Abstract

Insulin resistance is first and the most important feature in type 2 diabetes development. Initially, this disorder is compensated by enhanced insulin secretion from pancreatic β-cells, but prolonged need for insulin leads to pancreatic β-cells degeneration and decreased rate of insulin synthesis and secretion. Numerous studies have shown, that insulin resistance has genetic background. Disruption of genes that encodes proteins involved in insulin action and insulin signal transduction might have physiological consequences in insulin resistance state — proper amount of insulin is insufficient to develop biological response. In this review the genetic as well as environmental factors that might lead to insulin resistance development are discussed. Numerous results obtained from knockout animal models studies as well as clinical studies are presented. These data provide evidences for the genetic predisposition to glucose tolerance impairment and insulin resistance development. Data presented in this review also emphasize strong correlation between obesity, as the main environmental factor, and the occurrence of insulin resistance. Besides, results of some studies give evidences for interaction between genetic and environmental factors, especially obesity, in insulin resistance development. Despite the fact that insulin resistance and type 2 diabetes has been widely studied, the pathogenesis of these metabolic disorders, the role of genetic and environmental factors, and their influence on development of the above disorders has not been yet elucidated.

key words: insulin resistance, type 2 diabetes, genetics, environmental factors

Genetic basics of insulin resistance and its role in type 2 diabetes pathogenesis

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Background

Type 2 diabetes is caused by a complex of pathophysiological processes, in which environmental factors, as well as genetic predispositions are responsible for developing of this condition [1]. According to study published in “Diabetes Care” in year 2000 the total number of affected subjects was 171 million and estimated number of people with this disorder in year 2030 will reach 366 million [2].

The raise in the incidence is certainly caused by environmental factors such as sedentary life style, inappropriate diet rich in fat and carbohydrates, also genetic predisposition has very strong influence. Type 2 diabetes is considered to be multigenetic disease, in which the genes disruption interacts with environmental factors. It is characterized by peripheral insulin resistance, impaired insulin secretion and increased hepatic glucose production [3]. Many experiments and studies performed so far, especially utilizing knockout animal models, helped to propose theoretical order of events that result in type 2 diabetes mellitus development. Numerous studies provide strong evidences that insulin resistance is the earliest and the most important event in type 2 diabetes development [4, 5]. Insulin resistance is defined as a state, in which physiological level of insulin is insufficient to develop biological response that is peripheral glucose uptake by insulin-dependent tissues (skeletal muscle, adipose tissue and liver) [6]. Moreover, insulin resistance in liver causes increased glucose production, what leads to rise in glucose concentration and further pancreatic \( \beta \)-cells stimulation [7]. This state is followed by: decrease in glucose uptake to insulin-dependent tissues, increase in hepatic glucose production, hyperglycemia and hyperlipidemia. In its early state, insulin resistance is compensated by enhanced insulin secretion from pancreatic \( \beta \)-cells, what enables to maintain proper glucose level, but prolonged increased demand for insulin results in impairment of \( \beta \)-cells function and reduction in insulin secretion from pancreas in respond to hyperglycemia, particularly in the first-phase insulin secretion [5].

Signaling pathways of insulin action

Insulin induces various physiological changes in cellular metabolism [8]. This hormone affects not only glucose metabolism, but lipid and protein metabolism as well. It also influences cellular growth and genes expression. In numerous studies the possible pathways of insulin action have been investigated. The pathway resulting in glucose uptake into cell is well known; nevertheless there are still unrevealed processes or alternative ways of signal transduction. The initial step of insulin action is binding the hormone to its receptor, in particular to its extracellular \( \kappa \) subunit. This causes activation of intracellular tyrosine kinase domain of \( \beta \) subunit and multiple transphosphorylation reactions of specific tyrosine residues, including insulin receptor substrate family (IRS 1, 2, 3, 4) [3, 9]. The tyrosine phosphorylation of these molecules creates further downstream signal transduction, dependent on the regulatory subunit p85\( \alpha \) of phosphatidylinositol 3-kinase (PI3K) [10]. There are several targets of phosphorylated PI 3-kinase (PI (3,4,5) P\(_3\)) that transduct the signal downstream the cell: serine/threonine kinase Akt, also known as protein kinase B (PKB) and two atypical isoforms of protein kinase C: \( \zeta \) and \( \lambda \) (PK \( \zeta /\lambda \)) [11, 12]. The terminal step of insulin action is activation of GLUT4, a specialized hexose’s carrier. GLUT4 constantly circulate between various intracellular compartments and the surface membrane. After insulin stimulation there is an increase in exocytosis of GLUT4 vesicles [13]. The molecular mechanism of insulin-stimulated GLUT4 vesicles exocytosis is not clear. There is some evidence that during GLUT4 translocation and glucose uptake the \( \nu \)-SNARE protein like VAMP2 and VAMP3, located in the vesicle interacts with t-SNARE protein (syntaxin-4, Munc-18c), inbuilt in plasma membrane [14]. The scheme of insulin action resulting in GLUT4 translocation and glucose uptake is presented in Figure 1.

The correct interaction of all these proteins ensures proper insulin signal transduction and response to increased glucose concentration.

Insulin resistance development in relation to genetic factors

Insulin resistance in knockout animals models

Insulin resistance is a condition genetically determined. Genes encoding proteins involved in insulin signal transduction should be accounted for candidate genes, that may be responsible for insulin resistance. There is much evidence that disruption in these genes causes impaired insulin action and decreased rate of glucose uptake. Strong evidence is provided by studies performed on animal models with gene disruption.

The first step of insulin action is binding to Insulin Receptor (IR) encoded by \( \text{INSR} \) gene. For revealing the consequences of disruption in IR knockout animals were created [15, 16]. Phenotype of these animals differs depending on the number of affecting alleles. Heterozygous mice (IR\(^{+/\text{–}}\)) did not show any alteration in glucose and insulin concentration and in glucose tolerance in com-
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Comparison to the wild-type mice. Despite this, heterozygous animals showed increased insulin concentration at the age of six months [17]. On the other hand, homozygous mice (IR<sup>–/–</sup>) rapidly developed diabetes and died within 3–7 days. What was interesting, these animals were born with an altered growth and glucose tolerance, what means, that IR was not responsible for glucose tolerance during prenatal life. It was also reported that IR<sup>–/–</sup> homozygous mice had lower mass of white and brown adipose tissue, revealing a great role of IR in lipid metabolism regulation. Besides, these animals showed postnatal growth disturbance and skeletal muscle hypotrophy [15, 16].

The substrate for Insulin Receptor belongs to IRS (insulin receptor substrate) family proteins. Knockout animals with disruption of these genes exhibit various glucose metabolism impairment [18, 19]. Insulin receptor substrate 1 seems to be the most important substrate for phosphorylation activity of IR. Homozygous IRS-1<sup>–/–</sup> mice, unlike IR-deficient ones showed abnormalities in uterine growth. Additionally, these animals exhibit impaired glucose tolerance and reduced glucose uptake. In these mice the tyrosine-phosphorylation activity of IR has not been abolished totally thanks to an alternative substrate — IRS-2 [20]. The IRS-2 has the ability to bind to PI 3-kinase and to transduct the signal downstream the cell through an alternative, IRS-1 independent pathway. These finding inspired scientists to create IRS-2 deficient mice. Surprisingly, mice with disrupted allele of IRS-2 gene turned out to be both insulin resistance and had impaired pancreatic β-cell function (there was lack of compensation of insulin resistance state by increase in insulin secretion) [21]. Furthermore, Burks et al. noted infertility, low gonadotrophins level, increased food intake and obesity in female knockout IRS-2 mice [22]. Multiple biochemical analysis including glucose, proteins, lipids metabolism, blood pressure allowed to conclude, that IRS-1 appears to have a major role in insulin signal transduction in muscle, whilst IRS-2 seems to be specific for liver, muscle, adipocytes, pancreatic β-cells and reproductive organs.

The tissue-specific activity of IRSs family encouraged scientists to create knockout mice for further IRSs genes (IRS-3 and IRS-4). The results of these studies revealed no alteration in glucose and insulin concentration and in glucose tolerance, suggesting an insignificant role of these two substrates in insulin signal pathway [23, 24].

For a comprehensive analysis of the role of IRSs family in insulin action Laustsen et al., created double-knockout mice: IRS-1/IRS-3 and IRS-1/IRS-4 [25]. The latter showed the same phenotype as IRS-1 single-knockout animals, whereas the former revealed more severe type of insulin resistane and early onset type 2 diabetes.

Unexpected results were obtained in studies on PIK3R1<sup>–/–</sup> knockout mice. It was assumed, that disruption in this gene should lead to severe insulin resistance, but results were different than expected. It was due
to the p50α alternative splicing isoform of the same gene, which was expressed in knockout animals. This isoform of regulatory subunit was associated with rise in PI 3-kinase activation and thus with increased GLUT4 translocation and glucose disposal in skeletal muscle and adipocytes [26, 27].

The final state of insulin signaling pathway is GLUT4 activation and its translocation to a surface membrane. Studies on animals with disruption in gene encoding GLUT4 revealed unexpected results. GLUT4−/− homozygous mice did not display a diabetic phenotype [28]. Female GLUT4+− mice exhibited slightly increased glucose level, whilst male GLUT4+− mice showed normal glucose concentration in either fasted or fed states. These animals displayed also hyperinsulinemia and impaired glucose tolerance in response to insulin tolerance test. Furthermore, these animals were born with significant cardiac hypertrophy and with mass deficiency. The heterozygous GLUT4+− mice however, showed increased glucose and insulin concentration, impaired glucose tolerance and hypertension [29]. In hyperinsulinemic-euglycemic clamp studies Rossetti et al. showed that heterozygous mice for GLUT4 gene developed peripheral but not hepatic insulin resistance [30].

Many studies implicate Protein Tyrosine Phosphatase 1B (PTP1B) in insulin resistance development by insulin receptor kinase domain dephosphorylation. To assess its role in glucose homeostasis deregulation, knockout animals models were created. The results revealed increased insulin sensitivity and glucose uptake in PTP1B−− mice. Furthermore, these animals were resistant to weight gain, even fed with a high-fat diet [31].

**Tissue-specific insulin resistance in knockout animals**

To assess the role of particular tissue in glucose homeostasis, tissue-specific animal models with disruption of particular gene were generated. It is well known, that skeletal muscles are the main target of glucose disposal. Two types of muscle-specific knockout mice were determined: GLUT4-deficient and IR-deficient (MIRKO — muscle specific insulin receptor knockout) mice [32, 33]. MIRKO mice showed decrease in IR expression of 95% in skeletal muscle; besides, they displayed increased triglycerides and free fatty acids level, but the glucose, insulin concentration and glucose tolerance were normal. Kim at al. analyzed glucose homeostasis in these animals in hyperinsulimemic — euglycemic clamp study [34]. They noted that glucose transport and glycogen synthesis in muscle were reduced, whilst glucose transport in adipose tissue was significantly increased. GLUT4 knockout mice exhibited reduced basal and after insulin stimulation rate of glucose uptake and these animals developed early onset diabetes, progressing with aging [33].

Liver plays very important role in maintaining proper glucose homeostasis. This organ is responsible for regulation of glucose concentration, mainly by gluconeogenesis and glycogenogenesis/glycogenolysis. Disruption in liver-specific insulin receptor (LIRKO — liver-specific insulin receptor knockout) led to severe insulin resistance [35], what caused in these mice constant glucose production via the processes of gluconeogenesis and glycogenolysis. Significant role in regulating glucose homeostasis is played by pancreas, in particular pancreatic β-cells. To assess its role in carbohydrates metabolism specific β-cell IR knockout mice were created [36]. These animals were characterized by a decrease of insulin secretion in response to glucose and impaired glucose tolerance. These data showed considerable evidence that selective β-cell disruption in IR might lead to type 2 diabetes development.

Adipose tissue is also involved in glucose metabolism, thus the FIRKO (fat insulin receptor knockout) mice were created by Bluher et al. [37] to assess its role in deregulation of glucose homeostasis. These mice exhibited reduction in adipose tissue mass, and they seemed to be protected against obesity and insulin resistance. Furthermore, they displayed increase in lifespan by about 18% [38]. On the other hand, Kim et al. created animals with adipose-specific disruption in GLUT4 [39]. These animals were characterized by impaired glucose disposal but there was no alteration in animals’ growth and fat mass.

All these data allow to conclude, that any disruption in genes encoding proteins involved in insulin signal transduction downstream the cell of insulin-dependent tissue causes defect in glucose homeostasis, impaired glucose tolerance followed by appearance of insulin resistance and consequently type 2 diabetes development.

**The knowledge of insulin resistance obtained from clinical studies**

Studies performed on knockout animals revealed theoretical order of events, which might have place in human organism, what initiated studies on human diabetics. Several mutations have been found in humans with severe insulin resistance in genes encoding proteins involved in maintaining proper glucose homeostasis.

**Mutations in insulin receptor gene**

Mutations in insulin receptor might have various consequences, depending on the region in which particular mutation occurs, but almost all of them charac-
terize severe insulin resistance in human carrying these mutations. Several mutations affecting receptor biosynthesis have been determined. Four nonsense mutations (at codons: 133, 672, 897, 1000) and two deletion mutations (exon 14 and exon 17) have been shown to correlate with insulin resistance in those patients. It has been determined that the nonsense mutation at codons: 133, 897 and 1000 caused 80–90% decrease in insulin receptor mRNA level [40], whilst the deletion mutations impaired insulin receptor synthesis either by reducing the insulin receptor mRNA level [40] or deletion of the important domains of the receptor [41]. Two mutations: Lys15 [42] and Ser735 [43] have been associated with reduced insulin receptor affinity to insulin. In addition, the former mutation impairs insulin receptor transport to the cell surface [42]. Mutations in insulin receptor might lead to reduction in tyrosine kinase activity of the receptor. The most known mutation that has been defined to reduce the tyrosine kinase activity of insulin receptor is a missense mutation Gly→Val in 1008 position [44]. Gly1008 causes changes in region responsible for ATP binding, thus it causes disruption in phosphorylation of the insulin receptor tyrosine kinase domain. Kusari et al., on the other hand, did not show any alteration in sequence of insulin receptor gene in these patients [51].

Mutations in insulin receptor substrates family (IRSs) genes

There are several mutations in insulin receptor substrates family that have been proven to be associated with insulin resistance and increased risk for type 2 diabetes development. What is interesting, it has been defined that some polymorphisms in IRS-1 and IRS-2 genes increase the risk when combined with obesity. In IRS-1, Clausen et al. [45] and Krolewski et al. [46] in unrelated studies have revealed that single nucleotide polymorphism at codon 972 causing amino acid substitution: Gly→Arg is associated with increased risk for type 2 diabetes when correlated with obesity. Very similar results have been obtained by Cama et al. [47] studying SNP polymorphism in IRS-2 gene at codon 1057 causing amino acid replacement: Gly→Asp. This SNP polymorphism has been associated with increased risk for type 2 diabetes development accompanied by obesity. There was no significant statistical association of this variant with increased risk for type 2 diabetes development in subjects with proper Body Mass Index; moreover, this variant seemed to be protective against type 2 diabetes in lean subjects and was associated with increased risk only in obese individuals. Genetic variants of IRSs are the best examples of interaction between two factors leading to insulin resistance: genetic and environmental.

Genetic variants of PI 3-K

The phosphatidylinositol 3-kinase is an enzyme essential for downstream transduction of insulin action pathway within the cell. It has been shown, that this process is disturbed in subjects with insulin resistance and with type 2 diabetes. Study performed by Andreelli et al. revealed, that the insulin stimulated increase of PIK3R1 mRNA level has been totally impaired in patients with type 2 diabetes compared with lean and obese subjects but with proper insulin tolerance [48]. There was also unnoticeable induction of PIK3R1 expression in skeletal muscle in response to severe diet in patients with type 2 diabetes, while in lean and obese subjects without diabetes and with normal insulin sensitivity the increase of PIK3R1 mRNA level in response to restrict diet was considerable [49]. These data suggest that regulation of expression rate in response to changeable environmental factors as diet or insulin concentration must be impaired. In studies searching for genetic variants two SNP variants have been associated with increased risk for type 2 diabetes. The first one is intronic polymorphism SNP42 that has exhibited increased risk for insulin resistance development and has been associated with greater BMI index in carriers of G variant. The second one: Met→Ile at codon 326, in previous studies did not show considerably increased susceptibility to type 2 diabetes [50, 51], but Wareham et al. have revealed statistically significant correlation with increased disease risk [52].

Genetic variations in SLC2A4

SLC2A4 encodes GLUT4 (facilitated glucose transporter, member 4). Studies performed on knockout animals displayed disregulated carbohydrates metabolism as well as protein and lipid metabolism leading to insulin resistance development in mice carrying disruption in GLUT4 gene. In clinical studies A Andersen et al. [54] showed reduced total amount of GLUT4 protein in patients with type 2 diabetes compared with healthy subjects. They revealed also decreased rate of SLC2A4 expression in response to insulin stimulation in patients with type 2 diabetic compared with controls. Furthermore, there was no statistically significant correlation between the basal level of GLUT4 protein and insulin stimulated glucose uptake in diabetics, while in healthy controls there was 3,5-fold higher drop in GLUT4 protein in abundance. In study performed on cultured human skeletal muscle cells, a reduced insulin stimulated glucose transport into skeletal muscle cells in NIDDM (non-insulin-dependent diabetes mellitus) patients comparing to healthy subjects has been reported [55], but the GLUT4 expression was not altered. The sequencing of SLC2A4 gene did not reveal any statistically significant correlation between polymorphism in this gene and impaired glucose uptake and insulin resistance [53]. The role of disruption in GLUT4 gene in glucose transport
into insulin-dependent tissues deregulation and insulin resistance occurrence needs to be further elucidated.

**Genetic variations of PTP1B gene — PTPN1**

PTPN1 (protein tyrosine phosphatase, non-receptor type 1) is negatively implicated in insulin resistance development by insulin receptor tyrosine kinase domain dephosphorylation. It has been shown that disruption in this gene develop insulin resistance in knockout mice. In clinical study the results obtained by different scientific groups are divergent and often contradictory. In skeletal muscle McGuire et al [55] showed increase in PTPase activity in patients with insulin resistance, what corresponds to results obtained from knockout animals. On the other hand, Kusari et al. [56] exhibited decrease in skeletal muscle PTPase activity in patients with NIDDM and in insulin resistance individuals without frank diabetes. Interesting results were obtained by Ahmed et al. [57] in study performed on three groups of subjects: lean, obese individuals and obese patients with NIDDM. They showed increased PTPase activity in lean and obese subjects, but with proper response to insulin, and decreased PTPase activity in diabetic patients. Worm et al. [58] did not show increased PTPase activity in skeletal muscles of NIDDM patients during hyperinsulinemic euglicemic clamp study, whilst in skeletal muscle in healthy controls the rise in PTPase activity was significant. Furthermore, the basal PTPase activity was markedly lower compared with healthy controls. These data allow speculating the possible mechanism leading from insulin resistance to type 2 diabetes in relation to PTPase activity and its expression. In the first state, increased glucose concentration leads to enhanced insulin secretion. The newly developing insulin resistance increases insulin synthesis and secretion, what subsequently stimulates PTPase activity impairing insulin sensitivity by insulin receptor dephosphorylation. When an organism is running out of compensating ability of pancreatic β-cells, the decreased insulin level does not stimulate PTPase activity, therefore in state of frank diabetes mellitus the PTPase activity is decreased.

Divergent results obtained from numerous studies did not provide straight data concerning the role of genes disruptions in impaired glucose transport into skeletal muscle and adipose tissue cells and insulin resistance development.

**Insulin resistance development in relations to environmental factors — obesity**

According to the above mentioned information, type 2 diabetes is caused by interaction of genetic and environmental factors. The main and the most important environmental factor leading to type 2 diabetes is obesity. It seems that prolonged increased in energy intake, in particular fatty acids, disrupts the insulin action pathway. It is well known that the excessive accumulation of adipose tissue, especially visceral type of obesity, causes disruption in glucose homeostasis. There are many ways in which visceral adipose tissue impairs glucose metabolism. The greatest influence on glucose metabolism impairment have circulating free fatty acids (FFA), which inhibit either glucose uptake and oxidation in skeletal muscle (mainly by inhibition of glycolysis and glucose transport), or insulin secretion from β-cells [59]. Suppression of GLUT4 translocation by fat metabolism seems to be the most important cause of insulin resistance development. Moreover, free fatty acids increase serine/threonine phosphorylation of IRS-1 and reduce its affinity to insulin receptor protein kinase and PI 3-kinase activity.

Adipose tissue produces many factors such as adiponectin, resistin, leptin, tumor necrosis factor (TNF-α), retinol-binding protein-4 (RBP-4) or cytokines, mainly IL-6, 13, 18. Some of them may impair glucose metabolism. Investigators suggest that obesity causes increased inflammatory cytokines production by adipocytes and thus insulin resistance development as a result of inflammatory process. It was seen that there is increased level of TNF-α in adipose tissue in obese and insulin-resistant humans and animals. TNF-α, similarly to FFA disrupts the insulin signaling pathway by increase in serine/threonine phosphorylation of IRS-1, what reduces the affinity of IRS-1 to the insulin receptor protein kinase activity and even causes its inhibition [60]. It was shown that neutralization of TNF-α positively correlates with improvement in insulin sensitivity in animals. Nevertheless, this study has not been performed on diabetic humans so far.

Another factor which may play a role in insulin resistance development is ROS (reactive oxygen species). In obesity the ROS production is elevated, causing inflammatory pathway activation. Several studies show that inflammatory cytokines such as TNF-α or dexamethasone increase the ROS level, what results in insulin resistance [61]. Additionally, ob/ob mice treated with antioxidant drugs improve their insulin sensitivity and glucose metabolism. Furthermore, oxidative stress, which accompanies obesity may be involved in pancreatic β-cell degeneration and thus in progression of the insulin resistance state.

Retinol-binding protein-4 is also implicated in inflammatory process of insulin resistance state [62]. Very important in maintaining proper glucose homeostasis is coordination of many compartments of the cell. Scientists suggest that RBP-4 is responsible for cross-talk between many cells and organs. Besides, in insulin resi-
stance in obese people increased RBP-4 level was observed. The role of RBP-4 in insulin resistance pathogenesis is based on induction of PEPCK (phosphoenolpyruvate carboxykinase) expression in liver and thus impairment in insulin signal transduction in muscles.

It is very important also to discuss the relation between mitochondrial oxidative metabolism and insulin resistance. As ATP-dependent potassium channels regulate the process of insulin secretion, any disorder in ATP production may have consequences in impaired insulin secretion. The inflammation state that accompanies insulin resistance and obesity may deteriorate ATP production. Decreased mitochondrial oxidative metabolism in muscles of insulin resistance individuals was observed [63]; furthermore, there was an increase in serine phosphorylation of IRS-1 and decrease in Akt stimulation in response to insulin. The mitochondrial oxidative metabolism might be defected by ROS or inflammatory cytokines, which levels are elevated in obesity.

Only the most important factors concerning obesity with relation to insulin resistance and increased risk for type 2 diabetes mellitus have been discussed. There are many other mechanisms concerning increased body mass and its involvement in impaired glucose and insulin metabolism and development of type 2 diabetes, but it was not possible to discuss them all in this review comprehensively.

**Conclusion**

Insulin resistance is a condition that accompanies multiple diseases: metabolic syndrome, obesity, PCOS or type 2 diabetes. It is defined as a state, in which proper concentration of insulin is insufficient to develop physiological response, that is glucose uptake into insulin-dependent tissue such as skeletal muscle, adipose tissue and liver. It can be induced by genetic disruption, what was shown in knockout animals studies. Thanks to these studies researchers were able to evaluate the theoretical order of events followed by progression of insulin resistance resulting in type 2 diabetes. According to the newest knowledge, insulin resistance is the first and probably the most important feature in type 2 diabetes development, initially compensated by enhanced insulin secretion, followed by β-cells degeneration and decrease in insulin synthesis and secretion. In clinical studies many disruptions in genes encoding proteins involved in insulin signal transduction correlated with insulin resistance. Furthermore, in many cases some variants of particular genes increased risk for insulin resistance only when accompanied by obesity, showing a great role of interaction between these two: genetic and environmental factors. It has also been observed that some variations in these genes change the rate of their expression, what has physiological consequences. All these data allow to conclude, that in insulin resistance development one has to take into account both: genetic (genetic variants or impairment in expression regulation) and environmental (mainly obesity) implications.

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