenses in the US, with $51.6 billion/year in direct costs and over $100 billion/year in both direct and indirect costs. Direct costs include the costs of personal health care, hospital care, physician services, allied health services, and medications. Indirect costs include the value of lost productivity from illness or premature mortality. The estimated direct cost of obesity is comparable to that of other prevalent, chronic diseases, such as type 2 diabetes and coronary heart disease, and is more costly than both hypertension and stroke. Moreover, obesity contributes to the development of other chronic diseases; it is estimated that 61% of the direct cost of type 2 diabetes, 17% of the direct cost of coronary heart disease, and 17% of the direct cost of hypertension are attributable to obesity (Wolf and Colditz, 1998, Hodgson and Cohen, 1999).

Increases in body mass index (BMI) are associated with considerable increases in total expected lifetime medical care costs for treatment of coronary heart disease, type 2 diabetes mellitus, hypertension, hypercholesterolemia, and stroke [Thompson et al., 1999]. For example, in men aged 45 to 54 years, total costs increase from $19,600 among lean men (BMI 22.5 kg/m2) to $36,500 in obese men (BMI 37.5 kg/m2). The cost difference between lean and obese persons also increases with age. Compared with lean persons, overweight (BMI 27.5 kg/m2) raises lifetime healthcare costs for these five diseases by 20%, class I obesity (BMI 32.5 kg/m2) raises them by 50%, and class II obesity (BMI 37.5 kg/m2) raises them by nearly 100%. These findings obtained in men are similar to those obtained in women.

Obesity is associated with increased outpatient and inpatient medical costs. There is a relative increase in the cost of healthcare services required by obese compared with lean members of a health maintenance organization (HMO) in northern California. These healthcare services can be divided into three categories: 1) outpatient healthcare visits, outpatient pharmacy services, outpatient laboratory services, 2) total outpatient services, total inpatient services, and 3) total cost of health care. Among the 17,118 members of this HMO, there was a 25% increase in total healthcare costs in those with class I obesity (body mass index [BMI] 30.0-34.9 kg/m2) and a 44% increase in total healthcare costs in those with class II or III obesity (BMI 35

kg/m2 or greater), compared with lean patients (BMI 20.0-24.9 kg/m2). The increased healthcare costs for obese patients were largely a result of costs related to coronary heart disease, hypertension, and diabetes (Quenesberry et al. 1988).

Regular physical activity is an important component of any weight loss program because it is associated with long-term weight maintenance and has beneficial health effects, such as decreasing coronary heart disease and diabetes that are independent of weight loss itself. The important physiological and clinical issues regarding the use of physical activity as part of obesity therapy will be reviewed in this section.

The relation of plasma triglyceride to LDL particle size and subclass pattern reflects the existence of differing forms of VLDL that give rise to larger and smaller LDL particles. Lower plasma triglyceride levels reflect VLDLs that are secreted with lower triglyceride content and are efficiently lipolyzed to larger LDL particles by the action of lipoprotein lipase (LPL). These LDLs have high affinity for LDL receptors (LDL-R). A higher level of plasma triglyceride is associated with larger VLDL particles that are lipolyzed less efficiently by LPL, giving rise to remnant particles. The properties of these remnants, including increased content of the apoprotein CIII, further slow lipolysis and also lead to reduced receptor-mediated plasma clearance. The remnants are further lipolyzed by the combined action of LPL and hepatic lipase (HL), and also undergo exchange of triglyceride for cholesterol derived from LDL and HDL, a process mediated by cholesterol ester transfer protein (CETP). The resulting triglyceride is, in addition delipidated and remodeled to form smaller, lipid-depleted LDL. These particles have lower affinity for LDL-R. Moreover, higher levels of remnant particles lead to increased exchange of triglyceride for cholesterol in both LDL and HDL, a process mediated by cholesterol ester transfer protein. Triglyceride-rich LDLs and HDLs are degraded further by HL, leading to yet smaller LDLs and to smaller and less stable HDLs that are more rapidly catabolized, resulting in reduced HDL cholesterol (Figure 4.1.)

Thus, pattern B LDL is associated with a cluster of interrelated metabolic abnormalities associated with increased risk for cardiovascular disease that has been designated atherogenic dyslipidemia. Factors leading to this dyslipidemia include abdominal adiposity, high dietary carbohydrate (especially simple sugars), insulin resistance, and genetic predisposition.

In the San Antonio Heart Study (Hanley et al. 2002), the higher the HOMA-IR quintile, the higher the insulin resistance and the greater the risk of cardiovascular disease even when adjusted for age, sex, and ethnicity. This association remained significant when adjusted for all other relevant variables.

The National Cholesterol Education Program (NCEP) has traditionally focused on high low-density lipoprotein cholesterol (LDL-C) as a risk factor for coronary heart disease (CHD). In the NCEP Adult Treatment Panel III (ATP III) recommendations published in JAMA in 2001, the NCEP suggested that the metabolic syndrome might independently predict the development of both type 2 diabetes and CHD. Note that in most definitions of the metabolic syndrome whether NCEP, WHO or AACE, diabetic subjects are included among those subjects who now have the metabolic syndrome.

Figure 4.1. *Model for Origins of AtherogenicDyslipidemia of Obesity and Metabolic Syndrome*
CETP, cholesteryl ester transfer protein; Chol, cholesterol; HDL, high-density lipoprotein; HL, hepatic lipase; IDL, intermediate-density lipopritein; LDL, low density lipoprotein; LDL-R, LDL receptor; MetS, metabolic syndrome; TG, triglycerides; VLDL, very-low-density lipoprotein.

Most papers examining the relationship of the metabolic syndrome to cardiovascular disease have excluded diabetic subjects with the metabolic syndrome since diabetic subjects are at high risk of cardiovascular disease whether they have the metabolic syndrome or not. Note also that the arrow pointing from the metabolic syndrome to type 2 diabetes refers to non-diabetic metabolic syndrome patients.

The prevalence of coronary heart disease was studies in subjects in the Botnia Study in Western Finland. This study showed that the prevalence of the metabolic syndrome increases as glucose tolerance worsens from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) to diabetes (DM). Note that this paper use the 1998 WHO definition, which is a slightly older version of the WHO definition discussed in this slide talk. Remember that IGT and diabetes are one of the components of the WHO definition (Isomaa et al, 2001). In the case of high-density lipoprotein (HDL), numerous studies, such as this early analysis from the Framingham Heart Study, have shown that is has an inverse relationship with coronary heart disease risk. This risk is independent of total and low-density lipoprotein (LDL) cholesterol, such that the risks due to lower HDL and higher LDL levels are additive (Gordon et al. 1977).

The ratio of total/high-density lipoprotein cholesterol is a good index of the relative contribution of atherogenic vs. antiatherogenic lipoproteins to coronary heart disease risk. As shown here in data from the Physician's Health Study, the risk associated with high levels of this ratio is further increased in the setting of increased plasma triglyceride. These results are

also consistent with data from other studies indicating that the impact of elevated triglyceride on cardiovascular risk is related to the levels of other lipoproteins (Stampfer et al. 1996). The presence of pattern B low-density lipoprotein (LDL), with smaller LDL particles, underestimates the risk for coronary heart disease as assessed by elevated LDL cholesterol. In this example, for LDL cholesterol of 130 mg/dL, subjects with pattern B can have a substantially larger number of cholesterol-depleted LDL particles. There is one molecule of apolipoprotein B (Apo B) per LDL particle; hence, for subjects with pattern B, Apo B provides a better index of atherogenic particle number than does LDL cholesterol (Berneis and Krauss, 2002).

While lifestyle measures (diet, weight loss, physical activity) should be the primary approach to improving the atherogenic dyslipidemia of obesity, those subjects at high risk for coronary heart disease (CHD), including those with existing vascular disease, require more aggressive intervention to meet current CHD prevention guidelines. In the subgroup of hypercholesterolemic CHD subjects in the 4S trial who had concomitant elevations of triglyceride and reductions in high-density lipoprotein (left panel), statin treatment was found to achieve a significant reduction in the CHD event rate, whereas there was no significant benefit to subjects with an isolated low-density lipoprotein (LDL) increase (right panel). Hence, statins may be of particular benefit in the treatment of patients with atherogenic dyslipidemia who are at high risk of CHD, and statins should be considered to be first-line treatment if non-pharmacologic measures are not successful in achieving LDL target levels (Ballantyne et al. 2001). This study also determined that non-Hispanic whites and individuals with normal glucose tolerance, hypertension, dyslipidemia and a low waist circumference have a lower risk for cardiovascular events. Furthermore, the interaction statistics are all non-significant, suggesting that the relationship of insulin resistance to CVDs does not differ among ethnic groups or gender (Hanley et al. 2002).

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This manuscript is meant for educational purposes. Parts of the manuscript are copied from the webpage of the Obesity Society (http://www.obesityonline.org/cme/index.cfm).

5. INSULIN RESISTANCE AND METABOLIC SYNDROME

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5.1 Introduction

Combination of metabolic abnormalities in patients with high vascular risk is well known since long time. In his Banting lecture Reaven used the term "Syndrome X" for the description of the coexistence of these metabolic diseases. Other names as e.g. "Deadly Quartet" and insulin resistance syndrome emphasised the consequences and the possible cause of this syndrome. Most of the authors accept that the syndrome in the patients induces serious complications and that in its background insulin resistance could be the major determinant.

Nevertheless, recently the existence of metabolic syndrome was questioned and an independent treatment of the components of the metabolic syndrome was suggested. Nowadays, in this "post metabolic syndrome" period newest papers are examining new and old questions, whether metabolic syndrome exists, what are the important components, is diabetes mellitus a component or a final result of the syndrome, which definition describes better the essence of the syndrome, which definition predicts the mortality, which components are better predictors for cardiovascular morbidity and mortality, etc. However, a relatively new estimation formula for insulin resistance using homeostasis model assessment (HOME) made possible to study the role of insulin resistance in big cohorts.

5.2 Insulin resistance and definitions of metabolic syndrome

The first international definition was given by the World Health Organization (WHO) in 1999. This was focusing on the glucose intolerance and insulin resistance. Other components as elevated blood pressure (> 140/90 mmHg), raised plasma triglycerides (> 1.7 mmol/l), decreased HDL cholesterol (for men < 0.9 mmol/l, for women < 1.0 mmol/l), obesity (waist-hip ratio for men > 0.9, for women > 0.85; and/or BMI > 30 kg/m^2), and microalbuminuria (urinary albumin excretion rate > 20 μg/min or albumin:creatinine ratio > 30 mg/g) were also involved.

The European Group for the Study of Insulin Resistance (EGIR) in 1999 proposed a definition to be used in non-diabetic patients only and put the insulin resistance in the centre, as well.

In 2001 the National Cholesterol Education Program – Third Adult Treatment Panel (ATP III) set a new definition concentrating rather on the obesity and dyslipidaemia. Three or more of the following risk factors are needed for the diagnosis of metabolic syndrome: fasting plasma glucose > 6.1 mmol/l, blood pressure > 130/85 mmHg, triglycerides > 1.7 mmol/l, HDL cholesterol for men < 1.03 mmol/l, for women < 1.29 mmol/l, waist circumference for men > 102 cm, for women > 88 cm.

In 2002 the American Association of Clinical Endocrinology (AACE) presented a position statement, again stressing the role of insulin resistance in the syndrome.
In 2005 the International Diabetes Federation (IDF) offered a consensus worldwide definition of the metabolic syndrome. This document shows the complexity of the problem of metabolic syndrome and divides the definition in four parts: part 1 contains definition for use of clinical practice, part 2 gives the additional metabolic criteria for research, part 3 details the recommendations for treatment, and part 4 suggests some future works. The definition for use in clinical practice is similar to the ATP III criteria focusing on the central obesity, but still keeping type 2 diabetes in the definition of the syndrome and strongly recommends the oral glucose tolerance test (OGTT). On the other hand, central obesity and insulin resistance were acknowledged equally important causative factors. Thus, IDF definition could be characterized as a "mixture" of WHO and ATP III criteria. According to this definition patients with metabolic syndrome must have central obesity defined as waist circumference > 94 cm for Europid men, and > 80 cm for Europid women plus any two of the following factors: raised triglycerides > 1.7 mmol/l or specific treatment for this lipid abnormality, reduced HDL cholesterol < 1.03 mmol/l in males and < 1.29 mmol/l in females or specific treatment for this abnormality, raised blood pressure > 130/85 mmHg or specific treatment for hypertension, raised fasting plasma glucose > 5.6 mmol/l or previously diagnosed type 2 diabetes mellitus. In the part of additional metabolic criteria for research are as follows: abnormal body fat distribution, atherogenic dyslipidemia (beyond elevated triglyceride and low HDL cholesterol), dysglycemia determined by OGTT, insulin resistance measured by fasting insulin/proinsulin ratio, HOMA, minimal model, elevated free fatty acids or clamp, vascular dysregulation (endothelial dysfunction and microalbuminuria), prothrombotic state, and hormonal factors. In the part of recommendation for treatment, management of insulin resistance and hyperglycemia was declared. Metformin, thiazolidindions, acarbose, orlistat, incretin mimetics, dipeptidyl peptidase IV inhibitors, protein tyrosine phosphates 1B inhibitors, and the endocannabinoid receptor blocking agents are listed as verified or future therapeutic possibilities.

In 2005, after the IDF definition, the American Heart Association and the National Heart, Lung, and Blood Institute (AHA/NHLBI) gave an up-to-date guidance for the diagnosis and treatment of metabolic syndrome in adults. It accepted the existence of the metabolic syndrome, kept all of the ATP III criteria except of the fasting plasma glucose, which was decreased to 5.6 mmol/l.

5.3 Pathogenesis of insulin resistance in metabolic syndrome

Confirmatory factor analysis of Spanish, Mauritian, and U.S. populations was carried out to search for a single common factor of metabolic syndrome. This study confirmed the current clinical components of the metabolic syndrome (insulin resistance, waist circumference, triglyceride, HDL cholesterol, blood pressure). Leptin and uric acid were suggested to be possible contributors, and a single latent, common factor underlying in the background was supposed.

Candidate causative factors are visceral obesity, elevation of circulating free fatty acids (FFA), subclinical inflammation, production of reactive oxygen species, endothelial dysfunction, decrease of the first phase insulin secretion, smoking, etc.

Role of visceral obesity and/or FFA was recently discussed. Computer tomography (CT) scans and MRI examinations verified intra-abdominal, predominantly mesenteric and

omental, adiposity as independent predictors of insulin resistance. On the other hand, contradictory data are available for the role of subcutaneous adiposity in insulin resistance. Removal of abdominal subcutaneous adipose tissue by liposuction, in turn, does not improve insulin resistance, and treatment using thiazolidindions (so called insulin sensitizers) increases subcutaneous fat mass and insulin sensitivity. Lower body obesity and Prader-Willy syndrome with normal visceral adipose tissue mass seems not to induce insulin resistance. Others rather stress the role of FFA in the development of insulin resistance suggesting that this could be the most important contributor, and visceral obesity might not be the major factor because it is responsible for only 20-25% of total FFA delivery to the liver and less than 5% for the systemic (extrahepatic) FFA availability.

Several lines of evidence support the pivotal role of subclinical inflammation and production of cytokines in the development of insulin resistance. One of the key features is the induction of intracellular formation of reactive oxygen species (ROS) by cytokines. Cytokines-induced ROS production decreases insulin-mediated glucose uptake of the fat cells. This could be prevented by antioxidants, e.g. N-acetylcystein, superoxide dismutase (SOD), and catalase. This effect could be mimicked by the overexpression of catalase in the cytosol and in the mitochondria, and overexpression of CuZnSOD and MnSOD in these fat cells. TNF-α decreases intracellular insulin signal transduction, using a ROS dependent pathway, at least partially by the inhibition of serin phosphorylation of Akt (protein kinase B, PKB), which is an intracellular mediator of the metabolic effect of insulin.

Akt is not only a transducer of the insulin effect in the fat cells, but it is a very important regulator of the vascular tone, as well. Insulin induces activating phosphorylation of Akt, and this phospho-Akt (P-Akt) evokes activating phosphorylation of endothelial nitric oxide synthase enzyme (eNOS), which produces nitric oxide and results in vasodilation. This way insulin dilates nutritive precapillary arterioles increasing circulation of parenchyma, and leading to the metabolic effect of insulin, and decreases blood pressure preventing target organ damage. In insulin resistant state due to the overproduction of cytokines and ROS, insulin is not able to induce full vasodilation anymore (endothelial dysfunction) because of the decrease of P-Akt. Diminished circulation in the nutritive capillaries and inhibited intracellular signalling in the parenchymal cells lead to insulin resistance and hypertension. In this stage secondary hyperinsulinemia due to the second phase insulin hypersecretion could augment sympatethic nervous system and renin-angiotensin system activity causing further elevation of blood pressure.

Some authors suggest the primary role of the decrease of the first (acute) phase of the insulin secretion in the insulin resistance, since this develops as the impaired glucose tolerance (IGT) or obesity are present, and decrease of first phase insulin secretion causes early postprandial hyperglycemia. This, in turn, results in the elevation of the second phase insulin secretion, mentioned before.

There are papers suggesting endothelial dysfunction as a primary abnormality in the development of insulin resistance. This is based on the processes detailed above, and on the observations proving that in some cases endothelial abnormalities precede insulin resistance.

The role of decrease of first-phase insulin secretion in the insulin secretion in the insulin resistance, blood pressure and target organ damage ispresented in Figure 5.1.).

Figure 5.1. *The role of decrease of first-phase insulin secretion in the insulin resistance, blood pressure and target organ damage.*

Current smoking is an independent predictor for metabolic syndrome. Even passive smokers have an elevated prevalence of metabolic syndrome compared to the never smoker population. Furthermore, adult offspring of smoker parents, exposed to their parent's smoking during their childhood have a significantly elevated risk for the development of metabolic syndrome. In smokers the value of HOMA is higher than in matched controls suggesting insulin resistance. In lean current smokers an increase of the visceral adiposity could be detected. According to our, not published, results smoke induces a concentration and a time dependent decrease of the phosphorylation of the Akt.

5.4 Cardiovascular risk and insulin resistance

In the last two years more publications compared the predictive value of the different definitions of metabolic syndrome or carried out a direct comparison of insulin resistance with metabolic syndrome. WHO-definition was supposed to be more representative for the insulin resistance, than ATP III or IDF.

In a prospective cohort study authors investigated the cardiovascular risk of metabolic syndrome and insulin resistance. Patients were examined by coronary angiography. The main outcome was the incidence of vascular events during 2.3 year follow up. Hazard ratio (HR) for vascular events was 2.74 in case of metabolic syndrome (ATP III) and 1.51 in case of insulin resistance (both were significant). They concluded that insulin resistance is a strong and independent predictor of vascular risk.

In the Uppsala Longitudinal Study of Adult Men the role of insulin sensitivity in the development of congestive heart failure (CHF) was examined. In a population of elderly men (> 70 years) the first hospitalization for CHF was detected during a follow-up of 8.9 years. Risk of CHF was associated in a multivariate analysis with 2-hour glucose, fasting serum proinsulin level, body mass index, and waist circumference. If clamp glucose disposal rate was added to the model, obesity variables were no longer predictors for CHF. According to these results authors supposed, that the earlier described association between obesity and CHF may be mediated by insulin resistance.

The DECODE Study Group compared WHO, ATP III, modified ATP III (fasting plasma glucose was lowered from 6.1 mmol/l to 5.6 mmol/l), and IDF definitions of metabolic syndrome in predicting cardiovascular disease (CVD). HR was higher in case of WHO compared to the others in the male patients, but all definitions had a low predictive value in the female population.

- In the British Women's Heart and Health Study the general finding was the same as in the DECODE Study.
- In a Canadian long-term (12.6 years) study in a mainly male (75.6%) population HR for all-cause mortality was 1.2 using ATP III and 1.56 using WHO definitions.
- In another prospective, population based cohort study ATP III gave a long term prognostic information about total and cardiovascular mortality.

Taken together, these studies suggest that the WHO definition may be better in predicting the cardiovascular diseases. This definition system is based mainly on the insulin resistance concept. On the other hand insulin resistance seems to be an independent predictor of the CVD morbidity and mortality.

5.5 Recent studies using insulin sensitizers

Thiazolidindions are agonists of peroxisome proliferators-activated receptor γ (PPAR γ). These drugs increase hepatic and peripheral insulin sensitivity, preserve insulin secretion and pancreatic β-cell function. There are some data about their beneficial effect on the subclinical inflammation, as well. Through all these effects these drugs could decrease the risk of development of diabetes mellitus and macrovascular complications. Troglitazone, the first member of this group of drugs was withdrawn because of hepatotoxicity. Pioglitazone and rosiglitazone are now on the market.

In the Diabetes Reduction Assessment with ramipril and rosiglitazone Medication (DREAM) Trial effect of rosiglitazone on the frequency of diabetes type 2 was examined. In this study patients with impaired glucose tolerance (IGT), with impaired fasting glucose (IFG) or both were involved. They were obese, hypertensive. These three parameters (carbohydrate

abnormality, obesity, and elevated blood pressure) indicate that these patients could be mainly metabolic syndrome patients. They were followed for 3.0 years. The rosiglitazone group was compared to a placebo group. The primary outcome was a composite of incident diabetes mellitus and death. Rosiglitazone decreased the risk of composite primary outcome by about 60%, the risk for incident diabetes mellitus by 62%, and increased the regression from IGT or IFG or both to the normal glucose tolerance by 71% (fasting plasma glucose < 6.1 mmol/l) and by 83% (fasting plasma glucose < 5.6 mmol/l). The drug was more effective in the Indian and in the more obese population. Rosiglitazone significantly decreased the blood pressure, by 1.7 mmHg of the systolic and by 1.4 mmHg of the diastolic blood pressure. These results support the general approach of the insulin resistance detailed above emphasizing the role of Akt, eNOS and the endothelium in the development of metabolic syndrome. There is another interesting conclusion of this trial, namely that the treated group had an increase of body weight, body mass index, waist-to-hip ratio and waist circumference. It could mean, that rosiglitazone decreases insulin resistance in spite of the increase of body weight, i.e. eliminates the relation between obesity and insulin resistance. Rosiglitazone did not influence the cardiovascular endpoints but the incidence of non-fatal CHF was increased.

In a secondary prevention study in the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) effect of pioglitazone on the macrovascular morbidity and mortality was investigated. In type 2 diabetic, high risk patients the primary endpoint was the composite of all-cause mortality, non-fatal myocardial infarction, stroke, acute coronary syndrome, endovascular or surgical intervention in the coronary or leg arteries, and amputation above the ankle. The secondary endpoint was the composite of all-cause mortality, non-fatal myocardial infarction, and stroke. The time of observation was 34.5 months. Pioglitazone treatment did not influence the primary endpoint, but significantly decreased the secondary endpoint by 16%. Systolic blood pressure was significantly decreased by 3 mmHg. On the other hand, in the treatment arm the incidence of non-fatal CHF significantly increased.

These two big trials with insulin sensitizers suggest that thiazolidindions could have a preventive effect in the progression of insulin resistance, but their cardiovascular effectiveness should be further investigated.

5.6 In conclusion

Metabolic syndrome definitions try to identify patients at high risk for cardiovascular diseases. WHO definition relies on insulin resistance, ATP III is based rather on the obesity. IDF definition is a mixture of WHO and ATP III. Insulin resistance could be in the centre of the pathogenesis of metabolic syndrome. Overproduction of cytokines and reactive oxygen species could play a role in the insulin resistance. Importance of decrease of first phase insulin secretion and endothelial dysfunction in the development of insulin resistance could be also not excluded. In the majority of cases cardiovascular endpoints are better predicted by the insulin resistance-based WHO definition, than by the others. Insulin resistance itself is a predictor of cardiovascular morbidity and mortality. Drugs affecting directly the insulin resistance (the so called insulin sensitizers) prevent the progression of insulin resistance and this way decrease the rate of development of type 2 diabetes in IGT and IFG patients. This effect of insulin sensitizers evolves in spite of their increasing effect on the body weight. Effects of insulin sensitizers on the cardiovascular morbidity and mortality remain to be elucidated.

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6. PRO-INFLAMMATORY AND PROTHROMBOTIC FACTORS AND METABOLIC SYNDROME

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The metabolic syndrome represents combined occurence of atherogenic dyslipidemia, insulin resistance, elevated blood pressure and central adiposity. Pro-inflammatory and prothrombotic state contributing to endothelial dysfunction is a common feature of those with metabolic syndrome. Increasing frequency of abdominal obesity, reaching epidemic proportions, enhances the prevalence of metabolic syndrome. Both, obesity and metabolic syndrome, have the potential to influence on the incidence and severity of cardiovascular disease with serious implications for worldwide health care systems.

Visceral obesity is a key component in the development of the metabolic syndrome. Increased central adiposity, particularly in visceral region, leads to greater free fatty acid flux and inhibition of insulin action. Adipose tissue in obesity is resistant to insulin which is associated with disturbed glucose metabolism in the muscles and liver. Even mild or moderate degree of obesity with concomitant insulin resistance may be associated with metabolic syndrome. On the other hand, excessive accumulation of abdominal fat may lead to the development of metabolic syndrome independently on degree of insulin resistance.

It is suggested that chronic mild inflammation constitutes an important underlying factor of metabolic syndrome. Pathogenesis of obesity associated metabolic syndrome is mediated by disturbed production of biologically active molecules by fat cells. In obese subjects synthesis of several bioactive compounds – adipokines, by either adipocytes or adipose tissue infiltrated macrophages, is dysregulated, secretion of pro-inflammatory adipokines is elevated while that of anti-inflammatory is reduced.

All identified adipokines form a network linking adipose tissue with skeletal muscle, liver, adrenal cortex, brain and sympathetic nervous system. All these compounds participate in regulation of apetite and energy homeostasis, lipid metabolism, insulin sensitivity, immunity, angiogenesis, blood pressure and hemostasis.

Pro-inflammatory cytokines have been reported to induce insulin resistance in fat tissue and muscles. Prospective studies have shown that elevated levels of pro-inflammatory indices (like CRP) or diminished levels of protective anti-inflammatory marker (adiponectin) are important predictors of the development of type 2 diabetes.

Thus low-grade inflammation constitutes the bridge linking atherosclerosis with metabolic syndrome and is associated with higher risk for acute cardiovascular syndromes.

Inflammatory state is an important component of wide range of the diseases also those associated with aging. Trayhurn and Wood proposed an explanation to the increasing inflammatory response of fat tissue with developing obesity. The authors suggest that in growing adipose tissue mass, poorly vascularized, hypoxia is a critical factor. Expression of some cytokines (leptin), chemokines and angiogenic factors (VEGF) to stimulate

vascularization may be induced by hypoxia that has been shown recently in different situations and in adipocyte cultures.

Adipose tissue has an important endocrine function involved in inflammatory and thrombotic pathways. Fat cells produce and release more than 50 different compounds into the circulation. These adipokines play multiple roles in a wide range of physiological processes such as insulin sensitivity (adiponectin, resistin, visfatin), lipid metabolism (CETP, apoE, NEFA), hemostasis (PAI-1), blood pressure regulation (angiotensinogen) and angiogenesis (VEGF). Adipocytes release also: hormones (steroids, leptin) and prostaglandins, growth factors (TNF-α, TGF-β), cytokines (IL-1β, IL-6, IL-10), chemokines (IL-8, MCP-1, MIF β – macrophage migration inhibitory factor) and some acute phase proteins (haptoglobin, SAA) (Figure 6.1.).

Figure 6.1. *Adipokines linked to inflammation and the inflammatory response; According to (4).*
NGF, nerve growth factor

Several, listed above, adipokines are associated to the immune system and inflammation. In obesity expression, synthesis and release of pro-inflammatory adipokines (TNF-α, IL-6, PAI-1, haptoglobin and leptin) is enhanced with concomitant decrease of protective adiponectin. It seems that in obese subjects the inflammation state reflects, at least partly, increased release of inflammatory peptides and proteins from adipose tissue as a major source. This is the case for PAI-1 but not for increased level of CRP in the blood. The latter is probably also expressed in adipocytes and released in low amounts but mostly derives from hepatocytes after IL-6 stimulation.

Adipokines play multiple roles in the inflammatory process. Leptin stimulates accumulation of cholesterol in macrophages, IL-6 stimulates liver production of CRP and through visfatin influence glucose tolerance, TNF-α increases expression of adhesion molecules (ICAM-1, VCAM-1) enhancing monocyte adhesion to the vessel wall, and induces endothelium function changes, PAI-1 stimulates formation of thrombus after atherosclerotic plaque rupture.

6.1 Leptin

Leptin, is a hormone with divergent activities**.** This 16-kD cytokine, not exclusively (stomach, ovaries, placenta etc) produced by adipocytes remains a key hormone responsible for the

regulation of apetite and energy balance by hypothalamus. Leptin, acting as a "starvation signal" is a central factor in the elevation of sympathetic activity found in obese hypertensive patients.

Moreover, it has been reported that leptin affects vessel wall. Leptin may act as angiogenic factor and in vitro stimulates production of reactive oxygen species by activated monocytes. Also, it may contribute to arterial thrombosis through a platelet leptin receptor.

6.2 TNF-α

TNF-α is a multipotential cytokine with several immunologic functions. It is produced and released from adipocytes and its enhanced expression associated to induction of insulin resistance was reported in obese subjects. In adipose tissue TNF-α is also engaged in stimulation of lipolysis and apoptosis. Probably TNF-α activates transcription factor NF-kappa β that leads to increased production of cytokines and increases oxidative stress while adiponectin inhibits this factor.

A substantial effect of TNF-α on the expression and release of pro-inflammatory adipokines was confirmed, up to now, only by in vitro studies. In human adipocytes differentiated in culture TNF-α increased IL-6, MCP-1 (monocyte chemotactic protein), NGF, VEGF while adiponectin, adipsin, haptoglobin and leptin were decreased.

6.3 Interleukin-6

Interleukin-6 is a cytokine having multiple effects, secreted by immune cells, fibroblasts, endothelial cells, skeletal muscle and adipose tissue. This pro-inflammatory cytokine is increased in subjects with obesity and insulin resistance and may be regarded as a predictive factor for type 2 diabetes and myocardial infarction. Fat cells produce only about 10% of total IL-6 and regional differences has been observed. Visceral adipocytes produce much more IL-6 than from the subcutaneous depot. Induction of insulin resistance by IL-6 could be mediated by suppression of insulin receptor signal transduction in hepatocytes.

6.4 Adipsin

Adipsin, a serine protease is known to stimulate glucose transport for triglyceride accumulation in fat cells and to inhibit lipolysis. Obese humans have substantially increased blood adipsin concentration but still it is not clear whether high concentration reflects increased activity or resistance to adipsin.

6.5 Resistin

Resistin was suggested to be a link between obesity and insulin resistance but until now its role is unclear. Resistin is poorly expressed in human fat cells. Since it is produced by blood monocytes its inflammatory activity and contribution to development of endothelial dysfunction has been suggested.

6.6 Visfatin

Visfatin, recently discovered in the human visceral fat was suggested to play a role in glucose homeostasis through stimulation of the insulin receptor (insulin-mimetic effects).

6.7 Adiponectin

Adiponectin has been considered as a key regulator of insulin sensitivity and tissue inflammation. This 30-kD protein synthesized exclusively by adipocytes (white and brown) is present at very high concentrations in the blood but its level inversely correlates with the amount of body fat. It means that adiponectin concentration is higher in non-obese than in obese people. Regional difference exists in adiponectin production in humans, omental adipocytes secrete higher amounts than subcutaneous. Adiponectin level may be a predicting factor of diabetes and cardiovascular disease risk.

In the circulation adiponectin exists as varying molecular weight forms. High molecular weight complexes have the predominant action in the liver.

Adiponectin may act as signaling molecule to regulate insulin action in the liver (improve hepatic insulin sensitivity) and skeletal muscle (increase fuel oxidation). Two adiponectin receptors have been identified: AdipoR1 is highly expressed in skeletal muscle and promotes lipid oxidation, AdipoR2 is mostly expressed in the liver and enhances insulin sensitivity, reduces liver steatosis via increased PPAR- α**.** PPAR-α is a nuclear transcription factor that regulates expression of genes involved in FA beta-oxidation and regulates energy homeostasis.

Adiponectin antagonizes many effects of pro-inflammatory TNF- α, that in turn suppresses adiponectin production.

In type 2 diabetics adiponectin is significantly reduced. It was shown that administration of adiponectin increased glucose uptake by muscles, improved insulin sensitivity and suppressed gluconeogenesis in the liver cells.

Protective role of adiponectin within the arteries results from suppression of the inflammatory processes such as adhesion, proliferation, phagocytosis and deposition of lipids in monocytes.

In obese people increased gene expression of inflammatory and thrombotic cytokines and decreased expression of protective adiponectin has been reported suggesting a close link between abdominal obesity and other underlying risk factors of metabolic syndrome.

It has been shown recently that in obese postmenopausal women visceral adipose tissue volume inversely correlated with leptin and tended to inversely correlate with adiponectin gene expression. Positive relationship between fasting insulin and visceral adipose tissue TNF-α gene expression was observed in the subgroup of non-diabetic women. Additionally, IL-6 gene expression tended to be positively related to fasting insulin in these women. Expression of adiponectin was much lower in obese women with metabolic syndrome than without. These results suggest that enhanced pro-inflammatory cytokine expression in fat tissue links abdominal obesity with its metabolic disturbances.

Interestingly, the inflammation state in obese people can be partly reversed after weight loss. It has been shown that CRP levels decline with weight reduction.

Whether improving metabolic syndrome by weight loss and physical exercise is a consequence of changes in adipose tissue cytokine gene expression still needs explanation.

Recent studies indicate that regular physical activity improves insulin sensitivity and correlates inversely with leptin and mild inflammation (IL-6) in adolescents, independently of fat mass and localization. However, in this study beneficial effects of regular physical exercise on metabolic syndrome features were not totally explained by adipokines (adiponectin, TNF-α- receptor1).

Apart from impaired glucose tolerance and insulin resistance, dyslipidemia and hypertension a typical feature in metabolic syndrome is a prothrombotic state. The metabolic syndrome is frequently diagnosed in patients with venous thrombosis. Recent study reported the presence of metabolic syndrome in 50% of patients with deep vein thrombosis.

The risk of thromboembolism is significantly increased in abdominal obesity that results from activation and changes of coagulation system. This is reflected by enhanced generation of thrombin (which converts fibrinogen to fibrin), diminished fibrinolysis and increased platelet aggregation. Increased levels of fibrinogen, factor VII and VIII that leads to hypercoagulability is characteristic of metabolic syndrome. Simultaneously, enhanced production of PAI-1 decrease fibrinolysis.

Abdominal obesity (but mainly accumulation of visceral fat) resulting in low-grade inflammation is related to increased fibrinogen levels. Pro-inflammatory state is also associated with increased levels of coagulation factors: TF and factor VII and thus the risk of activation of coagulation cascade.

There are few studies in which interrelations between procoagulant factors and anticoagulant proteins were investigated in humans with wide range of body fat. Godsland et al (2005) have found that procoagulant factors VII and X, anticoagulant proteins C and S and PAI-1 correlated directly with total and central body fat but inversely with insulin sensitivity. The authors suggested that procoagulant factors and anticoagulant proteins are the features of the intercorrelated disturbances of the metabolic syndrome.

Also other factors of metabolic syndrome such as: TNF-α and homocysteine has been suggested to contribute to procoagulant state.

Fibrin degradation (fibrinolysis) is a process controlled by t-PA (tissue plasminogen activator) and PAI-1 balance. Decreased t-PA paralleled by increased plasma level of PAI-1 associated with insulin resistance are common in metabolic syndrome (Figure 6.2.).

Chronic inflammation and enhanced lipolysis in adipose tissue, leading to increased FFA, stimulate PAI-1 expression and synthesis, decrease conversion of plasminogen to plasmin and in consequence fibrin degradation being the important contributors of hypofibrinolysis.

Figure 6.2. *Hemostatic risk factors and insulin sensitivity, regional body fat distribution and the metabolic syndrome. According to Godsland et all. J Clin Endocrinol Metab 2005, 90, 190-7.*

The relationship between PAI-1 activity, adiponectin and CRP levels, insulin resistance and lipoproteins was studied in overweight and obese women. Interestingly, it was found that, PAI-1 activity inversely correlated with serum adiponectin, (independently of the amount of visceral tissue).

The other characteristic feature of metabolic syndrome is endothelial dysfunction often present in insulin resistance and type 2 DM. Excessive lipolysis resulting in chronic elevations of plasma FFA may induce endothelial dysfunction. This is reflected by high levels of markers such as thrombomodulin.

Insulin-resistance in obesity and dyslipidemia are associated with excessive platelet activation and aggregation. High levels of VLDL stimulate synthesis of thromboxane A2 in platelets from FFA (through binding of CD 36 ligand to platelets). TxA2 is known to enhance platelet aggregation.

In routine medical laboratory the easiest way to detect a proinflammatory state in a person without other detectable causes is measurement of hsCRP. If CRP level, measured twice within a few weeks, is above 3 mg/L a pro-inflammatory state is defined.

Then there is a need for lifestyle changes like weight reduction by diet or exercise. Therapies addressing the treatment of obesity related disorders should focus on modifying the inflammatory profile. When other risk factors are present together with elevated CRP, statins, nicotinic acid, fibrates, ACE inhibitors or thiazolidinediones (glitazones) decreasing CRP level may be used for treatment.

6.8 Thiazolidinediones

Thiazolidinediones - peroxisome proliferator-activated receptor-γ agonists are known for their beneficial effect diminishing the risk of metabolic syndrome. PPAR- γ -peroxisome

proliferator-activated receptor-γ, a nuclear receptor, is a ligand-activated transcriptional factor expressed in various types of cells but highly in human adipose tissue-derived cells.

They influence changes in secretion of adipokines linked with regulation of insulin sensitivity and other signalling molecules. It was shown that adiponectin and resistin genes are regulated by PPAR-γ. Moreover, TNF-α and leptin genes are regulated by PPAR-γ agonists.

The effect of short-term treatment with pioglitazone on lipoproteins, CRP and adipokines, adiponectin and resistin, in adult patients with metabolic syndrome without diabetes was studied by Szapary et al. It has been found that this drug significantly increased serum adiponectin (by 111%) while significantly decreased CRP (by 31%) and resistin level (by 10%). Also slight increase in HDL-C (by 15%) and favorable effect on LDL particle size was observed. Thiazolidinediones also significantly diminish plasma CRP levels and increase adiponectin in type 2 diabetics.

6.9 Fibrates

Fibrates known as lipid-lowering drugs (decrease TG) are the agonists of PPAR-α another transcription factor that regulates expression of genes involved in fatty acids beta-oxidation and regulates energy homeostasis. Fibrates also have anti-inflammatory and anti-thrombotic effects in the vessel wall in patients with metabolic syndrome.

6.10 Niacin (nicotinic acid)

The lipid-lowering drug niacin may modify inflammatory profile. It has been found however, that treatment with niacin significantly rises serum adiponectin (by 56% after 6 weeks) and leptin (by 27%) but does not change resistin, TNF-, IL-6 nor CRP thus fails to improve atheroprotective function attributed to adiponectin.

Assessment of prothrombotic state typically found in subjects with metabolic syndrome is not so easy in routine medical laboratory. The level of fibrinogen can be easily determined automatically, however, coagulation factors such as PAI-1 are generally not routinely measured.

Again changes in the lifestyle and regular physical exercise may increase production of fibrinolytic proteins thus exerting anti-coagulant action.

Treatment with thiazolidinediones may decrease the risk of thrombosis in metabolic syndrome. It has been reported that secretion of PAI-1 stimulated by insulin is suppressed by glitazones. PPAR-γ activated by glitazones suppress lipolysis (reduce FFA release) thus improve endothelial dysfunction that is probably mediated by reduction of TNF-α level and action. PPAR-γ agonists tend to decrease CD 36 expression.

6.11 Aspirin

In primary prevention of arterial thrombosis, antiplatelet agents like low-dose aspirin may be used in the long-term approach. In patients with risk of atherosclerotic cardiovascular disease aspirin seems to be a good therapeutic possibility.

The better understanding of the molecular actions of adipokines is the key issue to the discovery of effective therapy. Weight loss and pharmacological treatment leading to decrease of pro-inflammatory adipokine level may prevent the metabolic syndrome and type 2 diabetes and in consequence the development of atherosclerosis complications.

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7. EVIDENCE BASED LABORATORY MEDICINE IN THE DIAGNOSIS OF METABOLIC SYNDROME

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7.1 Introduction

Evidence based medicine (EBM) is a formalized system helping medical community cope with numerous different medical information, whereby the end result helps doctors identify the best diagnostic tests and treatments. Medical knowledge is accumulating and changing with such a dizzying speed that medical community has found it needs new methods to cope with it all. EBM provides formal protocols that are applied to the latest data to determine what data best support the best outcomes.

The aims of EBM in laboratory medicine (or EBLM) are to advance clinical diagnosis by research and dissemination of new knowledge, and to combine methods from clinical epidemiology, statistics and social science with the traditional pathophysiological and molecular approach. The evaluation of diagnostic investigations as well as the clinical decision-making process can help in translating the results of good quality research into everyday practice.

EBLM has a few elements which have to be satisfied by order: audit practice, identifying the question, search for evidence, critically appraising the evidence, applying it to practice by modifying the practice, and constant practice audit.

7.2 Definition of metabolic syndrome (MS)

Until 1998, there was no initiative to develop an internationally recognized definition. World Health Organization (WHO) was the first to publish an internationally accepted definition for metabolic syndrome. Subsequently, third report of the USA National Cholesterol Education Program: Adult Treatment Panel (NCEP:ATP III) has also defined clinical identification of MS. The NECP:ATP III guidelines suggest a diagnosis of metabolic syndrome (previously known as syndrome X) when three or more of the following risk factors are present: central obesity, elevated triglycerides, low HDL, raised blood pressure, and raised fasting plasma glucose. More recently, the International Diabetes Federation (IDF) has defined criteria for MS where metabolic syndrome is diagnosed if the patient has a 'large waist' plus any other two risk factors.

Since several definitions of the syndrome are in use, it is difficult to compare its prevalence and impact between countries. Fortunately, there is a chance for a more rational approach. In 2004, IDF convened a group of experts to establish a unified definition for MS. Table 7.1. presents the comparison of different definitions of diagnostic criteria for metabolic syndrome.

The metabolic syndrome is also known as syndrome X, insulin resistance syndrome, and deadly quartet (upper body obesity, glucose intolerance, hypertriglyceridemia and hypertension).

Table 7.1. *Comparison of different definitions of diagnostic criteria for metabolic syndrome (MS)*

7.3 Laboratory indicators for the diagnosis of metabolic syndrome

Based on these data, general laboratory tests used in the diagnosis of MS include determination of lipid profile (concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) and glucose metabolism (total glucose concentration, oGTT, and glycated hemoglobin HbA_{1c}).

There are other laboratory tests that are not recommended for diagnosing MS but may be ordered by some doctors to provide additional information. Tests that can also be useful include measuring of lipoprotein particle size (measurement of small dense low-density lipoprotein particles in particular) and determination of high sensitive C-reactive protein (CRP) concentration.

There also are some tests that can be used in research setting, such as plasminogen activator inhibitor-1 (PAI-1), fibrinogen, proinsulin and homocysteine.

7.4 General laboratory tests

7.4.1 Glucose

Insulin as a hormone enables glucose to move into the tissue. The liver produces glycogen and/or fatty acids from glucose. Insulin resistance causes an additional release of insulin by

the pancreas. This can lead to increased glucose and insulin concentrations in the bloodstream. Blood glucose may be measured on the fasting basis (8- to 10-hour fasting), randomly, or as part of oral glucose tolerance test (oGTT), a series of blood glucose testing.

7.4.2 Triglycerides

In MS, high triglycerides are the consequence of increased glucose and insulin concentrations. Diminishing glucose entering the cells increases the production of fatty acids (supported by high insulin concentration) and triglyceride production.

7.4.3 Total cholesterol

Measuring of total cholesterol concentration in the diagnosis of MS is included in the lipid profile determination and is used to assess the risk of heart disease. As high blood cholesterol is associated with arteriosclerosis, heart disease and an increased risk of death from heart attack, cholesterol testing is considered a routine part of preventive health care as well as a tool to assess complications of some diseases such as MS.

7.4.4 High-density lipoprotein cholesterol

The concentration of HDL-cholesterol is decreased in MS, probably due to the increased triglyceride concentration. HDL particles become enriched with triglycerides and are more rapidly removed from the circulation. Triglyceride-enriched HDL particles are smaller and become better substrates for hepatic lipase. The test for HDL-cholesterol is used along with other lipid tests to determine the risk of heart disease.

7.4.5 Low-density lipoprotein cholesterol

Insulin resistance has an unfavorable effect on lipid production. It decreases HDL-cholesterol, increases triglycerides, and also VLDL and LDL. So, determination of LDL-cholesterol is also important in the diagnosis of MS as well as a prognostic factor for some complications such as cardiovascular diseases.

7.5 Alternative laboratory tests

7.5.1 Insulin

Fasting insulin values may be too variable to be clinically useful in the diagnosis of MS but if measured, they will usually be elevated in these individuals. The methods of determination are different immunoassays.

7.5.2 High-sensitivity C-reactive protein (hs-CRP)

Although determination of CRP is not useful in the diagnosis of MS, it may be tested as part of the cardiac risk assessment. People who have hs-CRP results at the upper normal limit have a 1.5- to 4-fold risk of sustaining heart attack found in those with CRP values at the lower normal limit. It may come from cells in the fatty deposits in arterial walls that reflect the process of atherosclerosis; however, it may also come from other tissues. So, CRP determination is not used for large-scale screening of the general adult population but is useful as an independent marker of the risk of cardiovascular disease to help determine the course of treatment. Methods of determination are different immunoassays.

7.5.3 Microalbumin

Increased urinary albumin excretion precedes and is highly predictive of diabetic nephropathy. Microalbuminuria is between normal and overt proteinuria. Microalbumin is an early indicator of kidney disease. This test is used to help monitor diabetics and is recommended under the WHO criteria. Detection of microalbuminuria can be accomplished by using semiquantitative rapid tests or by performing quantitative immunochemical determination of albumin. Test strips used for screening purposes fail to detect most cases of microalbuminuria.

7.5.4 Small dense LDL

LDL varies in size and the smaller denser particles, which tend to form when elevated triglycerides and VLDL are present in the blood, are thought to be more aggressive in causing atherosclerosis. Determination of small dense LDL particle can be useful because LDL cholesterol values can be misleading. Optimal levels of LDL cholesterol can mask an increased number of LDL particles.

7.5.5 Homocysteine

Elevated plasma homocysteine may cause or result from insulin resistance and could be actively involved in atherogenesis or is just an indicator of vascular risk. Numerous clinical studies have shown that total homocysteine is a risk factor for cardiovascular disease and stroke in humans and predicts mortality independently of traditional risk factors in patients with CAD. The possible cellular mechanisms by which homocysteine may contribute to cardiovascular disease include unfolded protein response, oxidative stress, and the induction of proinflammatory factors. Interpretastion of some laboratory test results in the diagnosis of metabolic syndrome are presented in Table 7.2.

Table7.2. *Interpretation of laboratory test results in the diagnosis of metabolic syndrome (MS)*

7.6 Laboratory tests used only in research setting

There are a few tests that are primarily used for research purposes. The molecular basis for the link between adipose tissue, metabolic disorders and cardiovascular diseases has not been fully clarified. Research on adipocyte biology has revealed that adipocytes produce and secrete a variety of bioactive substances named 'adipocytokines'; these include growth factors, cytokines and complement factors. Adipose tissue probably acts as an endocrine organ that may affect the function of other organs. The explanation of the molecular mechanism and their impact in the development of MS would in the future be helpful in the diagnosis.

7.7 EBM studies of metabolic syndrome and laboratory indicators

EBLM studies have confirmed some of laboratory assays as relatively simple metabolic markers in the diagnosis of MS. Evaluation of the ability of metabolic markers has shown that plasma triglyceride concentration, the ratio of triglyceride/HDL-cholesterol concentrations, and insulin concentration are most useful markers in identifying insulin resistant individuals ATP III criteria for the diagnosis of MS were used to identify insulin-resistant individuals among 258 nondiabetic, normotensive, overweight individuals. The optimal cut-off points were 1.47 mmol/L for triglycerides, 1.8 for triglyceride/HDL-cholesterol, and 109 pmol/L for insulin. The respective sensitivity and specificity for these cut-off points were 67%, 64% and 57%, and 71%, 68% and 85%.

Different EBM studies which include treatment of MS can be useful in the evaluation of laboratory tests in the diagnosis of MS. Sixty nondiabetic adults with NCEP defined MS from local metropolitan Philadelphia area, with HDL-cholesterol lower than 1.0 mmol/L in men and 1.3 mmo/L in women, were treated with pioglitazone-PIO (a synthetic peroxisome proliferator activated receptor γ ligand), which has been approved for the treatment of hyperglycemia in diabetes mellitus type 2. The concentration of HDL-cholesterol increased by 11% as compared with 4% reduction in those who received placebo. Small LDL particles were reduced significantly, and the level of hs-CRP was reduced by 31%. Insulin resistance showed a modest decrease.

Sixteen Finnish patients diagnosed with diabetes mellitus type 2 were on a strictly defined low energy diet. The treatment led to body weight reduction by 6±1 kg and BMI reduction by 6%. Laboratory tests showed a 14% reduction of blood glucose concentration and 13%-24% reduction in serum triglycerides. Serum cholesterol concentrations were unchanged.

CRP has also been confirmed as a clinically important prognostic parameter in the diagnosis of MS. Examples of EBLM studies:

1. Inflammatory markers modified by multitarget treatment were investigated in a prospective, randomized study. A series of 300 nondiabetic patients (of Grecian descent) with MS (according to NCEP definition), free from CVD were studied over a 12-month period and treated for hypertension, hyperlipidemia, impaired fasting glucose and obesity.

2. Increased CRP concentrations in obese men of Canadian descent with MS were significantly reduced with gemfibrozil, a lipid lowering drug.

3. A series of 179 men and 166 women (of Japanese descent) with MS according to NECP ATP III criteria were included in a study evaluating cut-off points. The optimal cut-off point of CRP for MS might be 0.65 mg/L in Japan and this value could be useful in routine clinical practice and studies of MS.

So, it is concluded that triglyceride concentration, the ratio of triglyceride/HDL-lipoprotein cholesterol concentrations, and insulin concentration as well as CRP and blood glucose concentrations are useful markers in the prognosis and treatment of MS.

As one of the characteristics of MS is insulin resistance, and the risk sequel is development of diabetes mellitus type 2, determination of glycated hemoglobin, HbA1c, plays a role in the diagnosis and prognosis of MS. Hemoglobin A1c (HbA1c) is considered a standard measure of long-term glycemic control, and HbA1c levels are strongly associated with complications of diabetes. Examples of EBLM studies:

1. A Swedish study which included patients with clinically diagnosed type 2 diabetes showed a combination of HbA1c, fasting plasma glucose and BMI to be effective in screening for individuals at risk of future clinical diagnosis of type 2 diabetes. This study also showed that oGTT or familial history of diabetes were not necessary.

2. French subjects with clinically diagnosed diabetes from the Epidemiological Study on the Insulin Resistance Syndrome were investigated. Incident diabetes was defined by fasting plasma glucose ≥ 7.0 mmol/L or treatment by antidiabetic drugs. Results of the study showed that HbA1c predicted diabetes, even though the diagnosis of diabetes was based on blood glucose. It could be used as a test if fasting blood sampling was not available or in association with fasting plasma glucose. In subjects with impaired fasting glucose, HbA1c is better than glucose to evaluate the risk of diabetes, and it could be used to select subjects for intensive early intervention.

There are few EBM studies that indicate the importance of homocysteine in assessing complications in subjects with MS. Examples of EBLM studies:

1. Italian patients with MS and diabetes mellitus type 2 were treated with different antidiabetic drugs. There was a statistically significant decrease in basal homocysteinemia in glimepiride-treated patients (-27.3%) but not in rosiglitazone-treated patients.

2. Weight-reduction diet as well as replacement meal (Slim-FastTM products) in overweight/obese Australians with raised triglycerides showed a significant decrease in homocysteine values.

3. Folate and vitamin B12 treatment improved insulin resistance and endothelial dysfunction, along with decreasing homocysteine levels in Italian patients with MS, suggesting that folic acid has several beneficial effects on cardiovascular disease risk factors.

It is very difficult to compare different EBM studies and to define standards in laboratory diagnosis of MS. At first, difficulties arise from different names and definition of MS (differences in concentration limits for general laboratory indicators of MS such as

triglycerides, HDL-cholesterol and glucose. Furthermore, the criteria used for obesity in Caucasians could be different from those used in Asians and other populations. And finally, significant diversity in the design of EBLM studies of MS laboratory indicators can be documented (e.g., inclusion/exclusion criteria for diabetes mellitus, age, sex, treatment, etc.).

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8. HYPERTENSION AND THE METABOLIC SYNDROME

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The overall importance of arterial hypertension relies on two facts. It is a very common condition in clinical practice and one of the most important risk factors in the development of cardiovascular disease, the leading cause of morbidity and mortality in the modern world. Hypertension frequently remains undiagnosed until relatively late in its course, leading to a variety of other life-threatening conditions, like kidney damage and heart failure.

The association of increased blood pressure and metabolic abnormalities with poor cerebrovascular outcome had been recognized long before the concept of the metabolic syndrome became popular.

However, until 1997, hypertension was defined as blood pressure value above 160/90mmHg. Over the last decade extensive randomized trials documenting that an increase in systolic or diastolic blood pressure of 5mmHg was associated with a concomitant increase in cardiovascular disease of 20-30%, have led to a revision of the definition of hypertension. For the first time, in 1997, the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (JNC VI) recommended a cut-off value of 140/90mmHg for the general population and 130/85mmHg for diabetic patients. JNS VII recommended a value of 130/80mmHg for diabetic patients in 2003. That same year, The European Society of Hypertension and Cardiology (ESH/ESC) recommended a new classification, defining an optimal blood pressure as a value under 120/80mmHg (Table 8.1.). They emphasized that there was no single value dividing normotension from hypertension. The threshold for the initiation of blood pressure treatment should be determined on the basis of global cardiovascular risk (associated risk factors, risk of future organ damage and target blood pressure values)!

Table 8.1. *Blood pressure classification *ESH/ESC*

*ESH/ESC: European Society of Hypertension/European Society of Cardiology
(From: Journal of Hypertension 2003,21:1011-53.)

Current guidelines suggest that the target for blood pressure lowering in diabetic patients is below that for the general population, at 130/80mmHg, or lower in the presence of nephropathy (Table 8.2.) Unfortunately, blood pressure goals stated in the current guidelines are difficult to achieve in clinical practice.

Table 8.2. *Guideline recommendations for BP goals*

*ESH/ESC: European Society of Hypertension/European Society of Cardiology
**JNC 7: Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, 7th report
(From: Guidelines Committee. *J Hypertens* 2003; 21: 1011-53., Chobanian AV, *et al*. JAMA 2003; 289: 2560-72.)

However, the establishment of hypertension as a component of the metabolic syndrome, previously named syndrome X, has enabled more insight into the condition and allowed earlier detection and treatment.

In the context of global cardiovascular risk, metabolic syndrome is indeed a high risk condition, involving three or more risk factors, often organ damage and diabetes.

The metabolic syndrome refers to the clustering of cardiovascular risk factors that include diabetes, obesity, dyslipidemia and hypertension. According to the World Health Organization (WHO) definition from 1999, the metabolic syndrome is present in a person with diabetes, impaired fasting glucose, impaired glucose tolerance or insulin resistance harboring at least two of the following criteria: waist-hip ratio >0.90 in men or > 0.85 cm in women, serum triglyceride >/=150mg/dl or HDL-C< 35mg/dl in men and < 39mg/dl in women, urinary albumin excretion rate > 20 mcg/min and blood pressure >/= 140/90mmHg

In 2001, the National Cholesterol Education Program- Adult treatment panel (NCEP –ATPIII) defined the metabolic syndrome as having at least three of the following abnormalities: waist circumference >102 cm in men and >88 cm in women, serum triglyceride >/=150mg/dl, HDL-C< 40mg/dl in men and < 50mg/dl in women, BP>/= 130/85mmHg and serum glucose >/= 110mg/dl.

Last year, the International Diabetes Federation (IDF) has proposed a modified definition, which includes the presence of visceral obesity and at least two of the following criteria: triglyceride> 1.7 mM, HDL -C <0.9 mM in men and <1.1mM in women, systolic blood pressure > 130 or diastolic >85mmHg or taking medication for hypertension and glucose > 5.6 mM or a diagnosed diabetes.

As the precise pathophysiology is unknown, the metabolic syndrome is still the source of medical controversy. Recently, the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) advised refocusing on the individual components of the syndrome without regarding the syndrome as an identifiable target. This statement was not accepted by the International Diabetes Federation (IDF), which emphasized that whatever the uncertainties of definition and etiology, it is advisable to regard metabolic syndrome as a whole.

In spite of a recent debate and controversy surrounding the definition and etiology of the syndrome, there is no doubt that hypertension is associated with the laboratory and anthropometric findings linked to type 2 diabetes and the metabolic syndrome. In fact, hypertension affects up to 80% of patients with type 2 diabetes and 40% of patients with metabolic syndrome. On the other side, patients with metabolic syndrome have a 5.5-fold higher risk of diabetes and a 2-fold higher risk of new hypertension compared with patients without metabolic syndrome.

Moreover, the study of hypertension in the context of the metabolic syndrome has provided significant insights into the etiology of the condition, known to be complex and multifactorial.

Although the cause of hypertension in the metabolic syndrome has not been completely understood, insulin resistance and central obesity have been recognized as the main factors involved in its pathophysiology (Figure 8.1.). Multiple studies were performed in order to elucidate the mechanisms of this association. These studies have shown that all of the elements of the syndrome contribute to increased blood pressure, which further promotes vascular damage in cardiac, renal, and brain tissue.

Figure 8.1. *Pathogenesis of hypertension in the metabolic syndrome*

Insulin resistance could be defined as the inability of insulin to produce its numerous actions, in spite of unimpaired secretion from the beta cells. It could be caused by various genetic and acquired conditions.

Except in a few rare cases involving antibodies against insulin receptor or mutations in the insulin receptor gene, insulin resistance of the metabolic syndrome results from impairments in cellular events distal to the interaction between insulin and its surface receptor. Metabolic abnormalities result from the interaction between the effects of insulin resistance located primarily in muscle and adipose tissue and the adverse impact of the compensatory hyperinsulinemia on tissues that remain normally insulin sensitive.

Insulin resistance and the resulting hyperinsulinemia induce blood pressure elevation by activation of sympathetic nervous system and renin-angiotensin-aldosterone system (RAAS) with a consequential sodium retention and volume expansion, endothelial dysfunction and alteration in renal function. Hyperinsulinemia stimulates the activation of RAAS in blood vessels and the heart, generating the production of angiotensin II and its pro-atherogenic effects. At the same time, hyperinsulinemia in insulin resistant subjects stimulates the mitogen-activated protein kinase (MAPK) pathway, which promotes vascular and cardiac injury. The local RAAS in the visceral adipose tissue exerts more powerful systemic effects compared with the subcutaneous adipose tissue. Angiotensin II acts through angiotensin 1 receptors, inhibiting the vasodilatatory effects of insulin on blood vessels and glucose uptake into the skeletal muscle cells by blocking insulin action on phosphatydilinositol-3 kinaze and protein kinase beta through free oxygen production. This leads to a decrease in nitric oxide (NO) production in endothelial cells and vasoconstriction in smooth muscle cells, and inhibits glucose transport (GLUT 4) in skeletal muscles. The second mechanism by which insulin resistance contributes to hypertension includes the overactivity of angiotensin 1 receptor which further leads to vasoconstriction and volume expansion.

Endothelial dysfunction is present early in the state of insulin resistance, even before other components of the metabolic syndrome appear.

Although adiposity has been traditionally defined as an increase in total body mass, cardiovascular risk is associated with visceral fat accumulation. Increased visceral fat accumulation is a strong predictor of arterial hypertension. One of the proposed mechanisms by which hypertension is linked with central obesity includes sympathetic nervous system overactivation. Chronic sympathetic stimulation facilitates energy balance and weight stabilization in chronic overeating, but at the cost of adverse consequences such as elevated blood pressure. It has also been suggested that chronic increases in portal venous fatty acid levels may be responsible for hypertension that accompanies visceral obesity. Increases in portal venous fatty acid concentrations have significant pressor effects, perhaps mediated by increased sympathetic tone.

Visceral fat, in comparison to subcutaneous tissue, represents a metabolically active organ, strongly related to insulin sensitivity. Moderating the secretion of various adipocytokines like leptin, adiponectin, plasminogen activator inhibitor 1 (PAI-1), tumor necrosis factor alfa (TNF-alfa), interleukin-6 (IL-6), resistin, it is associated with the processes of inflammation, endothelial dysfunction, progression of hypertension and atherogenesis (Figure 8.2.).

Visceral adipose tissue is a production depot for cytokines including TNFa, which stimulates IL-6 production, and further generates production of C-reactive protein (CRP), fibrinogen and PAI-1 resulting in pro-thrombotic state. Circulating levels of these cytokines are generally increased in obese subjects and in patients with diabetes.

On the contrary, visceral adiposity is a state with a relative deficiency of adiponectin, the adpocyte “good guy”, which increases insulin sensitivity, glucose uptake in muscle cells and free fatty acid oxidation. This cytokine exerts anti-diabetic, anti-inflammatory and anti-atherogenic effects. Many studies have shown increased plasma adiponectin values in patients with hypertension as compared to normotensive population, as well as a negative correlation between adiponectin and mean systolic and diastolic blood pressure values. For those reasons, adiponectin was proposed as a marker of arterial hypertension.

Figure 8.2. *The relationship between visceral adipose tissue and cardiovascular disease*

Therapeutic approach to patients with hypertension and metabolic syndrome include non-pharmacological therapy, as it is important to change the unhealthy lifestyle which aggravates the underlying pathology. This treatment includes sodium restriction, alcohol and calorie restriction, smoking cessation, weight reduction, increased physical activity. However, it is often not sufficient to obtain the target values. Between pharmacological agents, a particular emphasis is placed on the RAAS blockade with ACE inhibitors and angiotensin II receptor blockers, which exert additional beneficial effects.

Patients with metabolic syndrome require strict blood pressure control, which could be achieved in 2/3 of patients only with two or more antihypertensive drugs.

In summary, hypertension is more than just elevated blood pressure, it is intimately associated with the metabolic syndrome. The frequent association between hypertension and multiple risk factors for cardiovascular disease is more than a chance finding. In patients with metabolic syndrome a multitarget approach, based on the assessment of the overall cardiovascular risk, should be applied. Increased understanding of the mechanisms contributing to hypertension in the metabolic syndrome, as well as critical analysis of the results of antihypertensive trials in patients with diabetes is important to develop a logical, evidence-based treatment strategy.

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9. APPROACH TO THE TREATEMENT OF METABOLIC SYNDROME

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9.1 Introduction

The metabolic syndrome is a constellation of interrelated abnormalities that increase the risk for cardiovascular disease and type 2 diabetes. The prevalence of this syndrome is increasing because of the 'obesity epidemic'. The most effective therapeutic intervention in patients with the metabolic syndrome should focus on modest weight reduction and regular physical activity. Drug therapy may be needed to achieve recommended goals if therapeutic lifestyle changes are not sufficient.

9.2 Management of risk factors

9.2.1 Obesity

Abdominal obesity is the body fat parameter most closely associated with the metabolic syndrome. Effective weight reduction improves all risk factors associated with the metabolic syndrome, and it will further reduce the risk for type 2 diabetes. Weight reduction is best achieved by behavioral change to reduce energy intake and by increased physical activity to enhance energy expenditure. Caloric intake should be reduced by 500–1000 calories per day to produce a weight loss of 0.5–1.0 kg per week. The goal is to reduce bodyweight by about 7–10% over 6–12 months, followed by long-term behavior modification and maintenance of increased physical activity.

9.2.2 Physical inactivity

Current guidelines recommend practical, regular, and moderate regimens of physical activity (e.g. 30 min moderate-intensity exercise daily). Regular and sustained physical activity will improve all risk factors of the metabolic syndrome.

9.2.3 Atherogenic and diabetogenic diets

There is general agreement that persons with the metabolic syndrome should follow some important dietary principles: low intakes of saturated fats and cholesterol, reduced consumption of simple sugars, and increased intakes of fruits, vegetables, and whole grains. More controversial is the relative amounts of carbohydrate and unsaturated fats. Some investigators favour lower fat intakes, whereas others recommend higher fat diets. Low-fat diets have been advocated to promote weight reduction, whereas higher monounsaturated fat intakes diminish postprandial glycaemia, reduce serum triglycerides, and raise concentrations of HDL-cholesterol.

9.2.4 Atherogenic dyslipidaemia

This condition comprises elevations of triglycerides and LDL cholesterol, and low HDL cholesterol. Statins (3 hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors) reduce risk for major cardiovascular disease events in high risk patients with the metabolic syndrome. Fibrates mitigate atherogenic dyslipidaemia and appear to reduce the risk for cardiovascular disease in patients with the metabolic syndrome. Their use in combination with statins is particularly attractive, but carries some increased risk for myopathy.

9.2.5 Blood pressure

Mild elevations of blood pressure can often be controlled with lifestyle changes, but if hypertension persists despite such therapies, antihypertensive drugs are usually required. Current guidelines do not provide specific recommendation for pharmacological management of the hypertensive patients with metabolic syndrome. Recent trials have consistently shown that therapy involving beta blockers and diuretics may have some negative impact on the metabolic and haemodynamic disorders present in metabolic syndrome. Several lines of evidence support the use of angiotensin-converting enzyme (ACE) inhibitors or angiotension receptor blockers as the appropriate first-line therapy and the calcium channel blockers as the second in the patients with metabolic syndrome.

9.2.6 Insulin resistance and hyperglycaemia

Lifestyle intervention can reduce the risk for conversion of impared glucose tolerance to type 2 diabetes. Preliminary reports indicate that metformin or thiazolidinediones also reduce risk for type 2 diabetes in people with impared glucose tolerance. On the other hand, no clinical trial evidence indicates that these drugs will reduce risk for cardiovascular disease events in patients with the metabolic syndrome. Currently, metformin or thiazolidinediones are not recommended solely for the prevention of diabetes. The cost-effectiveness of this approach has not been established. Metformin and thiazolidinediones improve insulin sensitivity. The increase in weight in patients treated with insulin secretagogues (sulfonylureas and repaglinide or nateglinide) and insulin results mostly from improved glycaemic control and increases in caloric intake as a result of hypoglycaemia. With the exception of nicotinic acid, lipid-altering drugs do not affect insulin sensitivity or weight, whereas the effect of antihypertensive drugs is more complex. β-adrenergic blockers and thiazide diuretics might decrease insulin sensitivity but less so at low doses, whereas ACE inhibitors and angiotensin II receptor antagonists have variable effects. By uncertain mechanisms, ACE inhibitors and angiotensin II receptor antagonists seem to decrease the incidence of type 2 diabetes.

9.2.7 Prothrombotic state

Metabolic syndrome is accompanied by elevation in prothrombotic factors (fibrinogen, plasminogen activator inhibitor 1, and possibly other coagulation factors). The only available clinical approach to an increased risk for arterial thrombosis in patients with metabolic syndrome is low-dose aspirin or other antiplatelet drugs. These drugs are universally recommended unless contraindicated in patients with established cardiovascular disease. In other people with the metabolic syndrome, aspirin prophylaxis is a therapeutic option when the risk for cardiovascular disease events is judged to be relatively high.

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