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Cellular glucose transport disturbances in the pathogenesis and therapy of type 2 diabetes mellitus

Znaczenie zaburzeń dokomórkowego transportu glukozy w patogenezie i terapii cukrzycy typu 2

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Abstract

The current world epidemic of type 2 diabetes mellitus results from two general groups of causative factors. One is the influence of strong pathogenetic environmental pressures — also described as negative civilizational influence — on the very large subpopulation, assessed at 30% of the total world population, which is genetically predisposed to react to this external stress with the symptoms of type 2 diabetes mellitus. Such a pathogenetic reaction is based on the appearance of cellular and organ resistance to insulin. A second factor involves the beta cells of the pancreatic islets and their dysfunction.

For these reasons, studies on the aetiology of insulin resistance have significance, both theoretical and practical. There are many biological deviations that can produce cellular insulin resistance and underutilization of glucose. The mechanism that is always present is the decrease of cellular glucose transport. For this reason, it should be approached as a potential target for preventive and therapeutic actions.

These pathophysiological and clinical circumstances were the motivation for presenting a review of cellular glucose transport pathophysiology, which contributes to the aetiology of insulin resistance, cellular underutilization of glucose, and type 2 diabetes mellitus. They underline the significance of cellular glucose transport as a target for prevention and therapy of type 2 diabetes mellitus and other insulin-resistant conditions.

This review presents comments about the influence on cellular glucose transport of diet, physical exercise, and pharmacotherapeutic agents, based on the authors' studies. The review could contribute to an innovative approach to the pathogenesis, prevention, and therapy of type 2 diabetes mellitus and other conditions related to insulin resistance. **(Pol J Endocrinol 2010; 61 (3): 292–302)**

Key words: *insulin resistance, cellular glucose transport, type 2 diabetes mellitus pathogenesis and therapy*

Streszczenie

Epidemia cukrzycy typu 2 na świecie wynika z dwóch głównych grup czynników przyczynowych. Jedną z nich jest wpływ czynników środowiskowych, opisywany również jako negatywny wpływ cywilizacji. Szacuje się, że około 30% całkowitej populacji na świecie ma genetyczną predyspozycję do ujawnienia objawów cukrzycy typu 2 pod wpływem czynników zewnętrznych. Zjawisko to jest związane z komórkową i narządową opornością na insulinę. Druga grupa czynników jest związana z dysfunkcją komórek β trzustki.

W związku z powyższym badania etiologii insulinooporności mają znaczenie zarówno teoretyczne, jak i praktyczne. Mechanizmem, który prowadzi do wielu zaburzeń metabolicznych, a przede wszystkim do insulinooporności jest spadek dokomórkowego transportu glukozy. Z tego powodu dokomórkowy transport glukozy powinien być uważany jako potencjalny cel postępowania prewencyjnego i terapeutycznego.

W opracowaniu przedstawiono postępy badań nad dokomórkowym transportem glukozy w aspekcie patofizjologii insulinooporności i cukrzycy typu 2. Najnowsze doniesienia naukowe podkreślają kluczową rolę dokomórkowego transportu glukozy jako celu prewencji i terapii cukrzycy typu 2. W artykule opisano obecny stan wiedzy na temat wpływu diety, wysiłku fizycznego oraz terapii farmakologicznej na dokomórkowy transport glukozy. **(Endokrynol Pol 2010; 61 (3): 292–302)**

Słowa kluczowe: *insulinooporność, dokomórkowy transport glukozy, cukrzyca typu 2 — patogeneza i terapia*

Introduction

A bilayer lipid cell membrane is impermeable to monosaccharides, and therefore glucose needs a biological transport system consisting of specialized transport proteins. Those proteins provide physiological harmony of glucose cell supply and its further metabolism.

Cellular glucose metabolism is a complex, multistage process. Individual process stages are coordinated by specialized regulatory systems. In physiological conditions, a quantitative coordination exists between the

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activity of the molecular intracellular glucose transport system (CGT) and intermediate glucose plasma metabolism. Insulin belongs to the most important regulators of this relationship [1–3].

In diabetes mellitus, this coordination becomes impaired. Tissue glucose utilization is diminished despite the existence of hyperglycaemia. The primary reason for this impairment is the coexistence of insulin deficiency and insulin resistance [4, 5]. Both of these pathogenic disturbances cause a shortage of insulin regulatory action on the cells and decrease cellular glucose transport and the utilization of glucose. This always involves abnormalities in the insulin signalling pathway [6–10].

Insulin resistance — the role in the aetiology of clinical syndromes

The physiological relationship between glycaemia and serum insulin concentration has the character of a linear variable. The level and the course of the relationship between glycaemia and cellular glucose utilization is individually different but is kept within a relatively narrow physiological range in healthy persons. The dependence between glycaemia and insulinaemia, when shifted towards higher levels, is diagnostic for insulin resistance [11, 12].

The list of the clinical syndromes and disturbances with insulin resistant hyperglycaemia is long [13].

Examples are listed below:

— obesity, particularly visceral, disturbances in adiponectin and other cytokines secretion by adipocytes;
— hyperglycaemia coexisting with hyperinsulinaemia in type 2 diabetes mellitus;
— dyslipidaemia, mostly mixed with the elevation of "small dense" LDL of triglycerides and the decrease of HDL, non-alcoholic fatty liver;
— arterial hypertension, particularly associated with obesity;
— hypercoagulability with PAI-I and fibrinogen elevation;
— atherogenic disturbances in endothelial cell function;
— microalbuminuria;
— polycystic ovary syndrome;
— states with an increase of CRP and of low-grade inflammatory markers.

All of above clinical conditions constitute an indication for diagnostic tests permitting a quantitative assessment of insulin resistance using metabolic clamp or mathematical methods of assessing the resistance to insulin in an indirect way.

A diagnosis of insulin resistance consequently means an indication for therapy including modification of lifestyle and the use of insulin sensitizers such as biguanides, tiazolidinediones, acarbose, incretins, or incretin enhancers [14, 15].

Cellular glucose transport and insulin signalling pathway — new areas of aetiological research in diabetes mellitus

The medical practitioner is mainly interested in the achievements of the basic biological sciences that lead to a better understanding of the disease and create more efficient methods of diagnosis, prevention, and therapy. The elucidation of biochemical and molecular mechanisms of insulin resistance may lead to more effective practice. Due to epidemiological pressures, this is also the task of primary care physicians.

Insulin resistance can be caused by structural or functional changes in any of the insulin regulatory actions on intracellular signalling mediator molecules, from the insulin receptor to the genes [17]. The defect of glucose utilization may therefore be induced in any phase of the signalling process: from the binding of insulin to its receptor, through the activity of GLUT transporters and of proteins responsible for the translocation of these transporters to the cellular membrane, to the structural or functional abnormalities of other signalling molecules, and transcriptional factors on the route of the insulin signal to genes.

Disorders at the level of insulin receptor have been thoroughly examined, especially the mutations disturbing its function as a tyrosine phosphatase. The effects of such mutations can be seen in special forms of type 2 diabetes mellitus that are accompanied by this type of insulin resistance.

The utilization of glucose by the muscles and adipose tissue can be impaired by many other disturbances in the signalling processes. In patients with type 2 diabetes, different abnormalities concerning the signal transduction cascade below the insulin receptor have already been found. For example, in patients with insulin resistance (not necessarily with type 2 diabetes), impaired IRS 1 phosphorylation and impaired PI-3 kinase activation in skeletal muscles are frequently encountered [18, 19]. In the adipose tissue of patients with type 2 diabetes mellitus and obesity, a decrease in glucose transport is connected with changes in the expression of the GLUT4 protein. In such patients, up to 80–90% of GLUT4 function in adipocytes could be lost. These phenomena result from impaired insulin sensitivity in the peripheral tissues, mainly in striated muscles, which are responsible for 70–80% of insulin-stimulated glucose utilization, and in adipose tissue, which is responsible for 5–20% of the insulin resistant glucose underutilization. It should be emphasized that cellular glucose metabolic underutilization is always connect-

Table I. *Clinical significance of studies on GLUT4 activity [1–3]*
Tabela I. *Kliniczne znaczenie badań nad aktywnoscią GLUT4 [1–3]*

Metabolic state — clinical syndrome	Adipocytes	Myocytes
	GLUT4 transport protein	GLUT4 transport protein
Obesity	↓ ↓	↓ →
Diabetes mellitus type 2	↓ ↓ ↓ ↓	↓ ↓ →
Diabetes mellitus type 1	↓ –	↓ →
Physical exercise		↑ ↑
Metabolic syndrome Pregnancy Liver cirrhosis Hyperthyroidism Turner Syndrome	Insulin resistance related to a significant decrease of cellular glucose transport	

ed with impairment of cellular glucose transport. In this way, a decrease of the glucose transport contributes to hyperglycaemia.

The "transport" hyperglycaemia in diabetes mellitus may arise in two ways: 1) as a result of a decrease in the GLUT transporters' expression and concentration, or 2) as an effect of functional disturbances in the process of the GLUT transporters' intercellular translocation.

Studies of the impairment of cellular glucose transport in diabetes mellitus, particularly in type 2, should now be seen as a new potential sphere of clinical activity (Table I) [6–9].

In abdominal obesity — after normal body mass is restored the insulin receptor regains its lost tyrosine phosphatase activity. Disorders of the expression and translocation of glucose transporter GLUT4 could be normalized and insulin sensitivity restored.

GLUT4 expression and pre-diabetic state

Insulin resistance is more frequent in the offspring of diabetic parents. This is shown in Figure 1.

In our own studies, GLUT4 protein expression was observed in peripheral blood lymphocytes in subjects with pre-diabetes. The aim of the study was to compare GLUT4 quantitative expression in lymphocytes in a type 2 diabetes mellitus risk group pre-diabetes with healthy subjects. The study groups included 1) 15 pre-diabetic subjects and 2) 15 persons with normal glucose tolerance and a positive family history of type 2 diabetes mellitus (first-degree relatives). As a control group, 15 healthy persons with no family history of diabetes mellitus were enrolled.

The lymphocytes demonstrating expression of GLUT4 were labelled with the use of indirect immunofluorescence. The quantitative determination of GLUT4 was performed by flow cytometry. In the control group, GLUT4 expression was on the level of 12% ± 1.5% and was significantly lower when compared with both pre-diabetic subjects (18.2% ± 8.8%) and the positive family history group (17.9% ± 9%). GLUT4 overexpression in subjects with a positive family history of type 2 diabetes mellitus suggests cellular glucose transport disturbances prior to hyperglycaemia (20). Determination of GLUT4 expression, therefore, appears to be a possibly useful method of early detection in individuals at high risk of diabetes mellitus type 2.

Cellular glucose transport as a therapeutic target in type 2 diabetes mellitus

Diet and the function of GLUT4

It has been observed that insulin resistance caused by a high-fat, high-sugar diet is the result of a direct decrease of the ability of insulin to activate GLUT4 in muscles. GLUT4 overexpression in muscles (physical training) can successfully prevent the occurrence of hyperglycaemia in these experimental conditions.

The process of increasing body mass by overnutrition is regularly accompanied by a decrease of peripheral cell sensitivity to insulin and, at the same time, by cellular glucose transport efficiency. These phenomena can be reversed by body mass normalization [21].

Skeletal muscle function, muscle training, and cellular glucose transport

A deficit of GLUT4 in the muscles of experimental animals induces moderate hyperglycaemia and diabetes mellitus. Insufficient utilization of glucose in skeletal muscles is accompanied by GLUT4 translocation disorders such as docking or fusion of sacs containing glucose transporters in the cellular membrane or T channels. It is measured as a change of specific activity of glucotransporters expressed as the amount of glucose transported per glucotransporter concentration per time

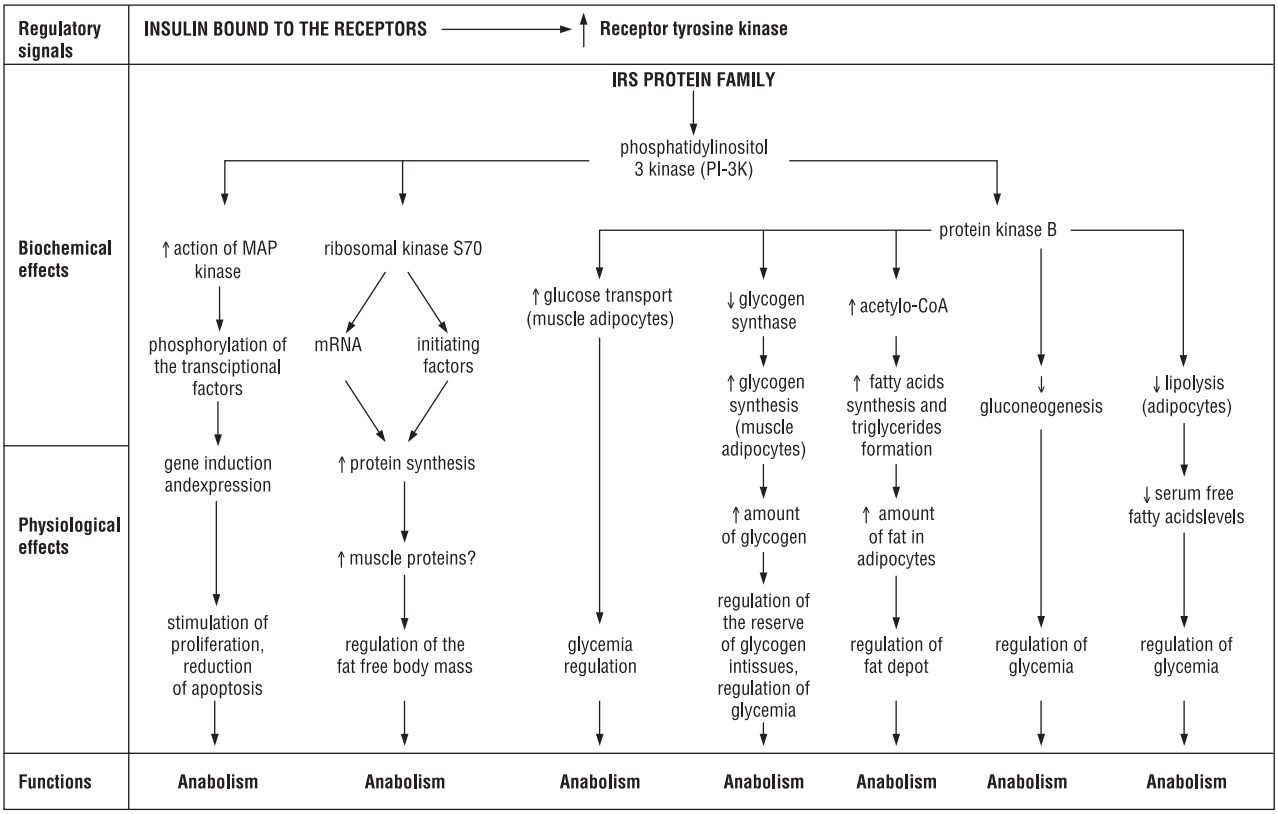


Figure 1. *The influence of insulin actions on intracellular metabolic processes. The biochemical reactions and physiological effects are successively harmonized and integrated [12]*

Rycina 1. *Wpływ aktywności insuliny na wewnątrzkomórkowe procesy metaboliczne. Reakcje biochemiczne i skutki fizjologiczne są skutecznie zharmonizowane i zintegrowane*

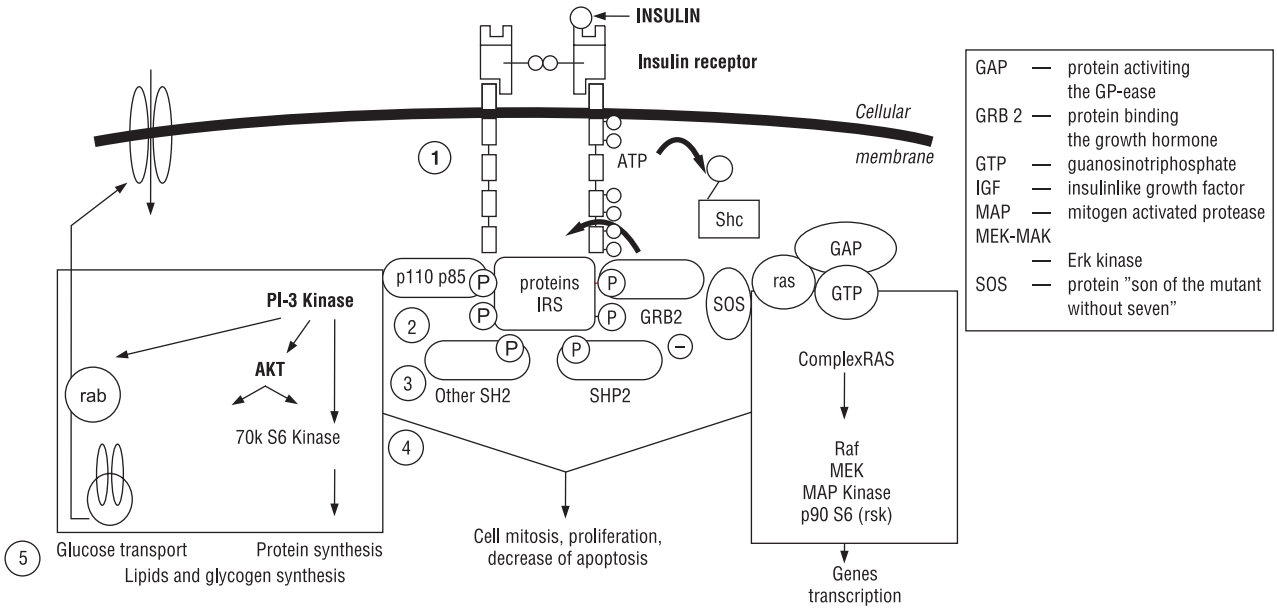
unit. It is suggested that the cause of these disorders is abnormal insulin signal transduction inside the cell, which primarily is related to improper activation of phosphatidylinositol 3-kinase. It is often, at least partially, the result of decreased expression of the p85 molecule responsible for the functional regulation of this kinase. Insulin-stimulated phosphatidylinositol 3-kinase function is impaired in insulin-resistant skeletal muscles. This has been found in experimental studies on animal models (rats with inherited obesity and hyperglycaemia coexisting with hyperinsulinaemia) and in obese patients with type 2 diabetes mellitus. Another cause of these abnormalities can be the storage of GLUT4 molecules in insulin-unresponsive compartments.

New research techniques like nuclear magnetic resonance spectroscopy have enriched the knowledge concerning the biochemical basis of molecular defects in the metabolism of skeletal muscles existing in type 2 diabetes mellitus. For example, it was shown with these methods that insulin resistance is often associated with a decrease of insulin-related glycogen biosynthesis in the skeletal muscle. This phenomenon is related to the impairment of the activity of GLUT4 and, respectively, cellular glucose transport (Fig. 2, Table II) [20, 21].

In the muscle and also in the liver, resistance to insulin can be caused by the inhibition of the phosphorylation of the insulin action signals by the metabolites of the lipid metabolism, as, for example, diacylglycerol (DAG) and acyl derivatives of fatty acids. It is clearly due to the inhibition by fat of the function of IRS proteins. This subsequently results in a decrease of PI -3K and cellular glucose transport. Disturbances of this kind are observed particularly in humans and in mice with lipodystrophy. In obesity, the decrease of adipose tissue mass reduces the accumulation of lipid metabolites in the myocytes and hepatocytes. At the same time, an increase of the sensitivity to insulin is observed. It takes place even without changing the concentration of the circulation in the blood of cytokines like interleukin 6, resistin, or leptin (Fig. 3).

The increased cellular accumulation of the lipid metabolites in persons without obesity and insulin resistance may be related to the genetic regulation of mitochondrial activities [1–3].

Many observations were made on the direct relationship between the insulin action and the functional efficiency of cellular glucose transport and whole body glucose homeostasis [3, 5]. The expression of GLUT4 in adipocytes of persons with type 2 diabetes mellitus is often signifi-



The whole signalling pathway could be divided into 5 levels:
1. Association of insulin molecule to the alpha subunit of the receptor and auto-phosphorylation (activation) if the tyrosine residues in the beta subunit of the receptor
2. Phosphorylation of the IRS proteins family
3. Action of the signals downstream on the mediating signals molecules SHP2 (sre homology 2) and on other mediators domaines recognizing the signals
4. Activation of serine and lipid kinases
5. Regulation of the final outcomes of the signalling on genes expression: cellular glucose transport, glycogen and lipids synthesis, mitogenesis

Figure 2. *Insulin signalling pathways. The signal of insulin association with the receptor stimulates insulin regulatory actions at the beginning by its interaction with insulin receptor substrate (IRS) proteins. Phosphorylation of IRS proteins results in a number of downstream effects. IRS-1 and IRS-2 have different functions. While IRS-1 has a predominant role in cell growth and insulin action in muscle and adipose tissue, the effects of IRS-2 influence the β-cell and liver, and also brain growth, reproduction and food intake*

Rycina 2. *Szlaki przekazu sygnału dla insuliny. Sygnał połączenia insuliny z receptorem stymuluje aktywność regulatorową insuliny początkowo przez oddziaływanie z białkami — substratem receptora insulinowego (IRS). Fosforylacja białek IRS powoduje liczne efekty; IRS-1 i IRS-2 charakteryzują odmienne funkcje, podczas gdy IRS-1 odgrywa głównie rolę w pobudzaniu wzrostu komórkowego i oddziaływaniu insuliny w mięśniach i tkance tłuszczowej, IRS-2 działa w komórkach β i wątrobie, a także wpływa na wzrost mózgu, reprodukcję i przyjmowania pożywienia*

Table II. ***Striated muscle and adipose tissue GLUT4 action in diabetes mellitus type 2 and obesity in human***

Tabela II. ***Działanie GLUT4 w mięśniach poprzecznie prążkowanych i tkance tłuszczowej u chorych na cukrzycę typu 2 i osób otyłych***

• The level of expression of GLUT4 in striated muscle in persons with diabetes mellitus type 1, 2 and with obesity is not significantly changed. However, cellular glucose transport is diminished. It could be caused by disturbances in the function of the molecules translocating GLUT4 to the cell membrane
• The muscle cellular glucose transport is significantly augmented by muscular work. Obesity significantly decreases the expression of GLUT4 in adipocytes. This disturbance is much more visible in persons with obesity and diabetes mellitus type 2. A similar perturbation was noted in women with gestational diabetes mellitus

cantly diminished [4–6]. At the same time, the level of GLUT4 in skeletal muscle may be normal. Insulin resistance in the striated muscle, therefore, may be related not only to the amount of GLUT4 but also to the disturbances in its translocation. The abnormalities may exist in the action of the mediator molecules signalling the insulin action on the translocation of GLUT4, which causes the absence of GLUT4 on the surface of cells. It was stated that the concentration of GLUT4 is increased in exercised muscle.

Holten et al. studied the influence of physical activity on insulin-mediated glucose uptake by GLUT4 and insulin signalling in the skeletal muscle of persons with type 2 diabetes mellitus. Aerobic training increased sensitivity to insulin in such patients [19]. In our own studies, the peripheral blood lymphocytes, as was established in the group of non-treated patients with type 2 diabetes mellitus, had increased expression of GLUT4.

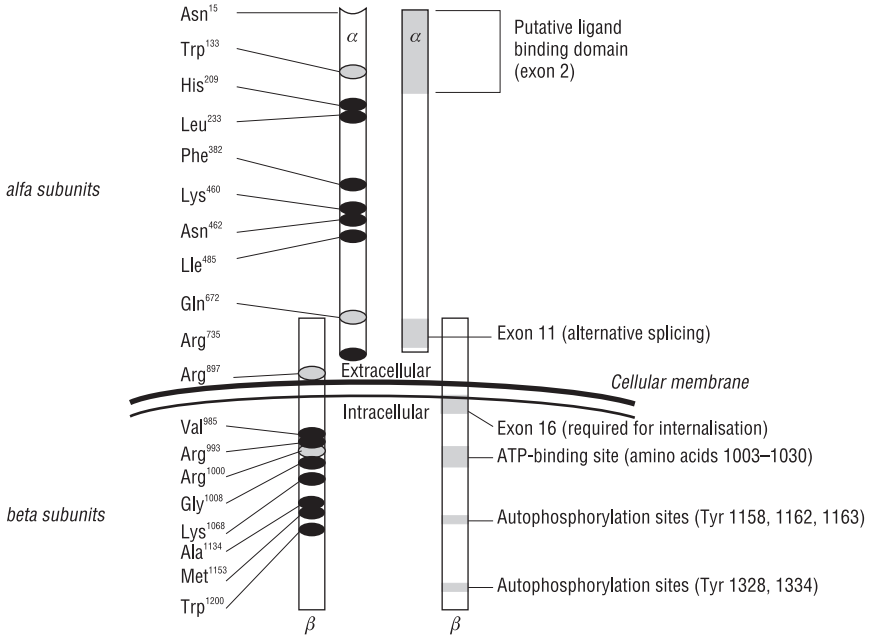


Figure 3. *Structure of the human insulin receptor with several known point mutations [5–8]*
Rycina 3. *Budowa ludzkiego receptora insulinowego z kilkoma znanymi mutacjami punktowymi [5–8]*

Cellular glucose transport and pharmacotherapy

Drugs which influence the activity of cellular glucose transport are mainly:
— insulin,
— sulphonylurea derivatives,
— metformin,
— thiazolidinediones,
— incretins,
— inhibitors of angiotensin-converting enzyme
— inhibitors of renal sodium glucose transport.

Insulin therapy

Insulin action primarily consists of three parallel pathways: 1) increase in cellular glucose transport; 2) stimulation of glucose utilization; and 3) enhancement of proliferative and antiapoptotic processes.

The state of knowledge of insulin influence on cellular glucose transport has been presented abundantly in literature [22–24].

Beta-cytotropic sulphonylurea derivatives and cellular glucose transport

Hypoglycaemic sulphonylureas exert an influence on cellular glucose transport [25–27]. In our studies, CGT was studied before and after gliclazide therapy in a group of type 2 diabetic patients who had previously not received any pharmacotherapy and, comparatively, in a group of healthy (control) subjects [2]. The study can be summarized as presented below [28].

Background and aims

Impairment of CGT is involved in the pathogenesis of diabetic hyperglycaemia and may therefore be regarded as a target for the action of antidiabetic drugs. Studies devoted to this process may create new pharmacotherapeutic possibilities and interpretations. In order to explore this hypothesis, CGT was studied before and after gliclazide therapy in a group of type 2 diabetic patients who had previously not received any pharmacotherapy, and in a group of healthy subjects.

Material and methods

The CGT of peripheral blood lymphocytes (PBLs) was assessed by timed incubation of cells with 2-[^{3}H(G)] glucose in basal conditions, and after the addition of gliclazide (in substantia) or gliclazide plus insulin. Incubation tests were performed at baseline in 28 newly diagnosed type 2 diabetics and 20 control subjects; in the diabetic patients, the tests were repeated after three months of therapy with gliclazide. PBLs were separated from whole blood by Ficoll-Isopaque gradient centrifugation. Incubation times were 15, 30, and 60 minutes. The scintillation of PBL lysate was measured with the Wallac 1450 MicroBeta counter.

Results

Treatment with gliclazide resulted in a statistically significant reduction in fasting plasma glucose of 1.77 mmol/L ($p < 0.001$) and in HbA_{1c} of 0.69% ($p < 0.001$). The magnitude of CGT in PBLs (pg/300 000 cells) in

Table III. *Cellular glucose transport (CGT) in pg of glucose per 300,000 of lymphocytes from the peripheral blood (PBL) of type 2 diabetic patients: a — incubation medium only, b — gliclazide added to the incubation medium and c — gliclazide and insulin (2 μj/mL) added to the incubation medium. CGT before therapy was significantly decreased, after 12 weeks of effective therapy with diet, and gliclazide was significantly higher (authors' own experiments)*

Tabela III. *Dokomórkowy transport glukozy (CGT) w pg na 300 000 limfocytów krwi obwodowej (PBL) chorych na cukrzycę typu 2: a — tylko medium, b — medium z dodatkiem gliklazydu, c — medium z dodatkiem gliklazydu i insulin (2 μj/ml). CGT przed terapią był istotnie obniżony, po 12 tygodniach leczenia dietą i gliklazydem zaobserwowano jego istotne zwiększenie (badanie własne autorów)*

Incubation model	Incubation time (min)	CGT intensity glucose transport in pg (300 000 lymphocytes)								
		Control subjects			CGT–PBL Type 2 diabetic persons before therapy			CGT–PBL Type 2 diabetic persons after 12 weeks of therapy with		
		Mean	SD	p	Mean	SD	p	Mean	SD	p
Lymphocytes, no	15	123.1	25.7		106.4	25.3		162.4	34.6	
drugs added — a	30	228.7	58.8		179.4	32.3		308.1	57.5	
	60	442.8	137.8		263.1	47.8		543.6	62.1	
Lymphocytes, gliclazide	15	203.6	49.1	< 0.001*	113.5	25.8	0.118*	210.3	36.7	< 0.001*
in substantia added — b	30	417.1	107.1	< 0.001*	199.0	36.3	< 0.001*	412.0	97.3	< 0.001*
	60	664.1	186.8	< 0.001*	316.2	57.8	< 0.001*	727.2	147.5	< 0.001*
Lymphocytes, gliclazide	15	308.8	87.2	< 0.001**	186.4	28.4	< 0.001**	349.3	54.5	< 0.001*
in substantia and insulin	30	647.7	181.5	< 0.001**	293.3	56.7	< 0.001**	689.6	145.2	< 0.001*
added — c	60	666.7	186.4	0.469**	306.0	57.1	< 0.001**	709.7	150.0	< 0.001*

* *v.* sample 1

** *v.* sample 2

healthy subjects and in type 2 diabetics before and after 12-week therapy with gliclazide (mean dose at the end of the study, 39.6 mg) is presented in the table. In all samples of PBLs, a significant increase was found in CGT as a result of treatment with gliclazide (Table III). All p values < 0.001.

Interpretation

When studied in the PBL model, CGT was found to be significantly decreased in type 2 diabetes mellitus. It was partially and significantly increased by the addition of gliclazide to the incubation probe, and it was further increased by the addition of insulin to gliclazide. The influence of gliclazide on the CGT process constitutes a pleiotropic action of this drug and underlines the importance of CGT as a target of pharmacotherapy in type 2 diabetes mellitus.

Hypoglycaemic therapy also induced significant changes in GLUT4 expression, as observed in our study in peripheral blood lymphocytes [28] (Fig. 4).

Metformin

This biguanide normalizes the impairment of GLUT4 translocation in adipocytes as examined in vitro. Administration of metformin to fa/fa Zucker rats increases cellular glucose transport. GLUT4 and GLUT l translocation to cellular membranes, and the transporter molecule activity.

In experimental conditions, administration of metformin increases the glucose transport without a change in the level of GLUT4 in rat muscles. This effect of metformin is ascribed to the influence of metformin on GLUT1.

In humans, metformin intensifies glucose absorption by striated muscle cells and increases cellular glucose utilization in type 2 diabetes mellitus, which is associated with a decrease in insulin resistance. Many studies suggest that the effect of metformin is the result of AMP kinase activation. It is connected with the phosphorylation of threonine in position 172 of the alpha subunit of the AMP kinase. It seems that metformin does not influence insulin signalling by the PI 3-kinase pathway.

Galuska et al. examined the effect of metformin on insulin action regulating cellular glucose transport in isolated, striated muscle specimens obtained from patients with type 2 diabetes mellitus [29]. Metformin lowered hyperglycaemia by increasing tissue sensitivity to insulin and glucose utilization, particularly in the striated muscle. It was shown that the transport of the glucose analog 3-0-methyloglucose, as determined in the biopsy muscle specimens, from the persons with type 2

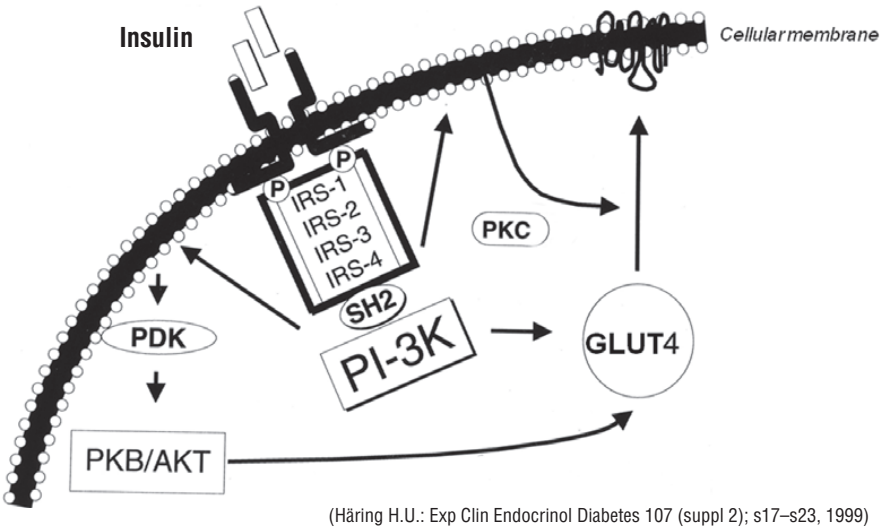


Figure 4. *Glucose transport stimulation by insulin. Molecular mechanisms of insulin-stimulated transport.The insulin-dependent glucose transporter 4 (GLUT4) is translocated by a phosphatidylinositol3-kinase (PI-3K) - dependent pathway including PKB/AKT and PKC stimulation downstream of PI-3K. PDK — phosphatidylinositol (3, 4, 5) — phosphate-dependent kinase; IRS, insulin receptor substrate.*

Rycina 4. *Stymulacja transportu glukozy przez insulinę. Mechanizmy komórkowe transportu stymulowanego przez insulinę. Insulinozależny transporter glukozy 4 (GLUT4) zostaje przemieszczony przez szlaki przekazu zależne od PI-3K (kinazy fosfatydyloinozytolu 3), w tym stymulację PKB/AKT i PCK przez PI-3K. PDK — fosfatydyloinozytolo (3, 4, 5)-fosforo-zależna kinaza; IRS — substrat receptora insuliny*

diabetes mellitus was not changed by the metformin in the concentration 0.01–0.1 mmol/L. For the increase of the 3-0-methyloglucose transport in some of the examined persons, a metformin concentration of 0.1 mmol/L was required. This concentration is higher than that routinely used in clinical therapy with metformin.

However, in other studies it was found that metformin in a concentration of 0.06 mmol/L increases the process of GLUT1 and GLUT4 translocation from the cytoplasm to the cellular membrane of adipocytes. Such effects are dose-dependent. In conclusion, it can be stated that the action of metformin on glucose transport may have different mechanisms. Besides the effect on cellular glucose transport, metformin may reduce insulin resistance in many other ways; for example, by the increase of the activity of the hexokinase and of the synthase of glycogen, the efficiency of glucose oxidation and glycolysis.

A typical example of the influence of metformin on the GLUT4 expression in patients with type 2 diabetes mellitus was observed in our own studies

Clinical observation — case description — **metformin therapy and GLUT4 expression in type 2 diabetes mellitus**. Male, 50 years of age, administration clerk. *Clinical data:* diabetes mellitus type 2, no clinical symptoms of metabolic decompensation, BMI — 35.3 kg/m^2, BP — 150/100 mm Hg, HbA_{1c} — 9.1%, glycaemia profile: 124—242 mg/dL, C-peptide, fasting — 2.86 ng/ml, cholesterol total — 262 mg/dL, LDL-cholesterol — 144 mg/dL, HDL--cholesterol — 46 mg/dL, triglycerides — 164 mg/dL.

Therapy: diet 1,500 kcal/244, metformin — 3 × 500 mg, simvastatin — 20 mg, perindopril — 5 mg.

Observations of the effects of therapy are presented in table 4. Metformin improved sensitivity to insulin (HOMA) and at the same time decreased GLUT4 expression (Table IV).

Thiazolidinediones

These medicines stimulate the activity of peroxisome proliferator-activated receptors α (PPAR-α), and increase the cellular absorption and utilization of glucose in patients with insulin resistance [30].

Administration of thiazolidinediones in insulin-resistant rats normalizes the GLUT4 translocation in their adipocytes. It has a similar influence on insulin resistance caused by administration of TNF-α. With the use of NMR, it has been shown that thiazolidinediones improve the cellular glucose transport in type 2 diabetes mellitus with insulin resistance. In such patients, thiazolidinediones intensified the stimulation of the PI 3-kinase by insulin and the activity of Akt by their positive effect on glucose transport both in muscles and in adipocytes. They increase GLUT4 translocation in cells. In muscles, this effect is only slight.

Table IV. *Case of diabetes mellitus type 2 (male, 56 years of age, BMI — 31 kg/m²) — comparison of glycaemia, insulinaemia, HOMA, and GLUT4 expression in peripheral blood lymphocytes before and after metformin therapy (see also the text)*

Tabela IV. *Chory na cukrzycę typu 2 (mężczyzna, 56 lat, BMI — 31 kg/m²) — porównanie wartości glikemii, insulinemii, HOMA i ekspresji GLUT4 w limfocytach krwi obwodowej przed i po terapii metforminą (patrz tekst)*

Glycaemia [mg/dL]	Insulin in peripheral blood [μj/mL]		Therapy — 3 months of metformin 3 × 500 mg	Glycaemia [mg/dL]	Insulin in peripheral blood [μj/mL]	
124	Fasting	28.0		98	Fasting	13.7
248 — 1 h	After meal (breakfast)	96.2		162	After meal (breakfast)	58.2
210 — 2 h		110.5		139		46.1
182 — 3 h		82.4		100		32.2
HOMA 4.5				HOMA 3.0		
GLUT4 expression in lymphocytes 16.2%				GLUT4 expression in lymphocytes 2.8%		

Incretin-mimetics and incretin-enhancers

The mucous membrane of the digestive tract, after coming into contact with products of food digestion, secretes about 30 hormones. A special place among these hormones is held by glucagon-like peptide l (GLP-1) and glycaemia-dependent gastric inhibitory polypeptide (GIP). Receptors of these peptides are located in β-cells and in the lungs, brain, liver, skeletal muscles, and kidneys. They have a stimulating influence, especially on postprandial biosynthesis and insulin secretion (about 50% of this reaction) [15]. In this way, they decrease hyperglycaemia in type 2 diabetes mellitus. Moreover, they exhibit a lot of other systemic effects such as a decrease of glucagon secretion, an increase of peripheral cells glucose utilization, a decrease of the gluconeogenesis and glucose production in the liver, stimulation of satiety, and an increase of stomach contractions. Therefore, GLP-l also regulates glycaemia, independently of insulin. Such an influence by GLP l is connected with its action on cellular glucose transport. It has been experimentally proven that GLP-l stimulates the expression of specific beta-cell genes. This stimulation involves not only the insulin gene, but also the GLUT l glucotransporter gene and the glucose phosphorylating enzyme gene of hexokinase-1. GLP-1 regulates the transcription of the GLUT l gene and hexokinase-l gene, as well as stabilizing insulin mRNA.

In studies on aging Wistar rats, it has been shown that GLP-l increases their glycaemia dependently on insulin secretion and normalizes the impairment of glucose tolerance related to age. It was connected with an increase in levels of mRNA for insulin, GLUT 2 molecules, and glucokinase of beta cells. The use of the GLP-l agonists gave similar results.

One of the important mechanisms increasing the concentration and action of GLP-l and GIP is inhibition of the activity of the enzyme that decomposes these hormones — dipeptidyl peptidase-4 (DPP-4). There are other substances of this kind which have also been offered in the form of incretin enhancers.

DPP-4 inhibitors have a positive influence on the size of the pancreatic islet mass and on the biological vitality of β cells — their morphology, their lifespan, and their resistance to apoptosis. They can also inhibit the decomposition of other biologically important peptides (*e.g.* aprotinin, bradykinin, endomorphin, GLP 2, NPY, prolactin, growth hormone, P substance). DPP-4 inhibitors decrease hyperglycaemia in individuals with type 2 diabetes by lowering GLP l and GIP catabolism and, therefore, increasing their blood concentration and action. It seems that the DPP-4 inhibitors action (sitagliptin, vildagliptin), decreasing insulin resistance, is combined with increased activity of glucose transporters [15].

Angiotensin inhibitors

Angiotensin decreases the influence of insulin on cellular glucose transport, especially in the heart. ACE inhibitors enhance the influence of insulin on GLUT4 translocation, probably by intensifying the action of bradykinin and NO.

The antagonists of angiotensin II receptors have similar effects (sartanes). These effects occur only in experimental animals with insulin resistance, but have not been proven in experimental animals with normal insulin sensitivity.

Inhibitors of renal sodium/glucose transport

Studies on the function of sodium–glucose cotransporters from the SGLT family became the basis for proposing their inhibitors as experimental drugs [32]. An example of such an SLGT inhibitor is dapagliflozin, a selective SLGT 2 inhibitor studied as a potential drug for decreasing glycaemia (particularly fasting) by the increase of renal glucosuria (EASD An. Meeting, 2007, Amsterdam, Komoroski B. J. et al.). This increase is proportional to the hyperglycaemia and results in its lowering.

Conclusions

Studies of cellular glucose transport create new cognitive ideas in the area of the pathogenesis of many diseases associated with insulin resistance. They have the potential of practical innovations.

In diabetes mellitus, hyperglycaemia in the extracellular space is associated with a deficit and a significant decrease of glucose utilization within the peripheral cells. These basic, pathogenetic phenomena occur as the result of a decrease of the regulatory action of insulin on the cells. The cause of such disturbances could be the insulin resistance and relative insulin deficit, or both of these pathophysiological conditions occurring simultaneously.

Knowledge of the structure and the mechanisms of glucose transporters action connected with insulin resistance has significantly enriched the understanding of the molecular pathogenesis of diabetes mellitus. It is already known that type 2 diabetes mellitus could be related to, among other factors, improper structure and/ /or function of the GLUT4 transporter. The molecular, pathogenetic possibility involves damage to molecules of the insulin signalling pathway through which insulin stimulates the exocytosis of GLUT4. Such damage can also involve the insulin receptor or molecules transmitting signals from the receptor to the sacs which contain GLUT4 and serve as the translocating GLUT4 vesicles.

The insulin regulatory action on glucose metabolism in peripheral cells begins with an intensification of cellular glucose transport. In diabetes mellitus, it is impaired. Cellular glucose transport abnormalities in the cellular glucose transport system are typically associated with insulin resistance.

Therefore, the structural and functional determination of glucose transport in practice may offer important clinical advantages for more precise diagnosis of metabolic pathology, classification of diabetes mellitus, and for inventing new therapies. Studies in this area are very active.

Pathologies related to cellular glucose transport disorders and insulin resistance also involve the pathogenesis of tumours, atherosclerosis, and many other diseases influenced by changes in this basic function of cells.

Studies of cellular glucose transport also provide new information about the biological mechanisms of the action of many hormones, regulating factors, and pharmacological agents in the area of insulin resistance.

References

1. Rhodes CJ, White MF. Molecular insights into insulin action and secretion. Eur J Clin Inverty 2002; 32 (Suppl. 3): 3–13.
2. Kahn BB. Lilly Lecture 1995. Glucose transport: pivotal step in insulin action. Diabetes 1996; 45: 1644–1654.
3. Kahn BB. Glucose transport: pivotal step in insulin action. Diabetes 1996; 45: 1644–1654.
4. Bergman RN, Finegood DT, Kahn SE. The evolution of β-cell dysfunction and insulin resistance in type 2 diabetes. Eur J Clin Investry 2002; 32 (Suppl. 3): 35–45.
5. Deeney JT, Prentki M, Corkey BE. Metabolic control of beta-cell function. Semin Cell Dev Biol 2000; 11: 267–275.
6. Shepherd PR, Kahn BB. Glucose transporter and insulin action- implications for insulin resistance and diabetes mellitus. NEJM 1999; 341: 248–257.
7. Kotani F, Peroni DD, Minokoshi Y et al. GLUT4 glucose transporter deficiency increases hepatic lipid production and peripheral lipid utilization. J Clin Investry 2004; 52: 1935–1942.
8. James DE, Piper RC. Mini-Review on the Cellular Mechanisms of Disease. J Cell Biol 1994; 126: 1123–1126.
9. Petersen K.E., Shulman G.I. Etiology of insulin resistance. Am J Med 2006; 119 (Suppl. 1): s10–s16.
10. Whithers DJ, White M. Perspective. The insulin signaling system — a common link in the pathogenesis of type 2 diabetes. Endocrinology 2000; 141: 1917–1921.
11. Petersen KF, Shulman GT. Etiology of insulin resistance. Am J Med 2006; 111 (Suppl. 1): 510–516.
12. Konrad D, Rudich A, Klip A. Insulin-mediated Regulation of Glucose Metabolism. In: Kumar S, O'Rahilly S (ed.). Insulin Resistance. John Wiley and Sons 2004: 63–86.
13. Savage D, O'Rahilly S. Syndromes of Severe Insulin Resistance. In: Kumar S, O'Rahilly S (ed.). Insulin Resistance. John Wiley and Sons, Chichester 2004: 511–534.
14. Matthaei S, Stumvoll M, Kellerer M et al. Pathophysiology and Pharmacological Treatment if Insulin Resistance. Endocrine Reviews 2000; 21: 585–618.
15. Holst JJ, Deacan CF. Coming of Age for the Incretins. In: Le Roith D, Vinik AI (ed.). Controversies in Treating Diabetes. Humanba Press 2008: 269–290.
16. Zhang BB, Moller DE. Drug Therapy for Insulin Resistance — a Look at the Future. In: Kumar S, O'Rahilly S (ed.). Insulin Resistance. John Wiley and Sons, Chichester 2004; 561–566.
17. Withers DJ, White M, Perspective. The insulin signaling system — a common link in the pathogenesis of type 2 diabetes. Endocrinology 2000; 141: 1917–1921.
18. Carvalho E, Kotani K, Peroni OD et al. Adipose — specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice locking GLUT4 selectively in muscle. Am J Physiol Endocrinol Metab 2005; 289: E551–E561.
19. Holten MK, Zacho M, Gaster M et al. Strength Training Increases Insulin--Mediated Glucose Uptake, GLUT4 Content and Insulin Signaling in Skeletal Muscle in Patients With Type 2 Diabetes. Diabetes 2004; 53: 204–305.
20. Bernat-Karpińska M, Czech A, Piątkiewicz P et al. Ocena ekspresji glukotransportera 4 (GLUT4) u osób ze stanem przedcukrzycowym i u osób metabolicznie zdrowych z dodatnim wywiadem rodzinnym w kierunku cukrzycy typu 2 — znaczenie patogenetyczne i kliniczne. Med. Metaboliczna 2009; XIII: 33–38.
21. Friedman JE, Dohm GL, Leggett-Frazier N et al. Restoration of insulin responsiveness in skeletal muscle of morbidly obese patients after weight loss. Effect on muscle glucose transport and glucose transporter GLUT4. J Clin Investig 1992; 89: 701–705.
22. Tatoń J, Czech A. Insulina, fizjologia, farmakologia, insulinoterapia cukrzycy. Wyd. Termedia, Poznań (w druku).
23. Tatoń J. Nowa strategia i zasady insulinoterapii — zwiększanie szansy uzyskiwania pożądanych, prewencyjnych celów leczenia cukrzycy. Med Metaboliczna 2008; XII: 23–34.
24. Rosenstock J, Owens D. Treatment of Type 2 Using Insulin. In: LeRoith D, Vinik AI (ed.). Controversies in Treating Diabetes. Humana Press, USA 2008: 69–94.
25. Szablewski L. Zaburzenia dokomórkowego transportu deoksy-D-glukozy do wybranych krwinek krwi obwodowej u osób chorych na cukrzycę typu 2.

(Disturbances of the cellular transport of deoxy-D-glucose into peripheral blood cells of persons with diabetes mellitus type 2). Wpływ choroby i sposobu leczenia na transportery glukozy w tych komórkach. Rozprawa habilitacyjna, Oficyna Wydawnicza Akademii Medycznej w Warszawie, 2006.
26. Imamura H, Morimoto L, Tanaka Y et al. Regulation of glucose transporter 1 expression by gliclazide in rat L6 myoblasts. Diab Nutr Metab 2001; 14: 308–314.
27. Pulido N. Sulfonylureas stimulate glucose uptake through GLUT -transporter translocation in rat skeletal muscle. Biochem and Biophys Research Communications 1996; 228: 499–504.
28. Tatoń J, Piątkiewicz P, Czech A. Gliklazyd zwiększa dokomórkowy transport glukozy (DTG) i reguluje ekspresję glukotransportera 4 (GLUT4). Badanie w cukrzycy typu 2 na modelu limfocytów krwi obwodowej. Med Metaboliczna 2007; XI: 10–19.
29. Galuska D, Nolte LA, Ziehrath JR. Effects of metformin on insulin — stimulated glucose transport in isolated skeletal muscle obtained from patients with NIDDM. Diabetologia 1994; 37: 826–832.
30. Lennon R, Welsch GI, Singh SC et al. Rosiglitazone enhances glucose uptake in glomerular podocytes using the glucose transporter GLUT1. Diabetologia 2009; 52: 1944–1952.
31. Al-Khalili L, Forsgren M, Kannisto K. Enhanced insulin-stimulated glucogen synthesis in response to insulin, metformin or rosiglitazone is associated with increased mRNA expression of GLUT4 and peroxisomal proliferators activator receptor gamma co-activator 1. Diabetologia, 2005; 48: 1173–1179.
32. Handlon AL. Sodium glucose co-transporter 2 (SGLT2) inhibitors as potential antidiabetic agents Expert Opinion on Therapeutic Patents 2005; 15: 1531–1540.