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RELATIVE CONTRIBUTION OF DECREASED INSULIN SENSITIVITY TO DETERIORATION OF GLUCOSE HOMEOSTASIS

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Summary

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Insulin resistance (IR) is defined as a state of decreased ability of insulin to stimulate the uptake and metabolism of glucose in target cells at physiological concentration. In men and cats, IR usually precedes the development of type 2 diabetes mellitus. The most consistent sign of IR is the impaired glucose tolerance. The present review aims at going over the main points and analyzing the available information about the role, nature, mechanisms (intra- and extracellular) and predisposing factors of IR as one of the primary factor for occurrence of diabetes in men and cats. An emphasis is put on the elucidation of mechanisms of disturbances in obesity, that, along with decreased physical activity and advanced age are among the essential risk factors of IR development. Hyperglycaemia, being one of the most important signs of IR, is resulting from both inhibited uptake and metabolism of glucose in muscles and from its enhanced production in liver.

Key words: cats, diabetes mellitus type 2, glucose homeostasis, insulin resistance, obesity

INTRODUCTION

Diabetes mellitus type 2 (DMT2) is a common endocrine disorders in men and, among domestic animals, cats are most frequently affected (Nelson *et al.*, 1990; Panciera *et al.*, 1990; Kahn, 2001, Rand *et al.*, 2004; Appleton *et al.*, 2005). In dogs, a form resembling human type 1 diabetes accompanied with autoimmune destruction of pancreatic β-cells is predominantly seen (Lendrum *et al.*, 1976; Baekkeskov *et al.*, 1982; Palmer *et al.*, 1983; Hoenig & Dawe, 1992; Hoenig, 2002).

The normal glucose tolerance depends mainly on three factors: 1. the secretion of insulin by β-cells; 2. the sensitivity of target cells to metabolic effects of insulin and 3. the glucose effectiveness, representing the ability of glucose to stimulate its own uptake by cells and at the same time, to inhibit the liver production of glucose (Cavaghan *et al.*, 2000; Kahn *et al.*, 2001; Kahn, 2003). The decreased insulin sensitivity or insulin resistance (IR) is defined as reduced capacity of insulin to stimulate the uptake and metabolism of glucose by cells (Gerich, 1998; Saltiel, 2001; Kahn, 2003). It is considered that IR is due to reduced amount of receptors or structural changes in them that result in decreased affinity to insulin (Miles *et al.*, 1998). Recently, more attention is paid on the so-called prediabetic

states. They are intermediate states between health and overt diabetes, known as impaired glucose tolerance (IGT). IGT is characterized with delayed elimination of glucose from the bloodstream after exogenous administration mainly due to development of IR in target cells (muscles, fat and liver cells). Thus, fasting blood glucose concentrations are generally close to upper reference values or slightly higher, as the secretory capacity of β-cells is yet preserved and IR could be compensated with increased insulin secretion (Kahn, 2001; Cavaghan *et al.*, 2000). It is accepted that the transition of IGT to overt diabetes occurs most commonly following exhaustion of the compensatory potential of β-cells (insulin hypersecretion) with regard to IR (DeFronzo *et al.*, 1992; Cavaghan *et al.*, 2000).

In animal species, including cats, the studies on this subject are still limited and data obtained in men and rats serve as baseline information. That is why, with the present review we have tried to sum up and analyze the available information about the role, predisposing factors and mechanisms (intra- and extracellular) of IR as one of the primary risk factors for occurrence and development of diabetes in cats.

NATURE OF INSULIN RESISTANCE

One of the main features of DMT2 is the resistance to insulin effects, resulting in increased liver production of glucose and disturbances in its uptake and metabolism in insulin-sensitive tissues (mainly in muscles and at a lesser extent in adipose tissue and the liver) (Petrus *et al.*, 1998). The available experimental data evidence that IR and the resulting hyperglycaemia are polyetiological in nature (Corcoran *et al.*, 2007; Lewis *et al.*, 2007; Weiss, 2007). Therefore, many factors, genetic and environmental, could influence the occurrence and development of IR.

The sensitivity of tissues to insulin is quantified by the amount of insulin, necessary to enhance the elimination of glucose from the bloodstream at the background of glucose effectiveness. The latter is defined as the ability of glucose to inhibit on its own the liver production of glucose and to stimulate the elimination of glucose from blood at basal insulin levels (Bergman, 1989; Del Prato *et al.*, 1995; Ader & Bergman, 1997).

The ability of insulin-resistant individuals to compensate for the reduced insulin sensitivity by increased secretion of insulin determines the extent to which a normal glucose tolerance could be maintained (O'Brien *et al.*, 1985; Nelson *et al.*, 1990; Rand *et al.*, 2004). The subjects that could no more maintain the balance between insulin secretion and insulin sensitivity develop an impaired glucose tolerance or DMT2 (Bergman *et al.*, 2002).

Although the etiopathogenesis of DMT2 is complex, in most cases there are disturbances in both the function of β–cells, respectively the secretion of insulin, and in peripheral insulin sensitivity, i.e. in the effect of insulin at cellular level.

In man, the interest during the last decade was primarily focused on revealing the role of IR as a major cause of impaired glucose homeostasis (DeFronzo & Ferrannini, 1991; Kruszynska & Olefsky, 1996). Despite the multiple studies in men, the localization of the primary defect is not completely known: whether in tissue sensitivity to insulin or in β-cells. The role and importance of genetic and environmental factors for the appearance and development of DMT2 are neither fully understood.

At present, the prevailing opinion is that the disturbances in β-cell function are genetically determined whereas the defects in insulin sensitivity are rater due to environmental factors (nutrition, decreased physical activity, obesity, advanced age etc.) without excluding entirely the role of heredity (Haffner *et al.*, 1997; Boden *et al.*, 1999; Boyko *et al.*, 2000; Fujimoto, 2000; Kahn & Filer, 2000.; Weyer *et al.*, 2001; Gerich, 2003). All these factors result in IR and when it happens in subjects with genetic predisposition to β-cell damage, an IGT is initially occurring that under a more prolonged action of adverse factors, progresses to overt diabetes (Cavaghan *et al.*, 2000).

There are data showing that both the β-cell function and the insulin sensitivity could be independent predictors of DMT2 (DeFronzo, 1997; Ferrannini *et al.*, 2005; Pratley *et al.*, 2000). There are still some unclear aspects about the relative contribution of IR and the impaired β-cell function in DMT2 development. The widespread view is that IR on its own is not sufficient for the occurrence of the disease (Weyer *et al.,* 2001). Most commonly, IR causes an evolution of the normal glucose tolerance into IGT. The further development of IGT into DMT2 however, requires always damage of β-cells, i.e. impaired secretion of insulin (Weyer *et al.,* 2001; Kahn, 2001).

Insulin exerts its effect after binding to specific receptors in target tissues. The hormone-receptor complex controls a number of intracellular processes, including the transmembrane transportation of glucose, the synthesis of glycogen and lipids, the expression of specific genes and suppression of the endogenous hepatic production of glucose (Alper, 2000). The sensitivity of target tissues to metabolic effects of insulin is one of the factors with primary importance for the maintenance of systemic glucose homeostasis.

Regardless of some unclear issues, a significant progress in the elucidation of molecular mechanisms responsible for the performance of insulin effects related to transmembrane glucose transport in insulin-sensitive tissues (skeletal muscles and adipose tissue) under normal conditions and in IR has been recently achieved. The insulin receptor has a heterotetrameric structure consisting of 2α and 2β chains and belongs to receptors with tyrosine kinase activity. Initially, insulin binds to the extracellular α subunit, then the signal reaches the intracellular end of the β subunit that actually manifests tyrosine kinase activity. Next, an autophosphorylation of tyrosine residues in the receptor's polypeptide chain occurs. Thus, the activated receptor binds and provokes phosphorylation of tyrosine residues of a protein, known as insulin receptor substrate (IRS). In reality, IRS proteins are more than 13, but IRS1 and IRS2 are the most important in mediating insulin signaling. IRS activates the phosphoinositide 3-kinase that catalyzes the formation of phosphoinositol 3-phosphate and consequently, a phosphoinositol 3-phosphate dependent protein kinase is activated. The latter phosphorylates another 2 protein kinases – the atypical protein kinase C (aPKC) and protein kinase B (PKB, a.k.a. Akt), that finally, mediate the translocation of the specific glucose transporter 4 (GLUT4) to the external wall of the cell membrane, where it binds glucose and transport it to the cytoplasm. The molecular mechanisms that accomplish the relationship between aPKC, PKB and GLUT4 are still not revealed. Furthermore, especially in muscles, PKB increases the activity of glycogen synthase, respectively of glycogeno-

genesis. In insulin-resistant states, the various corresponding triggering factors (increased free fatty acid levels, adipokines, cytokines etc.) interrupt insulin signaling pathway at specific locations, as will be explained below.

The most consistent feature of IR is IGT, resulting from delayed elimination of exogenously administered glucose (orally or intravenously) from the bloodstream. It is believed that the development of IR in muscles is the most important and that it causes disturbances in the transport of glucose through the cell membrane as well as in glycogen synthesis (Kahn & Flier, 2000; Shulman, 2000; Saltiel, 2001). Although less important, the IR in adipose tissue and the liver (Kahn & Flier, 2000; Saltiel, 2001), and the impairment of insulin-independent mechanisms involved in the maintenance of glucose homeostasis (Martin *et al.*, 1992), also play a role in this connection.

Despite the considerable advancement of molecular biology and the discovery of more and more molecules, participating in insulin-mediated processes, the cellular mechanisms responsible for IR development and the factors, involved in them are still unclear (Virkamaki *et al.*, 1999; Kahn, 2003).

It is shown that the synthesis of glycogen in muscles accounts for the intracellular metabolism of a major part of the glucose uptake by tissues and that it is virtually the most important process of non-oxidative glucose metabolism (Shulman, 2000). Therefore, the defective muscle glycogen synthesis is essential for the induction of IR. It is not yet quite clear where exactly these defects are located: whether in the glycogen synthase, hexokinase, resp. the phosphorylation of glucose or in sarcolemmal glucose transport. The recent investigations using NMR show that the impaired synthesis of muscle glycogen are rather resulting from disturbance of glucose transmembrane transport that could be due to impaired translocation of GLUT4 through the plasmic membrane of muscle cells or to the effect of free fatty acids upon the binding of insulin to its own receptors (Shulman, 2000). Post receptor disturbances resulting from IR have been also observed (Miles *et al.*, 1998).

INTRA- AND EXTRACELLULAR MECHANISMS OF INSULIN RESISTANCE

In this part we will accentuate upon the characteristic disorders occurring in obesity, as the most potent factor for the appearance and development of IR and consequently, DMT2. The cited data are primarily for small rodents (rats) and men, where the subject is considerably better explored than in other animal species including cats.

Regardless of the extensive research during the past years, there are still some unclear issues regarding the molecular mechanisms responsible for the disturbances in insulin-resistant states.

The most popular view states that the intracellular accumulation of triglycerides in skeletal muscles has the greatest impact upon IR development and this is explained by the considerably bigger mass of muscle tissue compared to that of other tissues (Yu *et al.*, 2002; Corcoran *et al.*, 2007; Lewis *et al.*, 2007). It is believed that the intracellular accumulation of triglycerides in skeletal muscles was genetically determined to a great extent (Weiss, 2007). Another hypothesis that gains recently recognition, is that the adipose tissue and more precisely the intraabdominal fat (the so-called visceral or

central obesity) are important for IR development and therefore, for accompanying hyperglycaemia (Kahn & Flier, 2000; Weiss, 2007; Stumvoll, 2007). Below we will discuss the role of aforementioned factors (intracellular accumulation of triglycerides and visceral obesity) for the occurrence and development of IR.

Under normal conditions, insulin suppresses the activity of lipoprotein lipase in muscles and increases it in fat stores, that leads to mobilization of free fatty acids (FFA) to fat stores where they are reesterified and stored as fuel under the form of triglycerides (Corcoran *et al.*, 2007). In obesity however, the activity of lipoprotein lipase in muscles increases on the account of fat stores and this results in release of FFA in myocytes, respectively in increased accumulation of triglycerides in muscles (Yu *et al.*, 2002; Corcoran *et al.*, 2007; Lewis *et al.*, 2007). The latter depends on several factors. The increased lipoprotein lipase activity together with the reduced activity of acylcarnitine transferase and the lower oxidative capacity of mitochondria create favourable conditions for inhibition of fatty acid oxidation and their utilization for synthesis of triglycerides (Corcoran *et al.*, 2007; Weiss, 2007). It is however considered that not triglycerides, but some of their precursors (long-chain fatty acyl-CoA and diacylgly-cerol) are more important for inhibition of insulin-stimulated glucose transport through the cell membrane to myocytic cytoplasm (Yu *et al.*, 2002; Corcoran *et al.*, 2007; Weiss, 2007). It was experimentally proved that these intermediate metabolites increase the activity of the so-called "classical" or "novel" protein kinases: protein kinase-θ and protein kinase-ε, that, on their part, provoke a phosphorylation of serine and threonine, but not of tyrosine residues in the molecule of IRS (Yu *et al.*, 2002; Corcoran *et al.*, 2007; Weiss, 2007). This, for its part, blocks the further transmission of insulin signaling pathway to phosphoinositide 3-kinase. All these events finally result in impaired translocation of GLUT4 to cell membrane surface and then, to inhibited glucose uptake in myocytes (Corcoran *et al.*, 2007; Weiss, 2007). This is believed to be one of the essential mechanisms in hyperglycaemia related to IR.

In revealing the role of obesity, issues of special interest are the mechanisms leading to IR development in the liver, as combined with the impaired glucose uptake and metabolism in muscles, they also contribute to the development of hyperglycaemia. These mechanisms in the liver are relatively less investigated and the obtained experimental data are rather contradictory.

As mentioned, the visceral obesity is essential for IR. The fat accumulated in the intraabdominal region has some specific features distinguishing it from subcutaneous fat. For instance, adipocytes in visceral obesity are more resistant to the antilipolytic effect of insulin and more sensitive to the lipolytic effect of catecholamines (Kahn & Flier, 2000). As the veins in the intraabdominal region are directly joining the portal vein, this results in increased FFA levels in portal blood, i.e. in the liver. It is established that increased FFA concentrations reduce the inhibiting effect of insulin upon the gluconeogenesis and glycogenolysis and therefore, an enhanced production of glucose is observed (the so-called endogenous glucose production – EGP) (Lam *et al.*, 2003). This also contributes to IR-related hyperglycaemia. The mechanisms of insulin signaling blockage is different from that described in muscles. The high FFA levels increase the activity of two ser-

ine/threonine kinases – protein kinase C-δ and kappa-β kinase inhibitor and decrease the activity of tyrosine kinase that results in enhanced phosphorylation of serine and threonine instead of tyrosine residues. This provokes blockage of insulin signaling pathways in hepatocytes, resp. enhanced EGP on the account of gluconeogenesis and glycogenolysis (Lam *et al.*, 2003; De Alvaro *et al.*, 2004; Boden *et al.*, 2005).

Among the factors, believed to be tightly related to obesity and namely, to FFA role in impaired insulin sensitivity, are the glucotoxicity and the lipotoxicity. The molecular mechanisms, by which IR is altered by these states, are under investigation (Sivitz, 2001).

The term lipotoxicity specifies the diabetogenic effect of increased blood FFA concentrations and the increased cellular content of triglycerides, mainly in the liver, muscles and pancreatic Langerhans islets.

Glucotoxicity is used to define the diabetogenic effect of elevated blood glucose concentrations (Sivitz, 2001). Similarly to lipotoxicity, glucotoxicity is manifested in the liver, muscles and pancreatic islets.

Lipo- and glucotoxicity could explain some of the commonest defects in insulin secretion and effects, observed in DMT2. The elevated FFA and glucose concentrations could act on some cells and tissues, disturbing the cellular glucose uptake, insulin secretion as well as to enhance hepatic glucose production (Sivitz, 2001).

Hyperglycaemia is able to induce IR autonomously, thus delaying the cellular glucose uptake (Yki-Jarvinen 1996; Richter *et al.*, 1988). It is thought that glucotoxicity is due to impaired transmembrane transport in cells (Sivitz, 2001). It is known that in insulin-sensitive tissues (heart, muscles, brown and white adipose tissues), the glucose uptake is mediated by the number and the functional activity of membrane glucose transporters. The primary, insulin-sensitive transporter in these cells is the so-called glucose transporter 4 (GLUT4) (Wilkes *et al.*, 1998) that is present in intracellular vesicles and the plasma membrane. Glucose could interfere with one or more steps in insulin-stimulated glucose transport (Sivitz, 2001). It is believed that the high glucose concentration induces IR through impaired mobilization of glucose transporters from the core of cells to the plasmatic membrane, where they exert its effect (Richter *et al.*, 1988). In this way, glucose toxicity could be viewed as a mechanism that preserved insulin-sensitive tissues from excess uptake of glucose and from complications related to diabetogenic state (Yki–Jarvinen, 1996).

Glucose resistance is a state with impaired insulin-independent removal of glucose (glucose effectiveness). It is also considered as a possible factor with a key role in view of its involvement in the maintenance of glucose homeostasis (between 70–78% on the background of basal insulin levels) (Christopher *et al.*, 1995; McArthur *et al.*, 1999). According to Sivitz (2001), the chronic exposure to high glucose concentrations deteriorates the ability of glucose to stimulate independently its uptake and metabolism by cells, which is also contributing to disturbances in glucose homeostasis and the occurrence of hyperglycaemia (Sivitz, 2001).

At a great extent, lipotoxicity could be explained by the so-called glucose-fatty acid cycle in skeletal muscles, a.k.a. Randle's cycle (Randle *et al.*, 1994). It shows that glucose oxidation competes with fatty acid oxidation, probably as a means of preserving cells from excessive

energy expenditure. The enhanced β-oxidation of fatty acids interferes with glucose oxidation by decreasing the activity of some key enzymes involved in that process (pyruvate dehydrogenase, phosphofructokinase and hexokinase) as well as in the transformation of glucose into glycogen (glycogen synthase) (Wititsuwannakul & Kim, 1997). All that results in accumulation of free glucose into cells, resulting in blockage of transmembrane glucose transport, respectively in increased blood concentrations. Therefore, Randle's cycle provides an explanation for the following disturbances in glucose metabolism due to increased concentration and oxidation of FFA: 1. reduced glucose oxidation; 2. reduced glycogen synthesis; 3. reduced glucose transport in muscle cells and 4. hyperglycaemia.

Another explanation of lipotoxicity that has recently gained a special significance is the impaired glucose uptake by cells caused by fatty acids via inhibition of one or more steps in insulin signaling cascade (Sivitz, 2001).

It was also demonstrated that FFA could suppress the gene expression of GLUT4 in muscles and in adipose tissue (Boden, 1997), as well as to contribute to disturbances in the mobilization of GLUT4 molecules from the interior of cells to their surface (Shulman, 2000; Hoenig *et al.*, 2003). This process is effective in muscles and the inhibition of one or more steps in the metabolism could result in impaired consumption of glucose and consequently, to hyperglycaemia. It is established that GLUT1 expression did not change significantly with obesity unlike GLUT4, allowing to assume that only GLUT4 transporters are affected early in obesity, respectively in FFA levels elevation and that these disturbances take place before any deviation in fasting glucose concentrations and in glucose tolerance has been occurred (Brennan *et al.*, 2004).

Liver occupies a central role in glucose metabolism. After carbohydrates intake, the own production of glucose in this glucostatic organ is inhibited and about 1/3 of glucose taken with food is utilized.

FFA could also provoke a hyperglycaemia through effects in the liver, where the impaired glycolysis results in the release of a greater amount of glucose by increasing the activity of pyruvate carboxylase and phosphoenolpyruvate carboxykinase – rate-limiting enzymes in the gluconeogenesis (Bahl *et al.*, 1997). Similarly, FFA exert an effect on glucose 6-phosphatase, the enzyme controlling the release of glucose from the liver.

Under normal conditions, the increased blood FFA concentrations stimulates whereas the reduced concentrations inhibit the gluconeogenesis (Chen *et al.*, 1999). The suppressing effect of insulin upon the hepatic glucose production is due mainly to its inhibitory effect on lipolysis and this is followed by lower FFA blood concentrations (Lewis *et al.*, 1997; Cherrington, 1999).

The relationship between the increased FFA concentrations, their oxidation and hepatic glucose production in obesity could be explained as follows: Under the influence of the high blood FFA levels, FFA utilization by hepatocytes is increased and thus, their oxidation and the accumulation of acetyl-CoA are enhanced. The high acetyl-CoA levels stimulate both the pyruvate carboxylase and glucose 6-phosphatase (Bahl *et al.*, 1997). The increased rate of FFA oxidation provides the necessary source of energy (under the form of ATP) and reduced nucleotides (NADPH) for continuation of gluconeogenesis pathway. FFA also provoke IR

in the liver by inhibiting some of the steps involved in the system of insulin-stimulated glucose transport in cells (Ellis *et al.*, 2000; Itani *et al.*, 2002).

All presented information makes clear that glucose and fatty acids compete to be utilized as a source of energy by cells. With this regard, the glucose metabolism is deteriorated not only by FFA oxidation, but the glucose itself could prevent the utilization of fats. The final product of the complete glucose metabolism is acetyl-CoA that is degradated to CO_2 and H_2O and ATP is generated via oxidative phosphorylation, In states with excess of energy however, acetyl-CoA is transformed into malonyl-CoA, responsible for the first step in fatty acids synthesis. As malonyl-CoA inhibits the transport of fatty acids from the cytosol to mitochondria, this leads to glucose-mediated suppression of FFA oxidation.

It is known that one of the most consistent signs of IR is hyperinsulinaemia (Miles *et al.*, 1998; Appleton *et al.*, 2005). It could be caused by both the enhanced pancreatic production of insulin in order to compensate for both the reduced insulin sensitivity and the decreased hepatic insulin clearance when IR is developing (Kahn & Flier, 2000). Hyperinsulinaemia by itself also induces IR via the so-called phenomenon of "down regulation of insulin receptors". It was experimentally determined that in this state, the disturbances are located in pre-receptor events and in the receptor apparatus of muscle cells (Miles *et al.*, 1998). The defects are both in the transendothelial insulin passage from capillaries to the interstitial fluid and in the hormone-receptor interaction, followed by reduction in tyrosine kinase activity in skeletal muscles. As a result, a number of intracellular reactions, specific for the metabolic effects of insulin, are hindered (Miles *et al.*, 1998).

During the last years, the adipose tissue is more and more considered not only as a store of fuel under the form of triglycerides, but as an endocrine organ as well, where apart the generation of considerable amounts of FFA, a number of biologically active substances are formed. The latter could be divided into two groups: tissue hormones called adipokines (leptin, resistin, visfatin, vaspin, adiponectin etc.) and cytokines (tumor necrosis factor-α – TNF-α, interleukin-6 – IL-6, interleukin-8, interleukin-10 etc.), some of which are shown to influence at a significant extent the tissue sensitivity to insulin (Kahn & Flier, 2000; Steppan *et al.*, 2001; Tsao *et al.*, 2002; Gerich, 2003; Boden *et al.*, 2005; Ischizuka *et al.*, 2007). It is determined that apart adipocytes, the adipose tissue contains a matrix of connective tissue, stroma and macrophages, where some of biologically active substances are also produced (Stumvoll, 2007).

As already stated, the intraabdominal accumulation of adipose tissue (visceral obesity) is an important factor for IR development. This is due to both the enhanced lipolytic activity, i.e. to higher FFA concentrations, as well as to the formation and release in the circulation of some of mentioned adipokines (resistin, visfatin, vaspin) and proinflammatory cytokines (TNF-α and IL-6), that play an essential role in the induction of IR (Hanif *et al.*, 2006; Stumvoll, 2007). However, the mechanisms by which adipokines induce IR are not yet clarified.

The inhibiting effect of TNF-α is due to stimulation of lipolysis and serine/threonine instead of tyrosine phosphorylation of IRSs and to the thus blocked transmission of insulin signal to phosphoinositol 3-phosphate, resulting in impaired translocation and expression of

GLUT4 to the surface of cell membrane, i.e. to inhibited glucose entry in cells (Kahn & Flier, 2000; de Alvaro *et al.*, 2004; Stumvoll, 2007). According to others (Ishizuka *et al.*, 2007) the inhibiting effect of TNF-α upon the transmission of insulin signal could be manifested only when the serine/threonine phosphorylation of IRSs is combined with induction of suppressor of cytokine signaling-3 (SOCS-3). It is also shown that IL-6 could reduce insulin sensitivity too, due to stimulation of SOCS-3 expression (Stumvoll, 2007).

An important fact in the development of IR in visceral obesity is that the production of some adipokines (adiponectin and leptin) having a beneficial effect on insulin sensitivity, is decreased (Weyer *et al.*, 2001; Hoenig, 2002; Stumvoll, 2007). For example, adiponectin is considered to play a major role in glucose and fatty acids metabolism in insulin-sensitive tissues (Weyer *et al.*, 2001). It suppresses the gluconeogenesis, i.e. the endogenous glucose production, enhances the uptake of glucose in muscle cells, inhibits the lipolysis and stimulates the oxidation of fatty acids (Stumvoll, 2007). All that improves blood glucose homeostasis. Leptin improves insulin sensitivity by decreasing the level of triglycerides in skeletal muscles via prompting of fatty acids oxidation, with simultaneous reduction of protein kinase-θ activity (Kahn & Flier, 2000; Dube *et al.*, 2007).

The elevated glucagon concentration is specific for obesity and DMT2 in some species and is considered to occur secondary to the reduced effect of insulin on α-cells (Hamaguchi *et al.*, 1991). In obese cats, glucagon concentration is also strongly elevated compared to cats with normal weight. It is believed that this is important for the progressive development of obesity towards diabetes as glucagon increases IR and could speed up the exhaustion of β-cells (Hoenig, 2002).

FACTORS PREDISPOSING TO INSULIN RESISTANCE DEVELOPMENT

The occurrence of IR depends on a number of factors, mainly related to the mode of living and the interrelationships between the organisms and environmental factors. Among of the commonest factors with impact on IR are genetic abnormalities, the gender, advanced age, the reduced physical activity, the nutrition, respectively obesity, hyperglycaemia, some diseases, medications etc. (Nelson *et al.*, 1990; Panciera *et al.*, 1990; Scarlett & Donoghue, 1998; Rand *et al.*, 2004; Appleton *et al.*, 2005).

According to some authors, mainly β-cells are genetically predisposed to disturbances in their function whereas gene mutations associated to IR are relatively rarely seen (Kahn & Flier, 2000; Saltiel, 2001; Kahn, 2003). Although gene mutations in insulin receptors have been reported, they are a very rare cause of IR and DMT2 (Haffner *et al.*, 1997). Thus, the prevailing view is that insulin-resistant states are not directly related to damage of some specific "diabetogenic genes" but are rather a consequence of obesity and more precisely, of mutations of genes, responsible for the distribution of fat in the body, that results in their deposition mainly in the abdominal regions (Gerich, 2003; Kahn, 2003).

Insulin resistance and genetic factors

The view that genetic disorders predispose mainly to obesity and the distribution of fat in the body than directly to IR is prevailing. In a certain percentage (25%), there is an IR without obesity or IGT. The studies on this subject in cats are rather

few. It is determined that cats, in which the insulin sensitivity is lower than its average values for the population, are at a three-fold greater risk for development of IGT in obesity and higher risk of diabetes, thus showing a certain effect of genetic factors upon IR development (Appleton *et al.*, 2001). The same authors showed that the high basal insulin levels correlated to increased risk of IGT development. These data evidence that some cats are predisposed to IGT consequently to reduced tissue sensitivity to insulin. In obesity, these cats are at higher risk for DMT2 compared to animals with no deviations in glucose tolerance (Appleton *et al.*, 2001).

Insulin resistance and nutrition

In men, the intake of refined, easy digestible carbohydrates and food rich in animal fats, in amounts exceeding the physiological norms, predisposes to obesity, respectively to IR (Olefsky *et al.*, 1973; Kolterman *et al.*, 1980). In this state, the demands to β-cells are sharply increasing in order to produce more and more insulin in order to compensate for the IR. When this is combined with other predisposing factors as reduced physical activity and/or genetic defects in β-cells, they become gradually exhausted and damaged and then, hyperglycaemia occurs due to the evolution of IGT in overt diabetes. This is one of the most popular theories of human DMT2 etiopathogenesis and it is highly valid for feline diabetes as well (Hoenig, 2002).

The cats are known to be true carnivores and their metabolism is functionally fitted to digestion and utilization of high-protein diet (Thiess *et al.*, 2004). During the last 20–30 years however, this diet is more and more replaced by commercial cat foods with high carbohydrate and lower animal protein content. The recently observed tendencies in cats are towards increased incidence of diabetes. This is probably resulting from the inadequate nutrition (low protein and high digestible carbohydrate content in commercial foods) combined with reduced physical activity and castration. Thus, obesity, IR and IGT are initially occurring, that very frequently evolve in diabetes. In cats, there are no data about the existence of a genetic predisposition to β-cell damage (Rand *et al.*, 2004).

Insulin resistance and obesity

The most important risk factor for DMT2 development is obesity-related IR (Nelson *et al.*, 1990; Rand *et al.*, 2004). Even the smallest changes in the body mass index and fat cell dimensions result in increased DMT2 risk. It was experimentally shown that for cats, obesity was also among the most important factors for DMT2 occurrence (O'Brien *et al.*, 1985; Nelson *et al.*, 1990; Panciera *et al.*, 1990; Scarlett *et al.*, 1998; Hoenig, 2002; Appleton *et al.*, 2005). This is due to obesity-induced IR mainly in muscles and at a lesser extent in the liver and the adipose tissue, as well as to hyperinsulinaemia (O'Brien *et al.*, 1985; Nelson *et al.*, 1990; Appleton *et al.*, 2001; Hoenig, 2002; Appleton *et al.*, 2005).

It was established that cats given a high-calorie food for 10 months, exhibited an increased body weight by 1.9 kg (44.2%), with simultaneous more than 2-fold reduction of insulin sensitivity (Appleton *et al.*, 2001). In two-thirds of these cats, insulin sensitivity was near the lower reference limit (Feldhahn *et al.*, 1999). These results correspond highly to findings in men that witness a reduction in insulin sensitivity by 44% to 72% in overweight compared to normal-weight individuals (Taniguchi *et al.*, 1995).

It is believed that fasting hyperinsulinaemia is among the most consistent

indices of IGT as a results of obesity (Rand *et al.*, 2004; Appleton *et al.*, 2005).

In the context of contemporary views it is determined that in both men and cats, the development of IR is directly related to fat distribution in the body – the higher the accumulation of abdominal fat but not peripheral subcutaneous deposition, the higher the insulin resistance (Peiris *et al.*, 1989; Despres *et al.*, 1990; Bjorntorp, 1993; Rand *et al.*, 2004).

Insulin resistance and physical activity

In men, the reduced physical activity increased the risk of DMT2 by direct decrease of insulin sensitivity as well as indirectly, helping the weight increase, i.e. the obesity. It is established that the regular exercise has a complex systemic effect and always results in improved sensitivity of receptors to insulin. This is thought to be due to stimulation of FFA oxidation in muscles and consequently, to decrease in the intracellular accumulation of triglycerides, respectively improvement of insulin signaling and enhanced glucose uptake by myocytes (Corcoran *et al.*, 2007). Shortly after the discontinuation of the physical effort however, IR is developing again (Horton, 1986; Prigeon *et al.*, 1995). This is also valid for domestic cats that receive their food in an effortless manner and are not forced to hunt for it. As reported by Giles *et al.* (2003) the increased level of physical activity in cats of 10 min per day has the same effect for the maintenance of normal weight as the feeding with low-calorie diet.

Insulin resistance and drugs

The veterinary specialists could assist in decreasing the insulin sensitivity in cats, using some medications for a long time or pharmaceutical forms with retarded effect. In cats, this could most commonly happen in using corticosteroids and progestins that are shown to produce IR in overphysiological concentrations (Rand *et al.*, 2004).

Insulin resistance and gender

It is confirmed that male cats develop diabetes more frequently than females (Panciera *et al.*, 1990; Rand *et al.*, 2004). It is evidenced that this is due to the following two factors: first, under normal conditions, males exhibit a tendency towards lower insulin sensitivity and in obesity, the difference with female cats becomes greater (Appleton *et al.*, 2001). Moreover, obese male cats have higher fasting insulin concentrations and as a whole, they secrete more insulin. Second, the same authors found out that male cats were more prone to obesity than females. Having a free access to food, males gain more weight (54%) than females (39%) for the same time, this being due mostly to the greater adipose tissue mass, thus resulting in reduced efficacy of insulin in decreasing blood glucose concentrations (Appleton *et al.*, 2001; Rand *et al.,* 2004).

The cited data about the lower insulin sensitivity and higher insulin levels, as well as for the lower insulin efficacy in males provide an explanation for the higher predisposition to IGT, respectively to diabetes, in male cats. Last but not least, neutered males with reduced physical activity, fed a diet inadequate as amount and quality, are prone at the highest extent to IR and therefore to diabetes. All these factors result in obesity that, as already stated, is the most potent risk factor for reduction of insulin sensitivity.

Insulin resistance and age

With advancing of age, the probability of decrease in insulin sensitivity is elevated in both humans and cats (Hoenig, 2002). This is probably related to structural al-

terations in insulin receptors or to reduction in their number and affinity.

METHODS FOR DIAGNOSIS OF INSULIN RESISTANCE

Recently, more attention is paid to the diagnostics of prediabetic states and IGT as its principal sign. In fact, this is a borderline state between health and clinical diabetes that is described mostly in men and carnivores and that develops consequently to IR in tissues (Nelson *et al.*, 1990; Appleton *et al.*, 2001; Appleton *et al.*, 2005). From the point of view of prevention and therapy of diabetes, the earliest possible detection of individuals with IGT prior to the occurrence of irreversible β-cell damage, when the troubles are mainly in the sensitivity and the affinity of insulin receptors and the synthetic capacity of β-cells is still preserved, is essential. For this purpose, objective criteria for as early as possible detection of subjects with IGT prior to β-cell injury and DMT2 development are necessary. In men there are such criteria for evaluation of the glucose tolerance status, some of them being used primarily with experimental purposes – the euglycemyc clamp technique called a "gold standard" and the minimal model analysis (Appleton *et al.*, 2005). In the euglycemyc clamp technique, the insulin sensitivity is determined as the amount of glucose, converted by one unit of insulin (Petrus *et al.*, 1998). In IR states, less glucose would be metabolized by one unit of insulin. The minimal model analysis is performed on the basis of intravenous glucose tolerance test, calculating the index of insulin sensitivity (Si) and glucose effectiveness (S_G) with specialized software (Bergman, 1989). Other criteria are utilized in the clinical practice such as the homeostasis model assessment (HOMA) and the quantitative check index (QUICKI), as well as the determination of changes in glucose and insulin concentrations after oral glucose tolerance test. HOMA and QUICKI are calculated only on the basis of fasting insulin and glucose concentration. Insulin sensitivity could be also assessed by calculation of the rate constant of glucose disappearance after intravenous insulin tolerance test (Appleton *et al.*, 2001).

According to the recommendations of the World Health Organization from 1999, the differentiation of people with normal glucose tolerance, IGT and overt diabetes should be done on the basis of both fasting blood glucose levels and after an oral glucose tolerance test.

Consistent with these criteria, diabetes is excluded in human subjects with fasting blood glucose levels equal to and under 6.1 mmol/L; these with 7.0 mmol/L and more are classified as diabetics and those with blood glucose between 6.1–6.9 mmol/L are classified as individuals with impaired fasting glucose. In the last group, an oral glucose tolerance test is indicated. The results of the test (glucose concentration on the 2nd hour after oral intake of 75 g glucose in 300 ml water) differentiate the subjects into: such without deviations from the normal glucose tolerance with < 7.8 mmol/L; such with diabetes– over 11.1 mmol/L and such with IGT with 7.8–11.0 mmol/L.

In animals however, the studies on this issue are limited and published data are mainly experimental and refer to the application of intravenous glucose tolerance test (Link *et al.*, 1997; Apletton *et al.*, 2001; Sarov *et al.*, 2004; Slavov *et al.*, 2005, Andonova *et al.*, 2006). There is neither a defined standardized procedure for performance of glucose-tolerance test nor reference values for glucose and insu-

lin concentrations at various intervals following its exogenous application. Moreover, the applied glucose dose varies within a broad range – from 0.05 to 1.0 g/kg. Our experimental data in a rabbit model (Georgiev I. Penchev *et al.,* 2006; Dimitrova *et al.*, 2006) showed that the determination of some kinetic parameters of glucose (plasma half-life and rate constant of glucose disappearance, area under the time-concentration curve and mean blood residence time), together with glucose dynamics after a glucose tolerance test, provides a detailed and objective information about the time and the rate of blood glucose levels reduction after exogenous application, i.e. about the glucose tolerance status, and could be used as reliable markers to distinguish between animals with normal glucose tolerance, IGT and overt diabetes. The developed model will soon be used in other animal species with high incidence of diabetes, mainly dogs and cats. The early detection of animals with IGT would allow at a significant extent to prevent or delay the development of diabetes and its specific clinical manifestations in prone subjects, whose potential for a successful therapy is significantly reduced, by adequate alteration of the rearing and feeding regimens.

Some authors propose to use the basal insulin concentrations after a 24-hour fasting as a reliable indicator of IR, as they find out a good correlation between this parameter and data calculated with the minimal model analysis and the euglycemyc clamp method (Appleton *et al.*, 2005).

In conclusion, it could be stated that IR is one of the main causes for DMT2. In cats, like men, the intraabdominal fat accumulation combined with feeding regimen, reduced physical activity and advanced age are potent risk factors for IR occurrence.

The mechanisms of hyperglycaemia, being the most important sign of IR, involve the increased blood FFA concentrations and the accumulation of triglycerides in tissues (mainly the skeletal muscles) as well as some tissue hormones synthesized in the adipose tissue – vaspin, visfatin, resistin and proinflammatory cytokines (TNF-α and IL-6 etc.) that cause blockage of insulin signaling pathways and inhibition of the insulin-dependent transport of glucose via the cell membrane.

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