Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 61; Numer/Number 3/2010
ISSN 0423–104X

Cellular glucose transport disturbances as a marker of the pre-diabetic state — pathogenetic and clinical significance of the assessment of GLUT4 expression

Zaburzenia transportu komórkowego glukozy jako marker stanu przedcukrzycowego — patogenetyczne i kliniczne znaczenie oceny ekspresji GLUT4

Małgorzata Bernat-Karpińska[1], Anna Czech[1], Paweł Piątkiewicz[1], Piotr Wierzbicki[2], Andrzej Górski[2]

[1]Chair and Department of Internal Medicine and Diabetology, II Medical Faculty, Warsaw Medical University, Brodnowski Hospital
[2]Department of Clinical Immunology, Institute of Transplantology, Warsaw Medical University

Abstract

Introduction: GLUT4 is a representative of the family of integral membrane proteins which facilitate glucose transport across cellular membranes. In the available literature there is no publication referring to the investigations of glucotransporter expression in pre-diabetic subjects. However, GLUT4 protein overexpression was shown in leukocytes of diabetic patients. The aim of this study was to compare GLUT4 quantitative expression in peripheral blood lymphocytes in type 2 diabetes mellitus risk groups to healthy subjects.
Material and methods: The study groups included 15 pre-diabetic subjects and 15 persons with normal glucose tolerance and positive family history of type 2 diabetes mellitus (first-degree relatives). As a control group, 15 healthy persons with no family history of diabetes were enrolled. The expression of GLUT4 on the surface of peripheral blood lymphocytes was investigated with the use of indirect immunofluorescence. Quantitative determination of GLUT4 was performed with the use of flow cytometry.
Results: In the control group, GLUT4 expression amounted to 12 ± 1.5% and was significantly lower in relation to both pre-diabetic subjects (18.2 ± 8.8%; p = 0.008) and the positive family history group (17.9 ± 9%; p = 0.001).
Conclusions: GLUT4 overexpression in subjects with positive family history of type 2 diabetes mellitus suggests that cellular glucose transport disturbances occur prior to hyperglycaemia. Determination of GLUT4 expression appears to be a possibly useful method of early detection in individuals at high risk of diabetes. **(Pol J Endocrinol 2010; 61 (3): 269–274)**

Key words: *type 2 diabetes, prediabetes, glucose transport, GLUT, lymphocytes*

Streszczenie

Wstęp: Glukotransporter 4 (GLUT4) jest przedstawicielem rodziny integralnych białek błonowych transportujących glukozę do komórek na drodze dyfuzji ułatwionej. W dostępnym piśmiennictwie stwierdzano zwiększoną ekspresję białka GLUT4 na powierzchni leukocytów osób chorych na cukrzycę; nie ma natomiast publikacji dotyczących badania ekspresji glukotransporterów u osób ze stanem przedcukrzycowym. Celem pracy było porównanie ilościowej ekspresji GLUT4 na limfocytach krwi obwodowej u osób należących do grup ryzyka cukrzycy typu 2 oraz u osób zdrowych.
Materiał i metody: Do badania włączono 15 osób ze stanem przedcukrzycowym oraz 15 osób metabolicznie zdrowych z dodatnim wywiadem rodzinnym w kierunku cukrzycy typu 2 (pokrewieństwo pierwszego stopnia). Grupę kontrolną stanowiło 15 osób zdrowych z negatywnym wywiadem rodzinnym. Do znakowania limfocytów wykazujących ekspresję białka GLUT4 użyto techniki immunofluorescencji pośredniej. Ilościowego oznaczenia GLUT4 na komórkach dokonano przy użyciu cytometru przepływowego.
Wyniki: Ekspresja GLUT4 w grupie kontrolnej wynosiła 12 ± 1,5% i była znamiennie niższa od ekspresji GLUT4 w grupie osób ze stanem przedcukrzycowym (18,2 ± 8,8%, p = 0,008) oraz z dodatnim wywiadem rodzinnym (17,9 ± 9%, p = 0,01).
Wnioski: Zwiększona ekspresja GLUT4 u osób z dodatnim wywiadem rodzinnym w kierunku cukrzycy typu 2 sugeruje istnienie zaburzeń dokomórkowego transportu glukozy w fazie przedhiperglikemicznej. Wydaje się, że oznaczenie ekspresji GLUT4 u osób z grup zwiększonego ryzyka może być wartościowym testem do wczesnego wykrywania cukrzycy.
(Endokrynol Pol 2010; 61 (3): 269–274)

Słowa kluczowe: *cukrzyca typu 2, stan przedcukrzycowy, transport glukozy, GLUT, limfocyty*

The study was a part of a project lead under the grant from MNiSW no 2P05B04330.

Małgorzata Bernat-Karpińska M.D., Chair and Department of Internal Medicine and Diabetology, II Medical Faculty, Warsaw Medical University (Brodnowski Hospital), Kondratowicza St. 8, 03–242 Warszawa, tel./fax: +48 22 326 58 17, e-mail: diabetologia@wum.edu.pl

Introduction

Glucose is a major energetic substrate for human cells with lipid cellular membrane impenetrable to monosaccharides. The cellular glucose transport is possible due to the special transport system created by two groups of proteins: glucotransporters (GLUT) and lesser-known sodium-dependent glucose cotransporters (SGLT). The presence of these transporters has been observed in nearly all human cells. While SGLT proteins are involved in the secondary active glucose transport (they draw the energy from the electrochemical Na^+ ion gradient), the cellular glucose transport by means of glucotransporters is based on the principle of facilitated diffusion, without energy, in accordance with the concentration gradient. Glucotransporters are a family of integral membrane proteins which significantly differ from each other in kinetic characteristics, substrate specificity, and tissue expression [1–3]. To date, 14 glucotransporters have been identified, the first of which was described over 20 years ago. These proteins are abbreviated as GLUT1–12, HMIT1, and GLUT14. The genes coding them are described in accordance with the established nomenclature (HUGO Nomenclature Committee) as SLC2A1-SLC2A14 [4–6]. Particular isoforms differ from each other in the amino-acidic sequences, but the general outline of their construction is similar. About 50% of the protein mass is constituted by a highly conservative intracellular domain, the segments of which create 12 α-helices formed into a hydrophilic channel allowing the sugar transport to take place. Other domains are less conservative — extracellular and cytoplasmatic, which contains the N-terminus fragment, C-terminus fragment and the large loop linking segments 6 and 7. Three classes have been determined within the glucotransporter family. Class I includes the earliest identified and best known isoforms — GLUT1–4. GLUT5 belongs in Class II along with GLUT7, GLUT9, and GLUT11, which are related to it. Class III gathers proteins characterized by the presence of the site of glycosylation in loop 9, not 7 as in the case of two other classes.

Expression of particular GLUT isoforms is specific for cells and tissues and is not constant but is modified by hormonal and environmental factors [7–10, 16–20]. In different diseases such as diabetes, tumours, and tissue ischaemia, varied expression of particular glucotransporter isoforms is observed. To date, studies have shown that glucotransporters 1, 3, and 4 are present on the surface of leukocytes, and the level of expression of the genes coding them, and the regulation of this expression, remains in a close relationship with the functions of particular cells [11–15]. As proven by earlier studies [13, 15, 21], the lymphocytes constitute an especially convenient experimental model for the assessment of cellular glucose transport, due to the ease of acquirement of the study material, which allows invasive procedures such as muscle or adipose tissue biopsy to be avoided. Furthermore, contrary to the other white blood cell populations, they are relatively easy to isolate from the peripheral blood and have a long in-vitro lifespan.

Aim of the study

The aim of the study was the quantitative comparison of GLUT4 protein expression — as a main glucose transporter sensitive to insulin — on the surface of peripheral blood lymphocytes in a group in pre-diabetic stage and in a group of metabolically healthy individuals with positive family history of type 2 diabetes mellitus, as compared with a group of healthy individuals with negative family history of type 2 diabetes mellitus.

Material and methods

The study was conducted on a group of 45 individuals of both genders, aged 35–75 years, who were qualified appropriately to one of the three equally populated groups: Individuals with impaired fasting glycaemia and/or impaired glucose tolerance (pharmacologically untreated); metabolically healthy individuals with positive family history of type 2 diabetes mellitus (first-degree relatives); and healthy individuals with negative family history of type 2 diabetes mellitus (control group) — selected on the basis of demographic and anthropometric features (Table I). All the participants of the study were informed about the aim and the course of the examinations, and were included in the study after they signed consent forms. The following individuals were excluded from the study:

— persons with renal failure (creatinine concentration $>$ 2.5 mg/dl);
— persons with liver impairment (transaminase activity $\geq 3 \times$ upper limit of the norm);
— persons with symptomatic heart failure;
— persons with neoplastic disorders in the course of last five years;
— persons using diabetogenic medicines (*e.g.* systemic corticosteroids, oral contraceptives, thiazide diuretics, miconazole);
— persons with alcohol or medicine abuse; addicted to medicines in the course of the last year;
— pregnant women, lactating women.

Patients were qualified to particular groups on the basis of medical examination, with special focus on medical history (standardized survey examination allowing the gathering of demographic, environmental, and clinical data) as well as laboratory examinations. The characteristics of examined individuals are given in Table I.

Table I. *Characteristics of the examined groups*
Tabela I. *Charakterystyka badanych grup*

Parameter	Control group	Possitive family history	IFG/IGT
Number of participants	15	15	15
SEX (M/W)	8/7	5/10	5/10
AGE (years)	48.6 ± 11.4	46.6 ± 8.78 SI	46.33 ± 5.58 SI
BMI [kg/m^2]	27.68 ± 2.44	28.13 ± 3.17 SI	30.55 ± 4.68 $p < 0.05$
SBP [mm Hg]	127.3 ± 13.3	132.0 ± 19.53 SI	144.0 ± 10.56 $p < 0.001$
DBP [mm Hg]	77.0 ± 9.2	84.33 ± 16.68 SI	95.33 ± 7.9 $p < 0.0001$

BMI — body mass index; SBP — systolic blood pressure; DBP — diastolic blood pressure; IFG — impared fasting glycaemia; IGT — impared glucose tolerance; SI — statistically insignificant

The following tests were performed in all volunteers: fasting glycaemia test (enzymatic method), oral glucose tolerance test (in accordance with WHO), glycated haemoglobin HbA_{1c} test (HPLC method), and tests for fasting insulin and fasting C peptide (radio immunological method).

Additionally, a fasting blood sample (10 ml) was drawn from all of the participants, from which the lymphocytes were isolated in order to mark the quantitative expression of glucotransporters on their surface. The lymphocyte isolation was performed with the use of Gradisol L ("Aqua-Medica" Poland) (2800 rpm., 20 min.). The gathered lymphocytes were rinsed twice with 0.9% NaCl solution (1800 rpm., 10 min.). After rinsing, the lymphocytes were suspended in such a measured dose of the so-called "transport solution" (Kaliman et al. 1995) as to ensure that the density was 10^6 cells/ml in each trial. The technique of single-colour indirect immunofluorescence was employed in the labelling of lymphocytes displaying the expression of studied GLUT proteins. To this aim, the monoclonal antibodies (MoAb) anti-GLUT4 were used along with the unspecific antibody labelling F(ab')2 immunoglobulin fragment, linked with the fluorescein isothiocyanate (FITC). The negative control was conducted in order to rule out the possibility of autofluorescence of the cells, and a positive control was conducted to eliminate the unspecific binding of the FITC-labelled antibody. Quantitative tests of glucotransporter expression were conducted with the use of the flow cytometry. The FACS-Calibur type flow cytometer with CellQuest software (Becton-Dickinson) was used for the acquisition and analysis of the data.

Statistical analysis

STATISTICA software was used in the analysis of the data. The acquired data are presented in the form of mean values and standard deviations. Quantitative parameters were compared using the t-Student test. $P = 0.05$ was assumed as the significance level threshold.

Results

The group of persons in the pre-diabetic stage and the group of metabolically healthy persons with positive family history of type 2 diabetes mellitus were characterized by higher BMI and systolic and diastolic blood pressure values in comparison to the control healthy subjects. The differences between the group of metabolically healthy persons with positive family history of type 2 diabetes and the control group were not statistically significant (Table I).

Mean values of the assessed biochemical parameters as compared with the control group (Table II). In the group of persons in the pre-diabetic stage a significantly higher (as compared with the control group) mean value of fasting glycaemia (6.05 mmol/L *v.* 5.13 mmol/L, $p < 0.0005$) and HbA_{1c} (5.95% *v.* 4.9%, $p = 0.0001$) was observed along with significantly higher indices of insulin resistance. In these groups, the fasting insulin level was appropriately 12.74 mU/L *v.* 7.92 mU/L ($p < 0.05$). The differences in the area of the HOMA-IR index were similar (3.38 *v.* 1.84, $p < 0.01$). In the group of metabolically healthy persons with positive family history of type 2 diabetes mellitus, the mean values of examined biochemical parameters were lower than in the group of persons with impaired fasting glycaemia (IFG) and/or impaired glucose tolerance (IGT), and higher than in the control group. The observed differences were not statistically significant, apart from the HbA_{1c}, in relation to the control group.

The study showed that mean expression of GLUT4 protein in the group of metabolically healthy persons

Table II. *Comparison of the results of laboratory examinations*
Tabela II. *Porównanie wyników badań laboratoryjnych*

Parameter	Control group	Positive family history	IFG/IGT
Fasting glycaemia [mmol/L]	5.13 ±0.66	5.25 ±0.37 SI	6.05 ±0.53 $p < 0.0005$
HbA_{1c} (%)	4.9 ±0.6	5.77 ±0.56 $p < 0.0005$	5.95 ±0.61 $p = 0.0001$
Fasting insulin [mU/L]	7.92 ±3.76	9.04 ±1.52 SI	12.74 ±7.65 $p < 0.05$
HOMA-IR	1.84 ±1.01	2.13 ±1.52 SI	3.38 ±1.9 $p < 0.01$

IFG — impared fasting glycaemia; IGT — impared glucose tolerance; SI — statistically insignificant

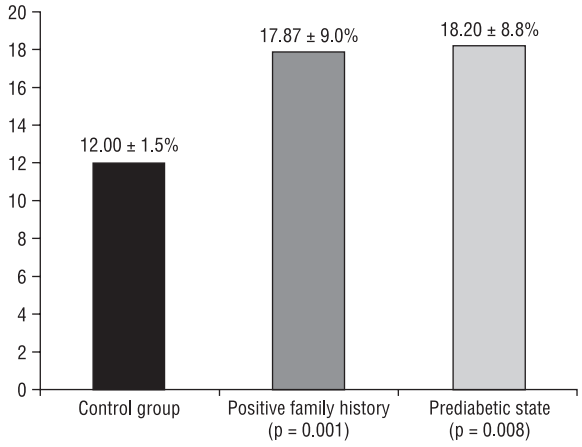

Figure 1. *Comparison of the quantitative expression of GLUT4 on the surface of peripheral blood lymphocytes in the control group, the group of metabolically healthy persons with positive family history of type 2 diabetes mellitus, and the group of persons in the pre-diabetic state*

Rycina 1. *Porównanie ilościowej ekspresji GLUT4 na powierzchni limfocytów krwi obwodowej w grupie kontrolnej, grupie osób metabolicznie zdrowych z dodatnim wywiadem rodzinnym w kierunku cukrzycy typu 2 oraz osób ze stanem przedcukrzycowym*

with positive family history of type 2 diabetes mellitus was 17.9% and was very close to the mean expression of this protein in the group in the pre-diabetic stage, in which it was 18.2%. These values were clearly higher than those observed in the control group (12%), and the acquired differences displayed statistical significance (Fig. 1).

Discussion

The growing incidence of diabetes mellitus constitutes a major socio-economic problem. The early identification of persons from the groups of increased risk for this disease allowing the introduction of preventive countermeasures, therefore, gains importance. It has been proven that the prevalence of abnormal glucose tolerance in subjects with at least one of the risk factors for type 2 diabetes mellitus is near 40% [22]. Moreover, it is well established that diabetes mellitus and IFG/IGT are observed more frequently in several other endocrinopathies (*e.g.* acromegaly) than in the general population [23]. Conventional biochemical methods (oral glucose tolerance test [OGTT], fasting plasma glycaemia [FPG]) allow the identification of persons in the prediabetic stage, *i.e.* with IFG and/or IGT. It has been proven that nondiabetic hyperglycaemia is related not only to a higher risk of the development of diabetes, but also to cardiovascular diseases [24]; therefore, the fasting glycaemia test and OGTT are insufficient for early detection of such persons. Furthermore, the OGTT is characterized by low repeatability and demands the use of advanced standardization, and its employment in the area of active diagnosis of diabetes is connected with organizational and financial difficulties [25]. In spite of the extreme complexity of the pathophysiological mechanisms that are the basis for the occurrence of diabetes, the only test used for the diagnosis of this disease is glucose concentration measurement. Glycaemia tests are insufficient for the appropriate earlier identification of persons at risk for the development of diabetes. Many studies have focused on investigations of the usefulness of other parameters in the risk assessment of diabetes development. It has been revealed that the presence of higher fasting levels of C-peptide in first degree relatives of the autoimmunological diabetic patients with no clinical symptoms of type 1 diabetes could be a prognostic factor to retard the clinical symptoms of the disease [26]. Numerous growth factors have been reported to be involved in the pathogenesis of diabetes mellitus and its long-term complications. It has been proven that the blood concentration of hepatocyte growth factor (HCG) is increased in patients with type 1 diabetes with proliferative retinopathy, and concentrations increase with the progression of retinopathy [27].

A major role in the pathogenesis of diabetes is played by cellular glucose transport disorders. It seems that their diagnosis in the prehyperglycaemic phase and the introduction of appropriate preventive measures could yield notable clinical and economic benefits. Impaired cellular glucose transport is mirrored by the altered expression of glucotransporters on the cellular surface. It has been proven that both in the case of chronic hyperglycaemia and in circumstances of lengthy hypoglycaemia, an increased expression of glucotransporters occurs on the surface of white blood cells. It was shown that hypoglycaemia induces the significant growth of GLUT3 protein expression on the surface of monocytes, and GLUT4 and GLUT3 on the surface of granulocytes [10]. In the mentioned study, the presence of glucotransporters on the surface of lymphocytes was not observed, which is contradictory to the results of later studies [13, 14, 28].

To date, the focus of studies has been on the examination of glucotransporters in patients with diabetes and the growth of their expression on the cellular surface, as compared with individuals with normal glycaemia [13, 15, 28, 29]. This phenomenon probably constitutes the adaptive mechanism allowing the adjustment of the immunocompetent cells to the altered metabolic circumstances. The complex system of cellular glucose transport is equipped with an autoregulatory mechanism, and the impairment of this mechanism leads to disorders of the function of particular populations of leukocytes, which can explain the more frequent incidence of tumours and immunity disorders in patients with diabetes compared with the general population [30–34].

Even though many studies have been concerned with the notion of cellular glucose transport in individuals with diabetes, in the available literature there are no publications concerning examinations of the expression of glucotransporters in the group of pre-diabetic individuals and the group of metabolically healthy persons with positive family history of diabetes mellitus.

In our study, a significantly increased (as compared with the control group) expression of GLUT4 protein on the surface of peripheral blood lymphocytes was observed in both the persons with IFG and/or IGT and in the metabolically healthy persons with positive family history of type 2 diabetes mellitus. This indicates that glucose metabolism disorders, including cellular glucose transport disturbances, occur in the early phase of diabetes development before the biochemical indices of this disease can be observed.

Conclusions

1. The increased expression of GLUT4 transport protein on the surface of peripheral blood lymphocytes in persons with proper glycaemia and positive family history of type 2 diabetes mellitus points to the existence of cellular glucose transport disorders already in the prehyperglycaemic phase.
2. Quantitative labelling of GLUT4, and probably of other proteins belonging in the GLUT family, gives hope for the development of diagnostic methods for the purpose of practical prevention of diabetes mellitus.

References

1. Joost HG, Thorens B. The extended GLUT family of sugar/polyol transport facilitators: nomenclature, sequence characteristics and potential function of its novel members. Mol Membr Biol 2001; 18: 247–256.
2. Saier MH Jr. Families of transmembrane sugar transport proteins. Mol Microbiol 2000; 35: 699–710.
3. Saier MH Jr. A functional-phylogenetic classification system for transmembrane solute transport proteins. Microbiol Mol Biol Rev 2000; 64: 354–411.
4. Phay JE, Hussain HB, Moley JF. Strategy for identification of novel glucose transporter family members by using internet-based genomic databases. Surgery 2000; 128: 946–951.
5. Joost HG, Bell GI, Best JD et al. Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am J Physiol Endocrinol Metab 2002; 282: E 974–E 986.
6. McVie-Wylie AJ, Lamson DR, Chen YT. Molecular cloning of a novel member of the GLUT family of transporter, SLC2a10 (GLUT10), localized on chromosome 20q13.1: a candidate gene for NIDDM susceptibility. Genomics 2001; 72: 113–117.
7. Phay JE, Hussain HB, Moley JF Cloning and expression analysis of a novel member of the facilitative glucose transporter family, SLC2A9 (GLUT9). Genomics 2000; 66: 217–220.
8. Reagan LP, Gorovits N, Hoskin EK et al. Localization and regulation of GLUT 1 glucose transporter in the hippocampus of streptozotocin diabetic rats. Proc Natl Acad Sci USA 2001; 98: 2820–2825.
9. Shikman AR, Brinson DC, Valbracht J et al. Cytokine regulation of facilitated glucose transport in human articular chondrocytes. J Immunol 2001; 167: 7001–7008.
10. Korgun ET, Demir R, Sedlmayr P et al. Sustained hypoglycemia affects glucose transporter expression of human blood leukocytes. Blood Cells Mol Dis 2002; 28: 152–159.
11. Estrada DE, Elliott E, Zinman B et al. Regulation of glucose transport and expression of GLUT3 transporters in human circulating mononuclear cells: studies in cells from insulin-dependent diabetic and nondiabetic individuals. Metabolism 1994; 43: 591–598.
12. Daneman D, Zinman B, Elliott ME et al. Insulin-stimulated glucose transport in circulating mononuclear cells from nondiabetic and IDDM subjects. Diabetes 1992; 41: 227–234.
13. Piątkiewicz P, Kłosowska D, Czech A et al. Investigations of Glucotransporter 4 (GLUT4) expression in lymphocytes of healthy subjects and type 2 diabetic patients. Med Metabol 2006; 3: 16–18.
14. Fu J, Mianu L, Melbert BR et al. Facilitative glucose transporter gene expression in human lymphocytes, monocytes and macrophages: a role for GLUT isoform 1,3 and 5 in the immune response and foam cell formation. Blood Cells Mol Dis 2004; 32: 182–190.
15. Piątkiewicz P, Czech A, Tatoń J. Glucose transport in human peripheral blood lymphocytes influence by type 2 diabetes mellitus. Arch Immunol Ther Exp 2007; 55: 119–126.
16. Carayannopoulos MO, Chi MM, Cui Y et al. GLUT 8 is a glucose transporter responsible for insulin-stimulated glucose up-take in the blastocyst. Proc Natl Acad Sci USA 2000; 97: 7313–7318.
17. Doege H, Schurmann A, Bahrenberg G et al. GLUT8, a novel member of the sugar transport facilitator family with glucose transport activity. J Biol Chem 2000; 275: 16275–16280.
18. Doege H, Bocianski A, Joost HG et al. Activity and genomic organization of human glucose transporter 9 (GLUT9), a novel member of the family of sugar-transport facilitators predominantly expressed in brain and leucocytes. Biochem J. 2000; 350: 771–776.
19. Watson RT, Pessin JE. Intracellular organization of insulin signaling and GLUT4 translocation. Recent Prog Horm Res 2001; 49: 175–193.
20. Benomar Y, Natur N, Aubourg A et al. Insulin and leptin induce GLUT4 plasma membrane translocation and glucose uptake in a human neuronal cell line by phosphatidylinositol 3-kinase-dependent mechanism. Endocrinology 2006; 147: 2550–2556.
21. Szablewski L, Piątkiewicz P, Oleszczak B et al. Wpływ leczenia insuliną chorych na cukrzycę typu 2 na pobieranie deoksy-D-glukozy przez limfocyty krwi obwodowej. Diab Dośw Klin 2004; 4,3: 217–223.

22. Strus A, Szepietowska B, Zonenberg A et al. Możliwości prewencji cukrzycy typu 2. Pol J Endocrinol 2008; 59: 6–12.
23. Stelmachowska-Banaś M, Zdunowski P, Zgliczyński W. Abnormalities in glucose homeostasis in acromegaly. Does the prevalence of glucose intolerance depend on the level of activity of the disease and the duration of the symptoms? Pol J Endocrinol 2009; 60: 20–24.
24. Levitan EB, Song Y, Ford ES et al. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. Arch Intern Med 2004; 164: 2147–2155.
25. Harris M. Frequency of oral glucose tolerance testing in US. Diabetes Care 1995; 18: 134–136.
26. Siewko K, Szelachowska M, Popławska-Kita A et al. Peptyd C jako czynnik ryzyka rozwoju cukrzycy typu 1 u krewnych I stopnia osób chorych na cukrzycę autoimmunologiczną. Pol J Endocrinol 2009; 60: 357–362.
27. Nowak M, Wielkoszyński T, Marek B et al. A comparison of the levels of hepatocyte growth factor in serum in patients with type 1 diabetes mellitus with different stages of diabetic retinopathy. Pol J Endocrinol 2008; 59: 2–5.
28. Szablewski L, Sobczyk-Kopcioł A, Oleszczak B et al. GLUT4 is expressed in circulating lymphocytes of diabetic patients. A method to detect early prediabetic stages? Diabetologia Croat 2007; 36: 69–76.
29. James DE, Piper RC. Insulin resistance, diabetes and the insulin-regulated trafficking. J Cell Biol 1994; 126: 1123–1126.
30. La Vecchia C, Negri E, Decarli A et al. Diabetes mellitus and the risk of primary liver cancer. Int J Cancer 1997; 73: 204–207.
31. Larsson SC, Orsini N, Wolk A. Diabetes mellitus and risk of colorectal cancer: a meta-analysis. J Natl Cancer Inst 2005; 97: 1679–1687.
32. Rousseau MC, Parent ME, Pollak MN et al. Diabetes mellitus and cancer risk in a population — based case — control study among men from Montreal, Canada. Int J Cancer 2006; 118: 2105–2109.
33. Chang FY, Shaio MF. Decreased cell-mediated immunity in patients with non-insulin-dependent diabetes mellitus. Diabetes Res Clin Pract 1995; 28: 137–146.
34. Nassaji-Zavareh M, Taheri R, Ghorbani R et al. Undiagnosed diabetes mellitus in patients with herpes zoster. Indian J Dermatol 2008; 53: 119–121.