Bulgarian Journal of Veterinary Medicine (2013), **16**, No 2, 65–80

RELATIVE CONTRIBUTION OF β-CELL DYSFUNCTION TO GLUCOSE HOMEOSTASIS IMPAIRMENT

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Summary

Ivanova, Zh. & I. Penchev Georgiev, 2013. Relative contribution of β-cell dysfunction to glucose homeostasis impairment. *Bulg. J. Vet. Med.*, **16**, No 2, 65–80.

The last decade has witnessed a remarkable progress in the elucidation of cell mechanisms of insulin resistance (IR), as well as in its importance for the appearance and maintenance of hyperglycaemia that accompanies type 2 diabetes mellitus (T2DM). At the same time, the role of impaired β-cell function for the development of T2DM remains insufficiently researched. That is why, the purpose of this review was to provide and analyse information about the role and significance of reduced functional activity of β-cells for T2DM onset and the pathogenetic mechanisms of impaired insulin secretion. The data presented clearly suggest that the transition of impaired glucose tolerance into overt diabetes absolutely requires a disturbance in β-cell function, resp. insulin secretion. Regardless of the numerous unclear issues about the mechanisms of β-cell dysfunction, it could be claimed that many factors provoking IR (obesity, increased free fatty acid concentrations, increased levels of some adipokines, free radicals etc.) results in disturbance of β-cell functional activity. The increased amyloid deposition in the pancreas is not less important. The currently prevailing opinion is that while IR is rather a consequence of adverse environmental effects, the β-cell impairment is genetically determined. Therefore, the risk of T2DM development increases extremely when genetic predisposition to impaired β-cell function is combined with harmful environmental effects resulting in IR development.

Key words: β-cell dysfunction, glucose homeostasis, obesity, type 2 diabetes mellitus

INTRODUCTION

The diabetological research during the last decade was mainly focused on elucidation of the significance of insulin resistance (IR) as a primary factor for the appearance and maintenance of hyperglycaemia accompanying type 2 diabetes mellitus (T2DM). At the same time, the role of pancreatic β-cells in this process remains insufficiently researched. So far there is no agreement on whether IR and reduced insulin release are independent factors for T2DM, and which of these two disturbances has a leading role. It is considered that IR alone, either induced by obesity or other factors, is not sufficient to cause T2DM, but it is rather a predisposing factor leading to impaired glucose tolerance (IGT) whereas impaired insulin secretion is probably determining whether IGT would progress to diabetes (DeFronzo, 1997; Pratley *et al.*, 2000; Weir & Bonner-Weir, 2004). In general, IR subjects have normal or slightly higher fasting blood glucose concentrations in the begin-

ning, because β-cells increase insulin release to compensate for reduced insulin sensitivity (Polonsky, 1999; Bergman *et al.*, 2002). This state results primarily in impaired glucose tolerance, which could be detected by glucose tolerance tests (oral or intravenous) (Nathan *et al.*, 2007). Yet, the factors leading to impaired synthesis capacity of β-cells, and depletion of their compensatory potential respectively, are poorly studied. That is why, the purpose of this review was to provide and analyse information about the role and significance of reduced functional activity of β-cells for T2DM onset and the pathogenetic mechanisms of impaired insulin secretion. Cited evidence are mainly from studies conducted in humans and laboratory animals (rats and mice), while data on the role of impaired β-cell function in domestic animals developing diabetes (cats, dogs and rabbits) are still few.

It is believed that β-cell dysfunction depends on numerous factors – genetic, environmental and life mode-related, consequent to obesity in particular – dyslipidaemia, amyloid deposits in the pancreas, reduced concentrations of some adipokines (leptin, adiponectin etc.), increased production of proinflammatory cytokines (tumor necrosis factor-α, interleukin-6), oxidative stress, and to the direct effect of hyperglycaemia.

NATURE OF B-CELL DYSFUNCTION

The disturbed β-cell function is manifested in a number of ways: reduced insulin release in response to glucose and non-glucose secretagogues (mainly amino acids) (Ward *et al.*, 1984; Berggren *et al.*, 1992); altered pulsatile and oscillatory patterns of insulin secretion (Pørksen *et al.*, 2002); impaired conversion of proinsulin to insulin (Kahn & Halban, 1997) and impaired release of islet amyloid polypeptide (IAPP, amylin) (Kahn *et al.*, 1998; Höppener *et al.*, 2002).

The disturbances in β-cells response to exogenous glucose, especially during the first phase of secretion are at the background of metabolic disorders, which would later lead to serious troubles in glucose homeostasis, resp. to T2DM.

In healthy subjects (humans and animals), the exogenous introduction of glucose, either during the oral glucose tolerance test (OGTT) or intravenously during the intravenous glucose tolerance test (IGTT) provokes secretion of insulin, occurring at two phases. The first phase of insulin secretion occurs very rapidly and results in strong increase in blood plasma insulin levels during the first 3 to 5, maximum 10 min after glucose infusion (in IGTT) or by the 30^{th} min (in OGTT). The second phase of secretion takes place gradually, after the 10^{th} or the 30^{th} min in IGTT or OGTT, respectively, and could last as long as the exposure to the glucose stimulus (Kahn, 2001). Usually, glucose levels in healthy individuals return to normal after the 1^{st} or the 2^{nd} hour in IGTT or OGTT, respectively.

The first phase is due to insulin release from mature secretory granules, located near the β-cell plasmatic membrane. The second phase is consequent from the release of both stored secretory granules (Daniel *et al.*, 1999), as well as newly synthesised amounts of insulin in response to glucose (Sizonenko *et al.*, 1993).

The absence or delay in the first phase of insulin secretion is usually accompanied by impaired glucose tolerance and increased blood glucose concentrations (hyperglycaemia). A direct relationship between impaired first phase of insulin secretion, insulin resistance in the liver and

increased fasting blood glucose levels is shown (Bock *et al.*, 2007; Faerch *et al.*, 2008). In cases of insulin resistance in the liver, the inhibiting effect of insulin on endogenous production of glucose (gluconeogenesis and glycogenolysis) is usually impaired (Bock *et al.*, 2007).

A standard method for evaluation of the first and second phases of insulin secretion is the glucose tolerance test with assaying insulin and glucose concentrations at precisely specified intervals after loading. Some indices calculated from the IGTT or OGTT are also proposed for evaluation of the first (early) insulin secretion phase. The insulinogenic index presents the ratio between blood plasma insulin and glucose levels 5–10 or 30 min after IGTT or OGTT, respectively, while the acute insulin response to glucose (AIR) is determined as maximum insulin concentrations after glucose infusion (Bergman *et al.*, 2002; Larson *et al.*, 2003; Festa *et al.*, 2008; Slavov *et al.*, 2010). Both indices are strongly correlated in subjects with normal or impaired glucose tolerance, but the higher variability of the insulinogenic index makes it less reliable for evaluation of the first phase of insulin secretion than AIR. Other parameters used are the area under the insulin concentrations curve ($AUC_{ins.0\rightarrow5min}$) and the insulin/glucose ratio by the 5th min after i.v. glucose infusion (I_{5min}/G_{5min}) (Larson *et al.*, 2003; Georgieva *et al.*, unpublished observation). For assessment of the second (late) phase of insulin secretion, the area under the insulin concentrations curve between post infusion min 30 and 120 are used (Larson *et al.*, 2003).

According to our data, obesity induced by castration of rabbits or feeding a high-fat diet to dogs inhibited the first phase of insulin secretion. This was manifested by lower insulinogenic index values (Slavov *et al.*, 2010), as well as glucose tolerance disturbances and statistically significantly higher glucose levels until the 10th min after IGTT, indicating β-cell dysfunction (Slavov *et al.*, 2010; Georgiev *et al.*, 2011). These results could be regarded upon as a direct outcome of insulin resistance in the liver, as the inhibited first phase of insulin secretion correlated with a marked fatty infiltration in hepatocytes of obese animals (Georgiev *et al.*, 2011).

ROLE OF THE FIRST PHASE OF INSULIN SECRETION FOR INHIBITION OF LIVER GLUCOSE PRODUCTION

The liver, being the primary glucostatic organ, is the main source of glucose in the post absorption period consequently to the optimal insulin/glucagon ratio that controls the gluconeogenesis and glycogenolysis rates. The rate of hepatic glucose production depends on the glucose uptake by peripheral tissues allowing for maintenance of stable blood glucose concentrations. The liver is essential for maintenance of glucose homeostasis during the period of absorption and enhanced endogenous production of glucose is related to deviations in mechanisms keeping its levels within physiological ranges (Consoli, 1992; Mevorach *et al.*, 1998). The significance of the first-phase insulin secretion in inhibiting endogenous glucose production was demonstrated by selective inhibition of both insulin secretion phases (Elahi *et al.*, 1989; Luzi & DeFronzo, 1989*)*. Experimentally, endogenous glucose production in controls was reduced up to 90% 20 min after infusion of glucose, whereas in the group without first phase of insulin secretion (after somatostatin administration), it was increased and by about 50% higher vs basal levels and 60-min levels.

Our data demonstrated that castration-induced visceral obesity in rabbits resulted in liver fat infiltration accompanied by reduced first phase of insulin secretion – index of β-cell dysfunction (Georgiev *et al.*, 2011).

ROLE OF THE FIRST PHASE OF INSULIN SECRETION FOR INHIBITION OF LIPOLYSIS

Some experiments provide support for the thesis that inhibition of hepatic glucose production was not related only to glycogenolysis and gluconeogenesis suppression by insulin but rather depends on the effects of this hormone on adipose tissue. The reduced blood concentrations of non-esterified fatty acids (NEFA) due to the antilipolytic effect of insulin impede endogenous glucose production (Rebrin *et al.*, 1996; Del Prato *et al.*, 2002). The suppressed first-phase insulin secretion however increases NEFA levels consequently to enhanced lipolysis, resulting in blocking the inhibiting effect of insulin on endogenous hepatic glucose production, i.e. in hyperglycaemia (Del Prato *et al.*, 2002; Caumo & Luzi, 2004; Salgin *et al.*, 2009).

ROLE OF THE FIRST PHASE OF INSULIN SECRETION FOR GLUCOSE UPTAKE BY INSULIN-SENSITIVE TISSUES

Skeletal muscles and adipose tissue are the main sites of insulin-mediated glucose uptake during the absorption period (Corcoran *et al.*, 2007; Weiss *et al.*, 2007). The rate of uptake by muscles is critical for preventing postprandial hyperglycaemia (Corcoran *et al.*, 2007; DeFronzo & Tripathy, 2009; Samuel *et al.*, 2010). The early phase of insulin secretion plays a very important role for crossing the endothelial barrier, resp. allows insulin to rapidly reach its skeletal muscle and adipose tissue receptors resulting to rapid uptake and metabolism of glucose in these tissues (Getty *et al.*, 1998).

The weakened first phase of insulin secretion is an early marker of β-cell dysfunction, which is observed long before the occurrence of significant changes in absolute glucose concentrations (Cavaghan *et al.*, 2000). According to some studies, 50–75% of β-cell secretory capacity is lost before the onset of overt deviations in fasting blood glucose levels (Kahn, 2001).

Experimental data in men and animals with impaired glucose tolerance demonstrate disturbances both in qualitative and quantitative indices of insulin secretion, and inhibited first-stage insulin secretion specifically outlined as a constant sign (Kahn *et al.*, 1998). The initial insulin response after IGTT (OGTT) or after a meal is postponed, while during the second stage the secretion could be enhanced due to the hyperglycaemia resulting from the lack of early secretion phase (Kahn, 2001).

Other studies demonstrate that insulin secretion occurs following two patterns – pulsatile and oscillatory. In healthy individuals, spontaneous pulsatile release of the hormone is observed every 8–10 min (Matthews *et al.*, 1983a,b). It is suggested that pulsations are an intrinsic feature of pancreatic islets of Langerhans, because even when they are isolated, outside the nervous system control, the pulsatile insulin secretion is preserved (Bergstrom *et al.*, 1989). The loss of pulsations is probably important for the appearance of IR and later, of T2DM as the continuous infusion of insulin is accompanied by impaired tissue activity that is not present when the hormone is released in a pulsa-

tile manner (Matthews *et al.*, 1983a,b; Ward *et al.*, 1990).

Two other components related to β-cell function, which are always impaired in T2DM are the biosynthesis of insulin, and a novel β -cell specific peptide called amylin (Kahn, 2001).

Insulin production requires a cleavage of the hormone from its progenitor proinsulin, resulting in release of equimolar amounts of insulin and C-peptide. The process takes place in β-cell secretory granules in response to stimulation (Smeekens & Steiner, 1990; Halban, 1991). It is disturbed in subjects with IGT and T2DM and after β-cell stimulation, the amount of proinsulin molecules is increased up to 5–8% (Ward *et al.*, 1987; Kahn & Halban 1997). The extent of proinsulin molecules increase is proportional to the extent of hyperglycaemia, which means that the insulin/proinsulin ratio is a marker of the degree of β-cell dysfunction (Saad *et al.*, 1990; Rǿder *et al.*, 1998).

The cause for this excessive increase in proinsulin molecules is not yet established. One hypothesis attributes the event to a primary β-cell defect (Porte & Kahn, 1989), whereas another one believes that increased demand for insulin secretion in IR states results in release of immature secretory granules in the period when the conversion of proinsulin to insulin is not yet completed (Rhodes & Alarcon, 1994).

Amylin (islet amyloid polypeptide, IAPP) is a factor involved in the progressive β-cell damage (Höppener *et al.*, 2002). IAPP is produced by β-cells "packed" with insulin into secretory granules (Kahn *et al.*, 1998) and is released together with it in response to glucose or other stimuli (Kahn *et al.*, 1990). It is considered that this peptide is a precursor of amyloid deposits, frequently seen in T2DM patients (Johnson *et al.*, 1989a,b). The deposition of amyloid in islets is a diabetogenic factor, which is both a sequel of IR and a cause of β-cell damage during the progression of IGT to diabetes (Höppener *et al.*, 2002).

It is reported that in cats similarly to men, T2DM is accompanied by amyloid deposition in more than 90% of cases (Höppener *et al.*, 2002). At high levels, amyloid suppresses the secretion of insulin and contributes significantly for worsening of glucose tolerance (Howard, 1986). It is not yet clear whether amylin induces IR initially or impairs directly β-cell function. According to Hoenig *et al.* (2000), the hyperstimulation of β–cells, being a compensatory reaction to IR, leads to amyloidosis. Amyloid replaces the functioning mass of β-cells in a way such that they could not compensate existing IR by increased insulin secretion and as a result, hyperglycaemia occurs.

It is thought that after amylin is secreted, it is accumulated extracellularly near β-cells, exacerbates their function and possibly, causes their death by disturbing the transportation of nutrients from blood plasma to β-cells or by interfering with glucose sensitivity and/or insulin secretion (Johnson *et al.*, 1989a,b; Ohsawa *et al.*, 1989). Although that according to other researchers amylin does not suppress insulin secretion (Bretherton-Watt *et al.*, 1990; Ghatei *et al.*, 1990) more recent studies suggest that the combination of high NEFA concentrations and amylin hypersecretion could together worsen insulin production and damage pancreatic β-cells (Kahn *et al.*, 1999).

It is also shown that glucagon concentrations are considerably increased in obese cats and could play an important role in disease progression, because glucagon increases IR and thus, could exhaust β-cells (Hamaguchi *et al.*, 1991).

The sum effect of the progressively developing β-cell dysfunction is the gradual transition of normal glucose tolerance into abnormal and then, diabetes. The mechanisms responsible for these changes in β–cell function are still subject of extensive research and analysis (Kahn, 2001).

POSSIBLE MECHANISMS INVOLVED IN BETA-CELL DAMAGE

Numerous hypotheses attempt to explain the appearance and development of β-cell dysfunction, namely:

- exhaustion of β-cells consequently to increased demands for insulin secretion in conditions of IR (DeFronzo *et al.*, 1992; Cavaghan, 2000);
- toxic damage of β-cells by prolonged hyperglycaemia (glucotoxicity) (DeFronzo *et al.*, 1992; Yki-Jarvinen, 1992; Robertson *et al.*, 1994; Poitout & Robertson, 2008);
- β-cells damage from dyslipidaemia (increased NEFA and adipokine concentrations), which often accompanies T2DM (lipotoxicity) (DeFronzo *et al.*, 1992; Unger, 1995; Pitout & Robertson, 2008; Perez-Martinez *et al.*, 2010);
- reduction of β-cell mass, probably as a late consequence of amyloid deposition in Langerhans islets (Johnson *et al.*, 1989a,b; Kahn *et al.*, 1999);
- oxidative stress.

It is acknowledged that during the early phases of IR, the secretory function of β-cells increases to overcome the reduced sensitivity of peripheral tissues (muscle, adipose tissue and liver) (Kahn *et al.*, 1993). Some authors differentiate five stages in the development of human T2DT, each of them connected with specific changes in the amount and functional activity of β-cells (Weir & Bonner-Weir, 2004): 1) Stage of compensation, characterised with normal or higher β-cell mass, increased insulin secretion and concentrations compensating for the reduced insulin sensitivity following obesity and/or reduced physical activity, and genetic predisposition. During this stage, fasting blood glucose is near the upper end of the reference range (>5 mmol/L and <5.6 mmol/ L), and the first-stage insulin secretion is enhanced. It is not yet definitively clear whether increased plasma insulin concentrations result from increased β-cell mass or from their enhanced functional activity. 2) Stage of adaptation, during which β-cells could not any longer counteract IR, resp. maintain normal fasting blood glucose levels. Fasting blood glucose increases to about 7.3 mmol/L, along with β-cell mass reduction, shortened first-stage insulin secretion and impaired glucose tolerance. It is thought that first-stage insulin secretion is not altered when blood glucose is up to 5.6 mmol/L, but the further increase in concentrations leads to progressive reduction and after 6.4 mmol/L – cessation of secretion (Weir & Bonner-Weir, 2004). 3) Stage of early decompensation. It is regarded as a transition period of further reduction of β-cell mass and function and elevation of blood glucose. During stages 4 and 5, β-cell function is completely decompensated and β-cell mass – strongly reduced, with appearance of all signs of T2DM, the most important of which is the marked hyperglycaemia (>16–20 mmol/L). Although such criteria have not been established in animals, according to our results rabbits with visceral obesity are at the boundary between stages 1 and 2, as they had normal blood glucose concentrations and IGT, hyperinsulinaemia (except for the suppressed first stage of insulin secretion), higher homeostatic model assessment ($HOMA_{\beta\text{-cell}}$) index and enhanced insulin secretion rate during the first 2

hours after glucose infusion, quantitated by $AUC_{ins\ 0\rightarrow60min}$ and $AUC_{ins\ 60\rightarrow120min}$, respectively (Georgiev *et al.*, 2011; Ivanova *et al.*, unpublished observations).

For as early as possible detection of β-cell damage in men, the so-called "disposition index" reflecting the relationship between insulin sensitivity and acute stage of insulin secretion after IGTT (up to the 5^{th} min after glucose infusion) is used (Bergman *et al.*, 2002). It is shown that under normal conditions, the relationship appears graphically as a hyperbola and the index is a constant variable because the lower insulin sensitivity entails a compensatory enhancement of insulin secretion. Defects in β-cells however make the values of this index lower. So far, in animals, the calculation of this index is not widely used as the procedure for precise determination of insulin sensitivity is very labourous and requires special software (minimal model analysis) (Bergman *et al.*, 2002).

This increased needs for biosynthesis and secretion of insulin resulted in the suggestion that over a more prolonged period of time, the higher demands toward β-cells on the background of IR would lead to their gradual exhaustion and loss of function (DeFronzo *et al.*, 1992).

Research data of several scientists however argue on the primary role of these mechanisms for the onset and development of IGT and T2DM. First, IR is observed in almost all obese subjects but at the same time, only a small proportion of them develop T2DM (Kahn *et al.*, 1993; Rewers & Hamman, 1995). Second, IR with enhanced β-cell function is accompanied by enhanced insulin secretion along with lower proportion of plasma proinsulin (Kahn *et al.*, 1992).

It is believed that the lack of rapid and adequate adaptation to IR could be a consequence of genetically determined disturbances in β-cell function which make impossible the increase in their secretory capacity and then, defects in β-cell function in condition of increased demands become overt (Poitout & Robertson, 2008). On the other side, β-cells of subjects without similar genetic troubles could adapt to compensate IR and prevent the onset of hyperglycaemia, resp. diabetes mellitus.

Glucose not only stimulates β-cells, but could have a negative impact on their function when its concentrations remain high over a prolonged period (Poitout & Robertson, 2008). This effect is known as "glucotoxicity" (Sivitz, 2001). The negative effect of hyperglycaemia is manifested through reduction of the gene expression of insulin and lower rate of insulin secretion in response of typical secretagogues, incl. the glucose itself (Leahy *et al.*, 1992; Poitout & Robertson, 2008). *In vitro* experiments have shown that increased glucose concentrations suppress insulin secretion and the expression of special genes (PDX-1 genes), responsible for the regulation of β-cell replication (Sharma *et al.*, 1999). Newer evidence demonstrate that supraphysiological glucose levels lead to functional disturbances in β-cell endoplasmatic reticulum and to development of oxidative stress, which are significantly involved in their damage (Robertson, 2006; Marchetti *et al.*, 2007; Poitout & Robertson, 2008). It is believed that some molecular mechanisms responsible for the inhibited insulin secretion are associated to degradation of mRNA coding for insulin synthesis and to lower levels of two important transcription factors (MafA and PDX-1) binding insulin gene promoter in β-cells (Robertson, 2006; Robertson & Harmon, 2006; Pirot *et al.*, 2007). It is also demonstrated that hyperglycaemia and the related metabolic troubles enhance the

production of reactive oxygen species (ROS) and free radicals, which could interfere with expression of genes coding for proteins associated with insulin synthesis on the background of low β-cell antioxidant defense (Poitout & Robertson, 2008). Nevertheless it is hypothesised that glucotoxicity is not the only factor for β-cell function loss in subjects during their progress from high risk to fasting hyperglycaemia or even early T2DM (Kahn, 2001).

Another factor involved in the onset of β-cell dysfunction is associated to altered lipid metabolism – the so-called lipotoxicity (Unger, 1995). The high dietary fat content is followed by weaker *in vivo* and *in vitro* insulin secretion (Kaiyala *et al.*, 1999; Mittelman *et al.*, 2000). It is acknowledged that obesity is the commonest cause for reduced insulin sensitivity. When the β-cell function is disturbed, impaired glucose tolerance and/or T2DM occurs (Kaiyala *et al.*, 1999; Mittelman *et al.*, 2000). Regardless of the increasing number of reports, the pathogenetic mechanisms responsible for obesity-induced β-cell dysfunction are not fully elucidated. Obesity is often accompanied by accumulation of intraabdominal fat, which is regarded upon as a metabolically active depot (Bjorntorp, 1996). Some factors produced by intraabdominal adipocytes could play the role of mediators of impaired β-cell function. Among them are nonesterified or free fatty acids (NEFA) whose high levels impair the β-cell function (Zhou & Grill, 1994; 1995; Perez-Martinez *et al.*, 2010). This adverse effect is manifested not only by lower insulin secretion rate, but also by delayed conversion of proinsulin into insulin (Zhou & Grill, 1995).

A number of mechanisms explaining how increased NEFA concentrations could provoke β-cell dysfunction and impaired insulin production have been proposed. One of them is the impaired intracellular metabolism of glucose, in particular its oxidation consequently to the reduced activity of pyruvate dehydrogenase complex, which results in lower rate of pyruvate conversion to acetyl-CoA (Pighin *et al.*, 2003). Another mechanism is the enhanced expression of the transcription factor sterol regulatory element binding protein 1c (SREBP 1c), induced by higher NEFA levels. This factor is known to stimulate hepatic lipogenesis via stimulation of specific lipogenetic enzymes synthesis. According to some research teams however, the activation of SREBP 1c in β-cells also contributes to inhibition of insulin secretion (Takahashi *et al.*, 2005).

It is believed that in order to exert its adverse effect on β-cells, increased fatty acid concentrations should be combined with hyperglycaemia (Briaud *et al.*, 2001). Recently, hyperglycaemia is even considered as a precondition for the negative influence of lipids on β-cell function (Poitout & Robertson, 2008). On the basis of their own and others' data, the two authors proposed to replace the terms "glucotoxicity" and "lipotoxicity" used so far with the collective term "glucolipotoxicity" that defines more precisely the pathogenesis of impaired β-cell function.

Lately, bioactive substances other than fatty acids (adipokines, incl. cytokines etc.) produced by adipose tissue are also considered important for the disturbance of β-cell function in obesity. These are leptin and proinflammatory cytokines TNF-α, IL-6, deemed to act as β-cell function inhibitors (Kulkarni *et al.*, 1997; Poitout *et al.*, 1998; Seufert *et al.*, 1999).

The ectopic lipid deposition in chronically obese subjects outside the adipose depots, including within the pancreas,

influences negatively the β-cell function and insulin secretion via different mechanisms. For instance, the data of Perez-Martinez *et al.* (2010) suggested that the excessive fat accumulation in the pancreas results in their non-efficient oxidation and stimulation of the synthesis of the so-called ceramides, which trigger β-cell apoptosis via increased nitric oxide production. The cascade of lipid-induced programmed cell death is termed "lipoapoptosis". Unlike the cells of other tissues, pancreatic β-cells have a very limited array of compensatory mechanisms to counteract lipoapoptosis and thus, they are extremely sensitive to the adverse effect of excessive lipid deposition in the pancreas in obesity (Perez-Martinez *et al.*, 2010).

Data are reported in support of the significance of reduced β-cell mass for lower insulin secretion rate in obesity and IR states (Clark *et al.*, 2001; Donath & Halban, 2004). Although the causes of β-mass reduction are not yet clear, it is hypothesised that the share of programmed cell death (apoptosis) as a sequel of hyperglycaemia, dyslipidaemia, increased proinflammatory cytokine and tissue hormone production by adipocytes (from the intraabdominal region in particular) is increased (Donath & Halban, 2004; Rhodes, 2005). The deposition of amyloid is a possible alternative mechanism explaining the reduced β-cell mass (Kahn *et al.*, 2000).

During the last years, increasing attention is paid to elucidate the role and significance of oxidative stress for a number of pathologies, including β-cell dysfunction (Pi *et al.*, 2010). The interest of scientists is triggered by recent facts confirming that minimum concentrations of ROS (for example H_2O_2 etc.) are absolutely necessary for many cell signalling pathways (Bashan *et al.*, 2009; Pi *et al.*, 2010). Thus, it is shown that ROS participate in the final stages of insulin signal transduction in target cells (Bashan *et al.*, 2009). A lot of evidence is now available in support of the thesis that ROS formed after glucose conversion act as signalling molecules for glucose-stimulated insulin secretion of pancreatic β-cells (Pi *et al.*, 2010). Of course, this beneficial effect is exerted by smallest ROS concentrations. When the disequilibrium between enhanced ROS production and reduced antioxidant defense in several states, including obesity is especially marked, ROS levels increase substantially and influence adversely the metabolism of carbohydrates, lipids, proteins, nucleic acid etc. At the same time, experimental data demonstrate that the levels of one of endogenous antioxidant enzymes – superoxide dismutase in β-cells are half the levels in the liver, while the expression of hydrogen peroxide inactivating enzymes (glutathione peroxidase and catalase) – only 1% of respective expression in the liver (Lenzen *et al.* 1996; Tiedge *et al.*, 1997; Pi *et al.*, 2010). The data confirm unquestionably that antioxidant defense mechanisms of β-cells are at a very low level which makes them rather vulnerable to the influence of higher ROS concentrations in disease states, including obesity.

The results of our research team established that the exogenous application of a combination of two antioxidants (vitamin E and d-limonene) in rabbits with experimentally induced obesity resulted in a certain normalisation of insulin sensitivity and β-cell function (Georgiev *et al.*, 2011; Ivanova *et al.*, unpublished observations). The observed effect remains however disputable in the light of the suggestions that stimulated endogenous antioxidant defense could block glucose-stimulated insulin secretion (Pi *et al.*, 2010).

The facts reviewed so far make clear that many factors inducing IR in obese subjects, provoke also impaired insulin secretion. It should be also emphasised that very often, these disturbances are manifested on the background of an existing genetic predisposition to β-cell damage.

It is believed that the risk of T2DM development is many times higher when a genetic predisposition to β-cell damage does exist (Poitout & Robertson, 2008). The consumption of high-fat food from subjects with genetic predisposition results in onset of β-cell dysfunction. The latter is further associated with the appearance of amylin-containing amyloid fibrils (IAPP). The progressive amyloid deposition reduces β-cell mass, followed by lower insulin secretion rate and aggravation of hyperglycaemia. From its part, glucotoxicity could worsen β-cell function via desensibilisation of cells to glucose.

The intake of glucose via the gastrointestinal tract has a potentiating effect on insulin secretion (Holst & Gromada, 2004). In such conditions, the insulin response of β-cells is almost twice more potent vs the same response after intravenous administration, despite the equivalent increase of blood glucose levels. This potentiating effect is due to the release of the so-called incretins by gastrointestinal endocrine cells – glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) etc. – which stimulate β-cell insulin secretion (Holst & Gromada, 2004). Thus, a deficiency of these incretins and/or the presence of "incretin" resistance could be involved in the pathogenesis of β-cell dysfunction (Drucker, 2001; Gang *et al.*, 2007). For instance, antibodies neutralising GIP and GLP-1 disturb glucose tolerance in different animal species, including primates (D'Alessio *et al.*, 1996). Although the amount of secreted GLP-1 is significantly lower compared to GIP, GLP-1 is a more potent stimulus for insulin secretion and is considered as the primary "incretin" (Drucker, 2001).

In conclusion, all data cited in the present review indicate that the progression of impaired glucose tolerance to overt diabetes requires the presence of β-cell dysfunction, resp. impaired insulin secretion. Notwithstanding the uncertain issues related to mechanisms of β-cell dysfunction, it could be affirmed that many factors inducing IR (obesity, increased concentrations of non-esterified fatty acids, some adipokines, free radicals etc.) impair β-cell function. The increased amyloid deposition in the pancreas is also an essential element of pathogenesis of impaired insulin synthesis. The currently prevailing opinion is that while IR results rather from the adverse environmental factors, β-cell damage is genetically determined. Thus, the risk of developing T2DM increases substantially when genetic predisposition to β-cell damage is combined with unfavourable extrinsic factors leading to insulin resistance.

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Paper received 27.02.2013; accepted for publication 10.05.2013

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