

THE IMPORTANCE OF DETERMINING THE PRESENCE AND DEGREE OF INSULIN RESISTANCE IN THE GENERAL POPULATION AND IN SOME CLINICAL STATES

Diana Muačević-Katanec, Velimir Profozić, Željko Metelko

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SUMMARY

Insulin resistance is the inability of insulin to produce its common biological effect at a concentration which is efficacious in healthy individuals. Insulin resistance can develop in all aspects of insulin action. As insulin resistance frequently occurs prior to the diagnosis of different clinical states, identifying and treatment of insulin resistance in the general population has a certain preventive significance. Insulin resistance is etiopathogenetically associated with type 2 diabetes mellitus and obesity (mainly of the central type), arterial hypertension, cardiovascular diseases, dyslipidemia, polycystic ovary syndrome, and some other disorders. Insulin resistance should be suspected in patients with a family history of diabetes mellitus in closest relatives, patients with a history of gestational diabetes or impaired glucose tolerance, arterial hypertension, dyslipidemia, elevated liver enzymes, in obese persons and those with the abdominal type of obesity in particular as well as in those with polycystic ovary syndrome. A separate group of clinically very interesting diseases are inherited syndromes characterized by extreme insulin resistance, contributing significantly to reduced insulin action and insulin resistance. Several methods for determining the presence and degree of insulin resistance such as

clamp technique, frequently sampled intravenous glucose tolerance test (FSIVGTT), continuous infusion of glucose with model assessment (CIGMA), and homeostasis model assessment (HOMA) are described. The most frequently used method today is HOMA, a simple method for the evaluation of the function of beta cells and the degree of insulin resistance from basal (fasting) glucose values and insulin concentrations.

INTRODUCTION

Definition

Insulin resistance is the inability of insulin to produce its common biological effects at a concentration efficacious in healthy individuals (1). In addition to regulating blood glucose level and its important role in the transport of amino acids, insulin has many other effects such as that on lipoprotein metabolism, coagulation process, and regulation of the autonomic nervous system. Insulin can also activate cytoplasmic and membrane enzymes, change the degree of synthesis and degradation of various proteins and specific mRNA, and influence the growth and differentiation processes (2). Insulin resistance can develop in all facets of insulin action.

Correspondence to: Diana Muačević-Katanec, MD, PhD, Vuk Vrhovac Institute, University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Dugi dol 4a, HR-10000 Zagreb, Croatia
E-mail: dmuacevi@idb.hr

DISEASES ETIOPATHOGENETICALLY ASSOCIATED WITH INSULIN RESISTANCE

Insulin resistance is frequently associated with type 2 diabetes mellitus (3,4). It is also etiopathogenetically associated with obesity (mainly of the central type) (5,6). Results of some studies have shown that almost half of the patients with arterial hypertension have insulin resistance and hyperinsulinemia (5). Insulin resistance also affects the development of dyslipidemia (7,8). The insulin-resistant state can also accelerate the development of atherosclerosis (9). Some other abnormalities associated with insulin resistance such as hyperuricemia and increased values of different cytokines can, according to the present knowledge, also increase the risk of coronary disease (9,10). The syndrome of polycystic ovaries is in practice frequently encountered in association with the problem of insulin resistance (11). There is a separate group of inherited diseases with syndromes characterized by extreme insulin resistance (type A insulin resistance syndrome), including leprechaunism, Rabson-Mendelhall syndrome, Werner's syndrome, pineal gland hypertrophy syndrome, Alstrom's syndrome, ataxia-telangiectasia, myotonic dystrophy, acanthosis nigricans, and various lipodystrophic conditions (2,12-16). An acquired state of resistance is type B insulin resistance caused by immunoglobulin G (IgG) insulin receptor antibodies (17). Of course, the most common acquired state of insulin resistance is obesity, although the two need not necessarily be associated.

THE KNOWN CAUSES OF INSULIN RESISTANCE

Genetic predisposition to insulin resistance

Low birth weight is often associated with insulin resistance in adult age. Although different studies carried out in children and adults have demonstrated consistent association between reduction in birth weight and insulin resistance, it remains unclear whether low birth weight is a consequence of disadvantageous intrauterine environment or genetic predisposition to insulin resistance.

Most extensive studies so far have been conducted on glucokinase gene polymorphism. Glucokinase is a glycolytic enzyme acting as a sensor of blood glucose (BG) level. The extent of insulin secretion from the beta cells of the pancreas is proportional to BG level.

Research on glucokinase gene polymorphism has revealed that mutations of this gene may cause mild dysfunction of beta cells with a consequential fasting glucose concentration of 5.5-8 mmol/l. This hyperglycemia persists in early childhood, with minimal changes during the person's lifetime. An association of the mutation of this gene with the development of type 2 diabetes in children and young persons has been observed (maturity-onset diabetes of the young, MODY). This has led to a presumption that polymorphism of genetic factors relevant for the secretion of insulin with a consequential growth retardation is in fact involved (18-21).

Genetic analyses of members of families with frequent occurrence of a severe insulin resistance syndrome have led to identification of a PPAR γ gene mutation (peroxisome proliferator-activated receptor gamma). A mutation in AKT2 gene, which is also associated with insulin resistance and diabetes mellitus, has recently also been established in these patients (22,23).

Type A insulin resistance syndrome

A separate group is a group of clinically very interesting genetically determined diseases characterized by extreme insulin resistance. The most important clinical syndromes with extreme insulin resistance are those caused by hereditary cellular impairment of insulin action, such as type A insulin resistance syndrome that occurs due to the lack or decrease in insulin receptor function (17).

Patients with type A syndrome usually developing in adolescence are almost exclusively female, also suffering from acanthosis nigricans, hyperandrogenism and polycystic ovary syndrome. It does not typically occur in diabetes, although impaired glucose tolerance or diabetes may develop at a later stage. In approximately 25% of cases, there is a mutation in a copy of insulin receptor within the tyrosine-kinase β -subunit. Type A syndrome variants involve insulin resistance and clinical features similar to acromegaly, but without the increase in growth hormone and insulin-like growth factor-1. It is possible that there is a defect in the insulin signaling transduction *via* metabolic pathways, but with intact growth stimulation, which is induced by compensatory hyperinsulinemia (17).

Other, much less frequent inherited diseases characterized by severe insulin resistance are leprechaunism, Rabson-Mendelhall syndrome, Werner's syndrome, pineal gland hypertrophy syndrome, Alstrom's syndrome, ataxia-telangiectasia, myotonic dystrophy, acanthosis nigricans, and various lipodystrophic conditions (2,12-16). The pathogenesis of extreme insulin resistance in these states has not been fully explained.

ACQUIRED STATES AS CAUSES OF INSULIN RESISTANCE

Type B insulin resistance

Type B insulin resistance is an acquired state caused by immunoglobulin G (IgG) insulin receptor antibodies. Many patients (80% female) show manifestations of autoimmune diseases such as arthritis, nephritis, vitiligo, alopecia areata and systemic lupus erythematosus. Fluctuating hyper- and hypoglycemias occur at times, as insulin receptor antibodies can be either inhibitory or stimulating (17).

Obesity

Obesity is a state of excessive accumulation of fatty tissue in the body. According to data of the World Health Organization, obesity threatens to become a pandemic. Body weight is determined by an interaction between genetic, environmental and psychological factors, acting through the physiological mediators of energy intake and utilization. Although obesity is a complex disorder, partially determined by various genetic factors, the imbalance between energy intake and utilization is the most important factor of getting weight. It causes a series of health problems, either independently or in combination with other diseases. It is still a question to what extent does the genetic background for insulin resistance syndrome influence the development of obesity, but it is well known that obesity, mainly of the central type, is strongly correlated with the worsening of insulin resistance.

Body mass index (BMI) values (the weight in kilograms divided by square of height in meters) have been used to classify obesity in populations because they correlate with the percentage of body fat as well as with mortality and morbidity.

As BMI >30 does not always correspond to excess adiposity, another simple measure for predicting excess visceral adipose tissue is waist circumference. It also strongly predicts insulin resistance and risk of metabolic syndrome.

Waist circumference ≥ 94 cm in men and ≥ 80 cm in women indicates a need for concern, and ≥ 102 cm in men and ≥ 88 cm in women are critical levels of intra-abdominal fat that indicate the need of action and intervention (6).

CONSEQUENCES OF INSULIN RESISTANCE

Diabetes mellitus

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by elevated blood glucose concentrations and disturbances in carbohydrate, fat and protein metabolism secondary to absolute or relative deficiency in insulin action and/or secretion. Insulin resistance is most frequently associated with type 2 diabetes mellitus, and in patients with type 2 diabetes mellitus it is characterized by the reduced ability of insulin to inhibit glucose synthesis in the liver, as well as by a reduced sensitivity of insulin receptors with a consequential reduced entry of glucose into striated muscles, leading to compensatory secretion of insulin (3,4). The severity of insulin resistance in these patients directly correlates with the degree of glucoregulation.

Dyslipidemia

Persons with insulin resistance often have decreased levels of high-density lipoproteins (HDL), a significant risk factor for cardiovascular (CV) disease. Increase in the free fatty acid influx, in addition to insulin resistance, leads to an increase in the production of triglycerides and triglyceride-rich low density lipoproteins (LDL), as well as a decrease in the concentration of HDL (7,8).

Both insulin resistance and dyslipidemia are determined by genetic and environmental factors. Depending on their expression and function, gene variants (mutations, polymorphisms) can primarily regulate either insulin action or dyslipidemia. Alternatively, evidence from population based-studies

suggests that dyslipidemia may affect insulin signaling; low levels of HDL-cholesterol, high levels of total triglycerides, and the occurrence of small dense LDL have been shown to predict insulin resistance and diabetes. Thus, the genes primarily regulating dyslipidemia may secondarily lead to insulin resistance in all target tissues (24).

Arterial hypertension

Hypertension is a common manifestation of insulin resistance. Results of some studies have shown that almost half of the patients with arterial hypertension, without the presence of diabetes mellitus type 2, have insulin resistance and hyperinsulinemia. It is estimated that 20% to 60% of persons who have diabetes also have hypertension (defined as blood pressure $\geq 140/90$ mm Hg). Hypertension is also a major factor for the microvascular complications linked to diabetes, including diabetic retinopathy and nephropathy. The exact etiopathogenetic mechanism of the development of arterial hypertension in persons with insulin resistance is, however, still unknown, as is the case with the possible influence of the degree of insulin resistance on the blood pressure level (5).

Hyperuricemia

Hyperuricemia is also associated with insulin resistance (9,10). Hyperuricemia and hyperuricosuria are defined as elevated serum and urinary levels of uric acid due to reduced excretion of uric acid or increased cell turnover. In patients with increased catabolism, such as those receiving chemotherapy or radiation therapy, or those with leukemia, lymphoma or other malignancies, an increased turnover of nucleoproteins is observed. According to the present knowledge, high levels of uric acid are often diagnosed in patients with metabolic syndrome, so it is considered to be its "associated member". Nowadays, it is known that it also increases the risk of coronary disease. In the atherosclerotic prooxidative milieu the antioxidant properties of uric acid paradoxically become prooxidant, thus contributing to the oxidation of lipoproteins. In such a milieu, an antioxidant-prooxidant redox shuttle exists (9).

Increase in cytokines

In the state of insulin resistance changes in several circulating factors contributing to vascular dysfunction are often present. Increases in cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, and plasminogen activator inhibitor-1 (PAI-I), render the endothelial cells less capable of secreting nitric oxide (NO). This results in vascular "stiffness," contributing to hypertension. Other atherogenic changes also result from endothelial dysfunction, including the elaboration of cell adhesion molecules, which leads to leukocyte migration into the vessel wall and the initiation of atherosclerotic plaque (1,5).

Atherosclerosis

Atherogenesis is the formation of atheroma-lipid deposits in the intima of the arteries. Atheroma produces swelling on the endothelial surface, which is a characteristic of atherosclerosis. In the prediabetic patient, the macrovascular system tends to be in a highly atherogenic state. The results of several recent studies of diabetes and CV disease implicate increased insulin resistance and its different, above mentioned components as the culprit in the enhanced risk of the prediabetic state (25). The insulin-resistant state can also accelerate the development of atherosclerosis by some other, as yet unexplained, mechanisms. Studies carried out more than 40 years ago showed that the infusion of insulin into the femoral artery of a dog resulted in accelerated formation of atherosclerotic plaques in the artery (5). The mechanism underlying these changes remains unclear.

Polycystic ovary syndrome

The syndrome of polycystic ovaries is in practice frequently encountered in association with the problem of insulin resistance. The polycystic ovary syndrome is a group of syndromes characterized by ovaries (enlarged or of normal size) with numerous follicular (watery) cysts, menstrual cycle disorders (menorrhagia or amenorrhea), increased hairiness, obesity, acne, impaired glucose tolerance, and reduced resistance of organs to insulin (insulin resistance). Not all of these symptoms need to be present to make the diagnosis. The problem of polycystic ovaries is not unique, which means that their onset might be due to

different reasons. The association of polycystic ovaries with an increase in the level of insulin has been observed in recent years. According to some authors, the latter represents the central event in the onset and development of polycystic ovaries. There has been an increasing body of evidence indicating that hyperinsulinemia results in the development of polycystic ovaries through an increase in ovarian secretion of the androgen hormones testosterone and androstenedione (11). An increase in androgen concentration interferes with the pituitary-ovary axis, leading to an increase in luteinizing hormone, anovulation, amenorrhea, and infertility. Testosterone is also responsible for the development of acne, increased hairiness, and possible hair loss (26). A precondition for the development of type 2 diabetes with all the related problems is also created. In case the patients are overweight, excess weight should be reduced at all events, as insulin resistance is thus decreased, and many symptoms are improved after body weight has been adjusted (26,27). If an increased level of insulin and impaired glucose tolerance are present, good results are achieved by using metformin in addition to a well controlled diet and exercise (28). It is still unknown why some women ultrasonographically diagnosed with polycystic ovaries have regular menstrual cycles, whereas others develop the polycystic ovary syndrome.

As insulin resistance frequently occurs prior to the development or diagnosis of any of the above states, identifying and treatment of insulin resistance in the general population has a certain preventive significance. Insulin resistance should be suspected in patients with a family history of diabetes mellitus in closest relatives, patients with a history of gestational diabetes, polycystic ovaries or impaired glucose tolerance, as well as in obese persons and those with abdominal type of obesity in particular.

METHODS FOR DETERMINATION OF INSULIN RESISTANCE

Several methods for the determination of insulin resistance *in vivo* have been investigated so far.

The clamp technique

The clamp technique is considered as a reference technique for the determination of insulin sensitivity in humans, because it directly measures the metabolic

effect of insulin in precisely set conditions. It can be applied in euglycemia, isoglycemia and hyperglycemia. However, it is also the most complex technique, as it requires simultaneous administration of glucose and insulin, multiple blood sampling, and an experienced operator (a physician or a technician) over a course of 3 and 6 hours, respectively.

This technique is used in clinical diabetology: (a) in clamp tests for the evaluation of peripheral insulin resistance; and (b) for the assessment of the relationship of the intensity of receptor/postreceptor defect in type 2 diabetes.

The euglycemic hyperinsulinemic clamp test is based on the following principle: the test is usually performed during morning hours. The patient presents for the test fasting, and is prepared by at least 2-hour bed rest before the beginning of the test. The preparation and calibration of the biostator is carried out simultaneously. Samples are obtained by introducing an intravenous cannula for insulin and glucose administration, and another one at the dorsal part of the hand for arterial blood sampling. The test procedure begins with continuous insulin infusion (40 $\mu\text{U}/\text{m}^2/\text{min}$). Based on the continuous measurement of glycemia, the biostator determines the quantity of glucose infusion required for maintaining the patient's glycemia at a level of 5.5 mmol/l. In order to achieve a 80%-96% suppression of the patient's own insulin secretion, insulinemia in the blood should be 50-60 $\mu\text{U}/\text{ml}$. Stable insulinemia of approximately 80-100 $\mu\text{U}/\text{min}$ is achieved by the clamp test infusion. The balance between stable insulinemia and glycemia fixed at 5.5 mmol/l is achieved in about 90 minutes. The steady state of the test is between 90 and 120 min, when the quantity of insulin required represents the quantitative expression of glucose uptake and usage in the peripheral tissue under hyperinsulinemic conditions. The quantity of metabolized glucose (M) is a measure of either a normal state ($M=10-12 \text{ mg}/\text{kg}/\text{min}$) or different degrees of peripheral insulin resistance, with M being 2-3 times lower than that in healthy persons (29,31-33).

Frequently sampled iv glucose tolerance test

Frequently sampled iv glucose tolerance test (FSIVGTT) is a somewhat simpler test, although it also requires iv administration of glucose and insulin, with multiple blood sampling within a 3-hour period

according to a method first used by Bergman and associates (34). The test is used to avoid individual differences in intestinal resorption of glucose. Intravenous cannulas are introduced in the cubital veins of both hands for the purpose of glucose or insulin and tolbutamide administration in case a modified, shortened version of the test is used, and for the collection of blood samples for insulin and glucose concentration determination. During the first minute from the beginning of the test, glucose is injected in a dose of 300 mg/kg, whereas insulin or tolbutamide are applied in a bolus after 20 minutes in order to increase the sensitivity of the test. Blood samples (3 ml) are collected at minutes 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90 and 180. The original test requires 30 blood samples in total collected over a period of 3 hours; however, if a modified test with tolbutamide or insulin is used, it is considered that 12 blood samples, as mentioned above, enable precise information on insulin sensitivity. Insulin resistance and sensitivity to insulin can be calculated by means of a model of minimal changes based on plasma insulin and glucose concentrations after intravenously applied glucose bolus. Two basic parameters that can be determined by the model of minimal changes are sensitivity to insulin (S_1) and "glucose efficiency", i.e. the ability of glucose to influence its own elimination from the blood (S_G).

Equation 1 describes the rate at which plasma glucose is accumulated in the cells and at which its concentration decreases to reference values after intravenously administered glucose. Equation 2 includes factors influencing insulin concentration in the tissue.

$$1) \frac{dG(t)}{dt} = -[SG + X(t)] G + C$$

$$C = S_G G_b \quad (35)$$

$$2) \frac{dX(t)}{dt} = K_a I(t) - K_b X(t), \text{ where}$$

G =plasma glucose concentration, I =plasma insulin concentration, X =insulin effect, G_b =glucose basal value. Data obtained from glucose concentrations and insulin levels at different times enable calculation of K_a and K_b , while S_1 is calculated by the formula K_a/K_b (36). The test has good reproducibility and can be applied both in persons without diabetes and in diabetic patients. The sensitivity improves significantly with the increase in plasma insulin level obtained by intravenous application of tolbutamide (100-300 mg), or infusion of rapidly acting insulin in a dose of 4-8

mU/kg/min over 5 minutes (37). Tolbutamide and insulin are applied 20 minutes after glucose bolus. Although the test is rarely used today, when performed, modified protocols are used for more precise interpretation. Insulin and tolbutamide modification has been proven to yield similar results in both patients with insulin sensitivity and diabetes as well as in healthy individuals (38).

Continuous infusion of glucose with model assessment

Continuous infusion of glucose with model assessment (CIGMA) is a method for the assessment of glucose tolerance, insulin resistance and beta cell function of the pancreas. The test is performed after the determination of basal (fasting) values of glucose and insulin, by means of an infusion of 5 mg of glucose *per* kilogram of ideal body weight over 60 minutes. Plasma glucose and insulin levels are measured three times during the last 10 minutes of the test, the mean values of these concentrations being considered as the so-called "steady state". The values obtained reflect the dynamic interaction between the insulin released and its effect on blood glucose level (37). The degree of insulin resistance is established by a computer model, and the calculation is based on the actual plasma insulin value obtained and the standard values from the measurements in the so-called "healthy population". The method can also be used to determine the size of beta cell secretion from the pancreas by means of a formula used for the same purpose in calculating this parameter using HOMA index (36).

Homeostasis model assessment

Homeostasis model assessment (HOMA) is a simple method for the evaluation of the function of beta cells and the degree of insulin resistance from basal (fasting) glucose values and insulin concentration. HOMA index was first described in 1985 (39,40). It has become a widely used model in clinical and epidemiological studies and, when used appropriately, it can yield valuable data on insulin resistance and beta cell function. HOMA has been compared with other tests for the determination of the degree of insulin resistance. Although euglycemic and hyperglycemic

clamps are referred to as “the golden standards”, studies have demonstrated excellent correlation between HOMA and euglycemic clamp.

The original HOMA model (40) uses fasting glucose and insulin values in the calculation of the possible presence and degree of insulin resistance as well as in the determination of the degree of functionality of beta cells of the pancreas. These parameters can be used by means of the following formulas:

HOMA-IR = (FPI x FPG) / 22.5, where

FPI=fasting plasma insulin level; FPG=fasting plasma glucose level;

HOMA-IR-%B=(20xFPI)/(FPG-3.5);

%B=HOMA-IR-(20 x FPI)/(FPG-3.5);

%B=% of functional beta cells in the pancreas (36,41).

HOMA has proved to be an excellent method in prospective studies. The method was thus used to compare the effect of sulfonylurea and metformin on insulin resistance and beta cell function with that of diet alone. A study conducted for six years found an improvement in beta cell function in patients on sulfonylurea (from 46% to 78%) during the first year of treatment, gradually diminishing to 54% over the following five years. In patients on diet alone, the functional capacity of beta cells decreased by approximately 4% a year. Insulin sensitivity increased only in patients treated with metformin, from 51% to 62% in the first treatment year, remaining so until the end of the study (41).

HOMA-B% is a measure of beta cell activity, and not a parameter revealing health or disease of these cells. Hence, it can be used in patients treated with insulin secretagogues, but with a very careful interpretation. Data from UKPDS have pointed to an increase in beta cell activity from 46% to 78% during the first treatment year, and a subsequent decrease to 52% over the following 5 years.

Initial improvement in the function is really a reflection of the mechanism of action of secretagogues. The resumed decrease in the activity by about 5% a year, identical to the results obtained in persons on diet alone, demonstrated that treatment had no effect on the improvement in beta cell function. These data indicate that HOMA model reflects the degree of activity rather than the “health” of beta cells in the first place (41).

The purpose of the use of HOMA index in the healthy population

There are several reasons supporting the use of HOMA index in the healthy population:

- 1) the formation of a control group for comparison of beta cell function and insulin sensitivity with patients with abnormal glucose tolerance
- 2) the collection of longitudinal data on persons who will develop abnormal glucose tolerance

Insulin sensitivity measurement (HOMA-S%), in addition to determination of beta cell functionality, can provide an answer to the question on the predominance of one of the two disorders.

In spite of the simplicity of HOMA index application, several groups of authors have tried to compare some other, simpler parameters in diagnosing and assessing the degree of insulin resistance. Thus, the results obtained by clamp technique were used to compare insulin, fasting insulin/glucose, Bennett's index, and a combination of fasting insulin level, BMI and triglycerides. As any of these individual parameters may indicate the presence of insulin resistance, they were used in an attempt to identify the simplest method for determining insulin resistance in the general population (42).

CONCLUSION

Various studies on insulin resistance have been conducted due to a large number of clinical entities observed in association with it. As a growing body of evidence has shown that insulin resistance occurs prior to the development of arterial hypertension, cardiovascular diseases, dyslipidemia, diabetes mellitus and other clinical disorders, its presence should be determined in persons with a positive family history of these clinical disorders. This is also corroborated by the fact that a large number of beta cells are already impaired at the onset of impaired glucose tolerance and increased fasting glucose values (42). Hence, the simplest test for determining insulin resistance in the general population would be of value in the prevention and timely treatment of individual disorders which can significantly accelerate the development of atherosclerosis. The results from clinical trials have indicated that, in comparison with other methods, HOMA is the method of choice to determine the

presence and degree of insulin resistance because of its simple application in daily routine and high level of precision.

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