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The expression of particular glucose transporters and insulin resistance indicators in the risk groups of type 2 diabetes — a two-year follow-up

Badanie ekspresji wybranych glukotransporterów oraz wskaźników insulinooporności w grupach ryzyka cukrzycy typu 2 — obserwacja 2-letnia

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Abstract

Introduction: The proper expression of particular glucotransporter (GLUT) isoforms determines a sufficient supply of glucose to tissues. The impairment of cellular glucose transport observed in insulin resistance leads to glucose metabolism disturbances. The aim of this study was the estimation of insulin resistance indicators and the quantitative expression of GLUT-1, GLUT-3 and GLUT-4 on peripheral blood lymphocytes in prediabetic subjects and persons with a positive family history of type 2 diabetes during 24 months of observation.
Material and methods: The study included 25 prediabetic subjects (according to WHO criteria) and 24 normoglycaemic individuals with a positive family history of type 2 diabetes. Twenty three healthy subjects with no family history of type 2 diabetes, matched with BMI, served as a control group. All participants were recommended to perform physical activity for at least 140 minutes per week and to maintain a low calorie diet. The peripheral blood lymphocytes demonstrating expression of GLUT-1, GLUT-3 and GLUT-4 were labelled with the use of indirect immunofluorescence. The expression of GLUT isoforms was investigated by flow cytometry. Cells were stained by using anti-human GLUT antibodies and FITC-conjugated immunoglobulin. Flow cytometry was performed using a FACS Calibur (Becton-Dickinson). Additionally, we determined: fasting plasma glucose (FPG), insulin and C peptide concentrations, HOMA-IR, BMI and WHR. All the tests were performed at baseline, and after 12 and 24 months.
Results: At baseline, prediabetics and subjects with a positive family history of type 2 diabetes were characterised by a much higher expression of GLUT-4 compared to control subjects. Twenty four months of lifestyle modification resulted in significant lowering of the expression of GLUT-4 on the surface of PBL in both studied groups, with no differences in the expression of GLUT-1 or GLUT-3. Both prediabetic subjects and individuals with a positive family history of type 2 diabetes revealed no significant differences in determined insulin resistance markers after 24 months of the observation compared to the baseline values.
Conclusions: The estimation of typical GLUT isoforms present on the peripheral blood lymphocytes, as well as the evaluation of insulin resistance indicators, are obviously insufficient for monitoring the metabolic disorders progression in the risk groups of type 2 diabetes. The decrease in GLUT-4 lymphocyte expression may reflect a positive influence of lifestyle modification on a tissue redistribution of this crucial insulin-dependent glucotransporter. The determination of GLUT-4 on the surface of peripheral blood lymphocytes can be a useful tool for the evaluation of the efficacy of therapeutic actions in subjects at high risk of type 2 diabetes. **(Pol J Endocrinol 2012; 63 (3): 212–219)**

Key words: *pre-diabetes, positive family history, type 2 diabetes, insulin resistance, glucose transport, glucose transporters (GLUT), lymphocytes*

Streszczenie

Wstęp: Właściwa ekspresja poszczególnych izoform glukotransporterów (GLUT) warunkuje odpowiednie zaopatrzenie tkanek w glukozę. Upośledzenie dokomórkowego transportu glukozy obserwowane w warunkach insulinooporności prowadzi do zaburzeń metabolizmu glukozy. Celem badania była ocena wskaźników insulinooporności oraz ilościowej ekspresji GLUT-1, GLUT-3 i GLUT-4 na limfocytach krwi obwodowej osób ze stanem przedcukrzycowym oraz osób z dodatnim wywiadem rodzinnym w kierunku cukrzycy typu 2 w trakcie 24-miesięcznej obserwacji.
Materiał i metody: Do badania włączono 25 osób ze stanem przedcukrzycowym (wg kryteriów WHO) i 24 osoby z normoglikemią z dodatnim wywiadem rodzinnym w kierunku cukrzycy typu 2. Grupę kontrolną stanowiły 23 zdrowe osoby bez wywiadu rodzinnego w kierunku cukrzycy, dobrane pod względem BMI. Wszystkim uczestnikom badania zalecono zwiększenie aktywności fizycznej do co najmniej 140 min tygodniowo oraz stosowanie diety niskokalorycznej. Do znakowania limfocytów krwi obwodowej wykazujących ekspresję GLUT-1, GLUT-3 i GLUT-4 użyto techniki immunofluorescencji pośredniej z wykorzystaniem swoistych przeciwciał anty-ludzkich GLUT oraz immunoglobulin sprzężonych z FITC. Ekspresję poszczególnych izoform GLUT oznaczano za pomocą cytometrii przepływowej przy użyciu cytometru FACS Calibur (Becton-Dickinson) Dodatkowo oznaczano glikemię na czczo, stężenie insuliny i C-peptydu na czczo, wskaźniki HOMA-IR, BMI oraz WHR. Wszystkie oznaczenia były wykonywane wyjściowo, po 12 i po 24 miesiącach obserwacji.
Wyniki: W chwili rozpoczęcia badania osoby ze stanem przedcukrzycowym oraz osoby z dodatnim wywiadem rodzinnym w kierunku cukrzycy typu 2 charakteryzowały się znacznie wyższą ekspresją GLUT-4 w porównaniu z grupą kontrolną. W czasie 24 miesięcy od wdrożenia modyfikacji stylu życia zaobserwowano istotny spadek ekspresji GLUT-4 na limfocytach krwi obwodowej w obu badanych

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grupach ryzyka, przy braku różnic w ekspresji GLUT-1 i GLUT-3. Zarówno w grupie osób ze stanem przedcukrzycowym, jak i wśród osób z dodatnim wywiadem rodzinnym w kierunku cukrzycy typu 2 po 24 miesiącach nie stwierdzono istotnych statystycznie różnic w zakresie badanych wskaźników insulinopooorności w porównaniu z wartościami wyjściowymi.

Wnioski: Ani oznaczanie typowych dla limfocytów krwi obwodowej izoform GLUT, ani ocena wskaźników insulinooporności są niewystarczające do monitorowania progresji zaburzeń metabolicznych w grupach ryzyka cukrzycy typu 2. Spadek ekspresji GLUT-4 na limfocytach jest prawdopodobnie odzwierciedleniem pozytywnego wpływu modyfikacji stylu życia na tkankową redystrybucję tego kluczowego insulinozależnego transportera glukozy. Oznaczanie GLUT-4 na limfocytach krwi obwodowej może być wartościowym narzędziem do oceny skuteczności interwencji terapeutycznych w grupach ryzyka cukrzycy typu 2. **(Endokrynol Pol 2012; 63 (3): 212–219)**

Słowa kluczowe: *stan przedcukrzycowy, dodatni wywiad rodzinny, cukrzyca typu 2, insulinooporność, transport glukozy, glukotransportery (GLUT), limfocyty*

Introduction

Given the growing epidemic of obesity, diabetes prevention is becoming one of the most important and most difficult problems not only in diabetology, but the whole of modern medicine. According to WHO data, there are currently more than 220 million people worldwide who suffer from diabetes and it is anticipated that this number will have doubled by 2030 [1]. Sedentary lifestyles and high-calorie diets which lead to overweight and obesity are regarded as the fundamental causes of the increase in the incidence of pre-diabetic and diabetic state, not only among adults but also in children and adolescents [2]. It is estimated that in Poland about 2 million people have diabetes and approximately 4 million people are in pre-diabetic state [3]. According to recent studies, in USA in 2007 the pre-diabetes problem affected about 57 million American adults [4]. Proper identification of persons at high risk of type 2 diabetes is required to take appropriate strategic actions aimed at preventing the development of diabetes, or at least delaying this disease and its related complications.

Among all risk groups of type 2 diabetes, two seem to be exceptionally significant: pre-diabetic subjects and first-degree relatives of type 2 diabetic patients.

The concept of pre-diabetes means impaired fasting glucose (IFG), i.e. fasting plasma glucose values of 5.6–6.9 mmol/L, and/or impaired glucose tolerance (IGT), a condition in which blood glucose in the second hour of OGTT is in the range of 7.8–11.0 mmol/L). A relatively new criterion for pre-diabetes diagnosis, recognised by the American Diabetes Association (ADA), is the percentage of glycated haemoglobin HbA_{1c} corresponding to 5.7–6.4% [5]. Pre-diabetic state, which is an intermediate stage between normoglycaemia and diabetes, can manifest many years before the diagnosis of diabetes. It is believed that within 3–5 years as many as 25% of people with diagnosed impaired fasting glucose and/or impaired glucose tolerance can develop diabetes [6].

The constant increase in the prevalence of type 2 diabetes results in a growing number of subjects burdened with a family history of this disease [7]. For many years, this disease has been considered as a family occurring disease, which means an increased risk of the development of type 2 diabetes in relatives of type 2 diabetic subjects [8, 9]. It is commonly known that the risk of the development of type 2 diabetes depends on both the degree of relation (i.e. the higher the degree, the greater the risk) and the number of family members suffering from diabetes. It has been revealed that in families with a single case of type 2 diabetes, the relatives are characterized by 40% diabetes susceptibility during their lifetime. Furthermore, type 2 diabetes coexists more often in monozygotic twins than heterozygotic ones. The probability of type 2 diabetes development in monozygotic twins reaches as much as 95% [10]. Moreover, it has been proven that in the offspring of couples in which both parents are affected with type 2 diabetes, the insulin resistance syndrome precedes the onset of the disease for ten years [11].

Given the alarming incidence of pre-diabetes, taking effective primary prevention in this group of people would benefit a large number of individual patients, as well as reap huge social and economic benefits. As recommended by ADA and the Polish Diabetes Association (PTD) for the year 2011, if there are no symptoms of hyperglycaemia, the test for diabetes should be performed every three years for each person over 45, and regardless of age once a year in people with other risk factors [12].

These principles for screening diabetes are based on the assessment of glycaemic control, which means that diagnosis of the disease comes at a relatively late stage, since it is known that not only diabetes, but 'hyperglycaemia not related to diabetes' is associated with a significantly increased risk of cardiovascular disease, which has been proven in large clinical trials [13, 14]. This risk is even greater when there is coexistence of impaired fasting glucose and impaired glucose tolerance [15–17].

For many years, clinicians have sought measurable exponents of changes in the pre-hyperglycaemic phase hoping to develop more sensitive diagnostic tests in the future. In 2004, an increase in the amount of proteins transporting glucose by facilitated diffusion (glucose transporters) on peripheral blood

lymphocytes in patients with type 2 diabetes was observed [18]. The authors of a further work suggested that changes in the expression of glucose transporter may precede the laboratory exponent abnormalities in diabetes [19].

Therefore, with the above objective, it seems useful to assess the possible relationship between the expression of GLUT transporter family and biochemical markers of glucose metabolism disorders, as well as insulin resistance indicators, in patients at risk of diabetes.

Aim of study

The aim of this study was to compare biochemical markers of insulin resistance and quantitative protein expression of GLUT-1, GLUT-3 and GLUT-4 on the surface of peripheral blood lymphocytes in the risk groups of type 2 diabetes: pre-diabetic subjects and individuals with a positive family history of type 2 diabetes, at baseline and after 24 months of observation.

Material and methods

The study included 25 people with impaired fasting glucose and/or impaired glucose tolerance, diagnosed on the basis of an OGTT test according to WHO criteria and 24 normoglycaemic individuals with a positive family history of type 2 diabetes. The type of family relation in the group of subjects with a positive family history of type 2 diabetes is presented in Table I. The control group consisted of 23 normoglycaemic people with a negative family history of diabetes, matched for BMI. The study included people of both sexes aged 35–75 years. All participants received information on the aim and course of the experiment, and were included in the study after giving informed written consent.

The study excluded individuals:

— with renal insufficiency (serum creatinine > 2.5 mg/dL);
— with liver disease (transaminase activity ≥ 3 times upper limit of normal);
— with symptomatic heart failure;
— with cancer in the last five years;
— using diabetogenic drugs (e.g. systemic corticosteroids, oral contraceptives, thiazide diuretics, miconazole);
— abusing drugs or alcohol, and those addicted to drugs in the past year;— pregnant women, lactating women.

Patients were enrolled in the study on the basis of an internal examination, with particular emphasis on interviews (standardized survey to gather demographic, environmental and clinical data) and laboratory tests. The characteristics of participants are shown in Table II.

Table I. *Type of family relation in the group of subjects with a positive family history of type 2 diabetes mellitus*

Tabela I. *Rodzaj pokrewieństwa w grupie osób z dodatnim wywiadem rodzinnym w kierunku cukrzycy typu 2*

First degree relative with type 2 diabetes mellitus	Number of cases	Percentage of cases (%)
Father	7/24	29.16
Mother	11/24	45.83
Brother/sister	3/24	12.50
Father + brother/sister	1/24	4.16
Mother + brother/sister	1/24	4.16
Father + mother	1/24	4.16

Table II. *Characteristics of the studied groups at baseline*

Tabela II. *Charakterystyka badanych grup w chwili rozpoczęcia badania*

Parameter	Control group	Positive family history	Prediabetes
Number of participants	23	24	25
Sex (M/F)	10/13	11/13	9/16
Age (years)	45.0 ± 8.0	46.6 ± 9.7	50.6 ± 8.0
BMI [kg/m^2]	30.7 ± 3.0	29.5 ± 4.6	30.1 ± 5.1
WHR	0.88 ± 0.09	0.87 ± 0.11	0.87 ± 0.06

All participants were recommended to perform physical activity for at least 140 minutes per week and to maintain a low-calorie diet with a minimal energy reduction of 500 kcal per day. These recommendations were notified every six months during the observation period. The verification of introduced lifestyle modification was based on a standardized questionnaire consisting of questions referring to the kind, frequency and length of performed physical activity. In all subjects at baseline and after 24 months, fasting plasma glucose determination (enzymatic method), serum fasting insulin and fasting C-peptide (radioimmunoassay) were assessed and the HOMA-IR indicator was calculated [20]. During the first and the last visit, the oral glucose tolerance test according to WHO procedures was also carried out.

Lymphocytes were isolated to determine the quantitative expression of glucose transporters (GLUT) on their surface from fasting blood samples (10 mL), taken from all participants. Lymphocyte isolation was performed using Gradisol L (Aqua-Medica, Poland) (2,800 rpm, 20 min.). The harvested cells were washed twice in 0.9% NaCl solution (1,800 rpm, 10 min.). After this procedure, the cells were suspended in a volume called 'Transport solu-

tions' [21] so that the density in each sample was 10^6 cells/mL. To label the cells that express the surveyed GLUT proteins, the single-colour indirect immunofluorescence technique was used. For this purpose, monoclonal antibodies (MoAb) anti-GLUT-1, anti-GLUT-3, anti-GLUT-4, and non-specific antibody marking F(ab') 2 immunoglobulin fragment conjugated with fluorescein isothiocyanate (FITC) were used. A negative control to exclude cell autofluorescence, and a positive control to exclude nonspecific binding of FITC-labelled antibodies, were also carried out. The quantitative estimation of the glucose transporters was executed by using flow cytometry. For data acquisition and analysis, a FACS Calibur flow cytometer with CellQuest programme (Becton-Dickinson) was used.

Statistical methods

The obtained data was presented as mean values and standard deviations. Quantitative parameters were compared using the Aspin-Welch test. The significance level threshold was p = 0.01.

Results

Individuals with a positive family history of type 2 diabetes

At the beginning of the study, individuals with a positive family history of type 2 diabetes compared to the control group were characterised by insignificant differences in fasting glucose (5.19 ± 0.3 v. 5.27 ± 0.04 mmol/L, p = ns) (Fig. 1), glucose at 120 min. of OGTT (5.8 ± 1.06 v. 5.66 ± 1.0 mmol/L, p = ns) (Fig. 2), and also in serum C-peptide levels (2.35 ± 0.84 v. 1.96 ± 0.56 ng/mL, p = ns) (Fig. 3), but significantly higher levels of fasting insulin (10.13 ± 4.74 v. 3.49 ± 2.51 mU/L, p < 0.01), and HOMA-IR (2.34 ± 1.14 v. 0.82 ± 0.62) (Figs. 4, 5).

The two-year study of the subjects burdened with a positive family history of type 2 diabetes showed no significant changes in fasting glucose (5.19 ± 0.3 v. 5.38 ± 0.58 mmol/L) or glucose at 120 min. of OGTT (5.8 ± 1.06 v. 5.99 ± 1.38 mmol/L) (Figs. 1, 2), as well as fasting insulin (10.13 ± 4.74 v. 9.61 ± 4.08 mU/L), serum C-peptide (2.35 ± 0.84 v. 2.57 ± 0.63 ng/mL), or HOMA-IR (2.34 ± 1.14 v. 2.34 ± 1.14) compared to baseline values (Figs. 3–5). There were also no statistically significant differences in BMI (29.5 ± 4.55 v. 30.8 ± 5.61 kg/m^2) or WHR (0.87 ± 0.06 v. 0.9 ± 0.07) (Figs. 6, 7).

During the two-year follow-up, there were no significant differences in expression of GLUT-1 (29.7 ± 10.36 v. 30.62 ± 11.72%) or GLUT-3 protein (7.54 ± 3.3 v. 8.24 ± 8.24%) (Figs. 8, 9). However, a significant decrease was observed with the expression of GLUT-4 protein (18.93 ± 12.71 v. 9.35 ± 6.07%, p < 0.01) (Fig. 10).

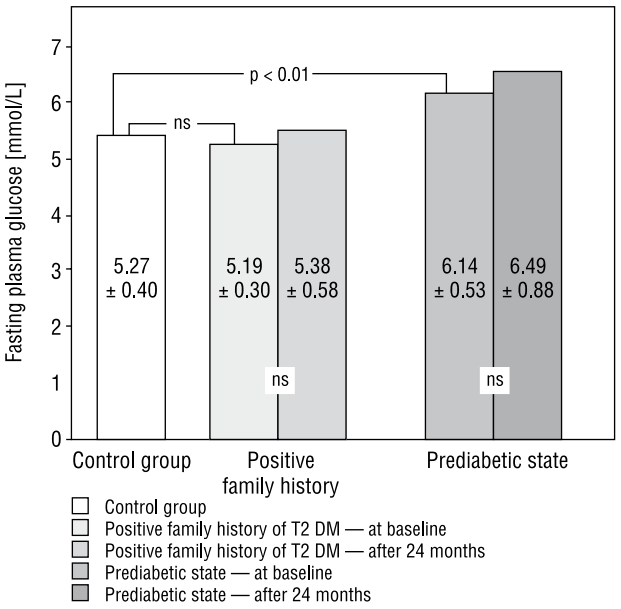

Figure 1. *Comparison of fasting plasma glucose in the studied groups at baseline and after 24 months with the control group*

Rycina 1. *Porównanie glikemii na czczo w badanych grupach wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

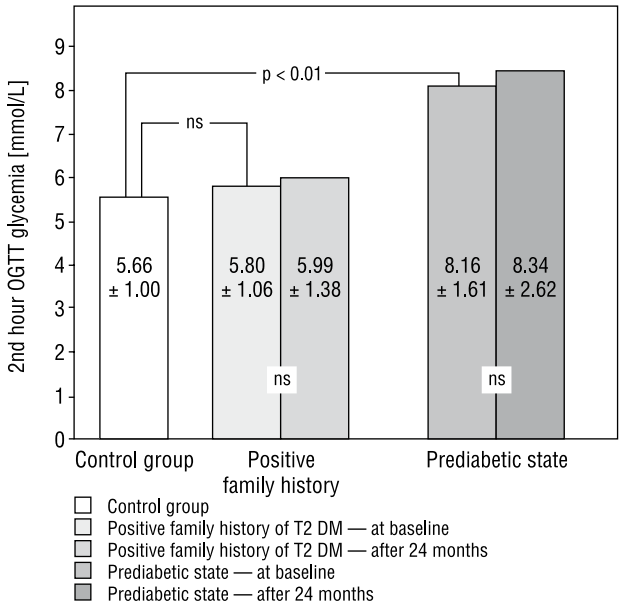

Figure 2. *Comparison of glucose in 120 minutes of OGTT in the studied groups at baseline and after 24 months with the control group*

Rycina 2. *Porównanie glikemii w 120. minucie OGTT w badanych grupach wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

Prediabetic subjects

At the start of the study, a group of people with pre-diabetic state in comparison with the control group was characterised not only by significantly higher fasting glucose (6.14 ± 0.53 v. 5.27 ± 0.04 mmol/L, p < 0.01) (Fig. 1) and glucose at 120 min. of OGTT (8.16 ± 1.61

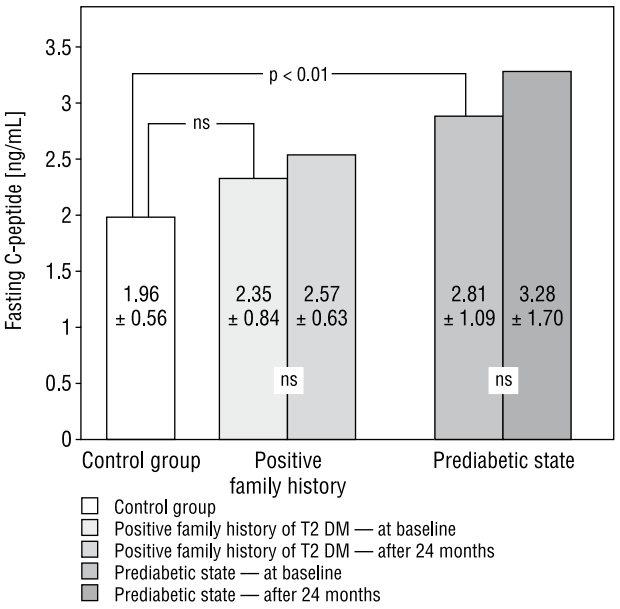

Figure 3. *Comparison of fasting C-peptide in the studied groups at baseline and after 24 months with the control group*

Rycina 3. *Porównanie stężenia C-peptydu na czczo w badanych grupach wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

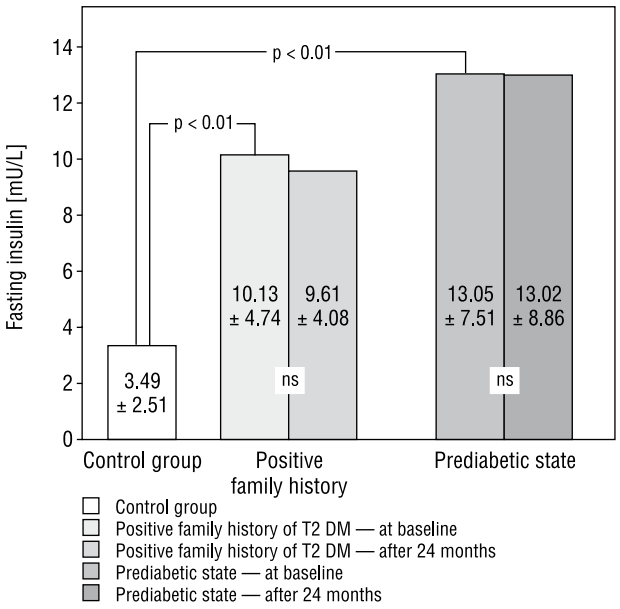

Figure 4. *Comparison of fasting insulin in the studied groups at baseline and after 24 months with the control group*

Rycina 4. *Porównanie stężenia insuliny na czczo w badanych grupach wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

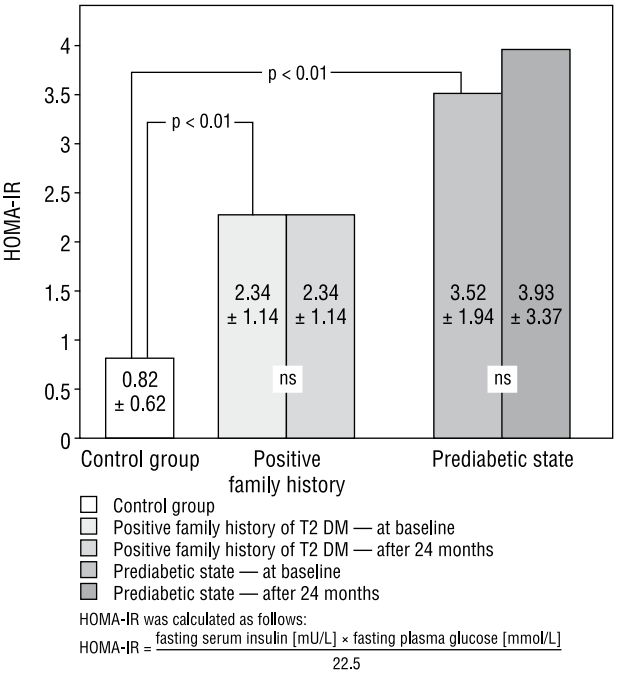

Figure 5. *Comparison of HOMA-IR in the studied groups at baseline and after 24 months with the control group*

Rycina 5. *Porównanie wskaźnika HOMA-IR w badanych grupach wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

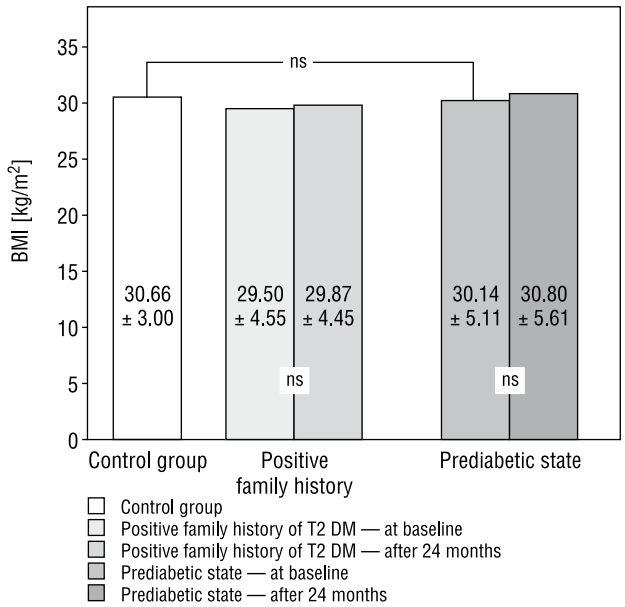

Figure 6. *Comparison of BMI in the studied groups at baseline and after 24 months with the control group*

Rycina 6. *Porównanie BMI w badanych grupach wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

v. 5.66 ± 1.0 mmol/L, $p < 0.01$) (Fig. 2), but also significantly higher rates of insulin resistance: fasting insulin (13.05 ± 7.51 v. 3.49 ± 2.51 mU/L, $p < 0.01$), serum C-peptide (2.81 ± 1.09 v. 1.96 ± 0.56 ng/mL, $p < 0.01$), and HOMA-IR (3.52 ± 1.94 v. 0.82 ± 0.62) (Figs. 3–5).

Similarly to the previously mentioned group, a two-year observation of the pre-diabetic state showed no significant increase in fasting glucose (6.14 ± 0.53 v. 6.49 ± 0.88 mmol/L) or glucose at 120 min. of OGTT (8.16 ± 1.61 v. 8.34 ± 2.62 mmol/L) (Figs. 1, 2), as well as

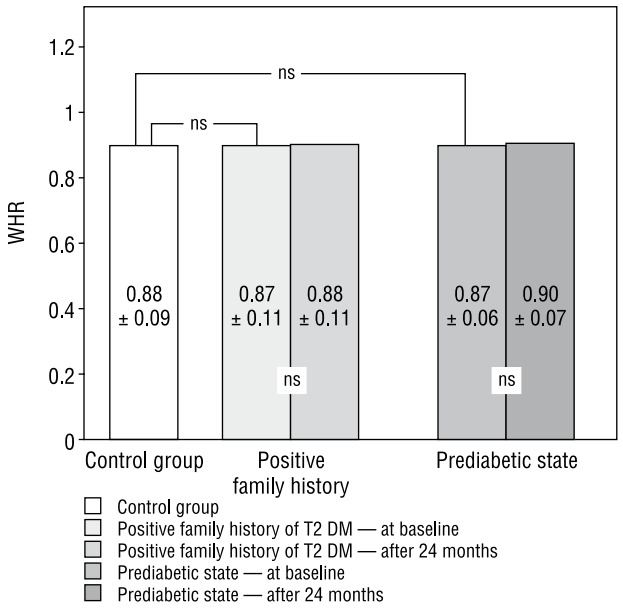

Figure 7. *Comparison of WHR in the studied groups at baseline and after 24 months with the control group*

Rycina 7. *Porównanie WHR w badanych grupach wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

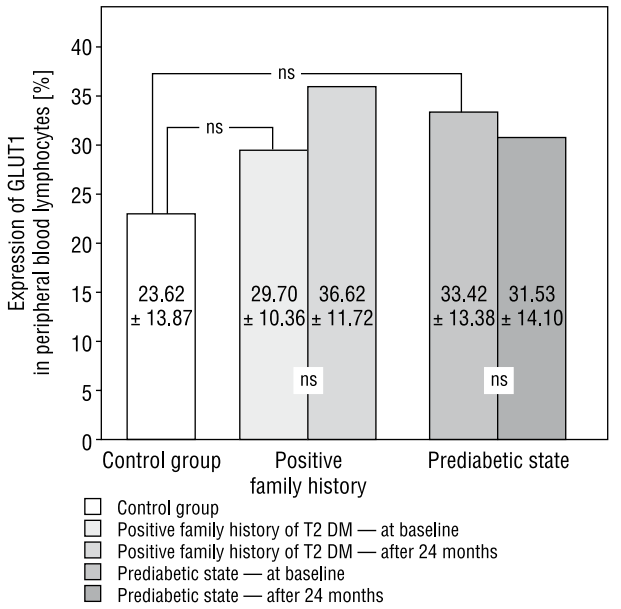

Figure 8. *Comparison of the quantitative expression of GLUT-1 on the surface of peripheral blood lymphocytes in the studied groups at baseline and after 24 months with the control group*

Rycina 8. *Porównanie ilościowej ekspresji GLUT-1 na limfocytach krwi obwodowej w badanych grupach wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

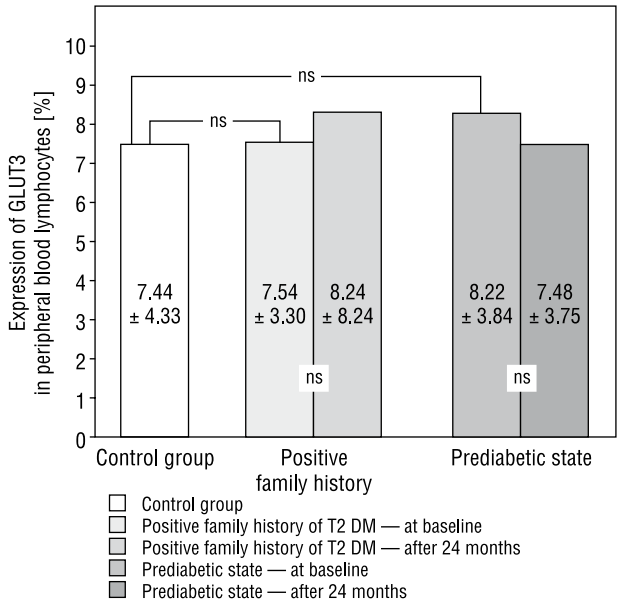

Figure 9. *Comparison of the quantitative expression of GLUT-3 on the surface of peripheral blood lymphocytes in the studied groups at baseline and after 24 months with the control group*

Rycina 9. *Porównanie ilościowej ekspresji GLUT-3 na limfocytach krwi obwodowej w badanych grupach wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

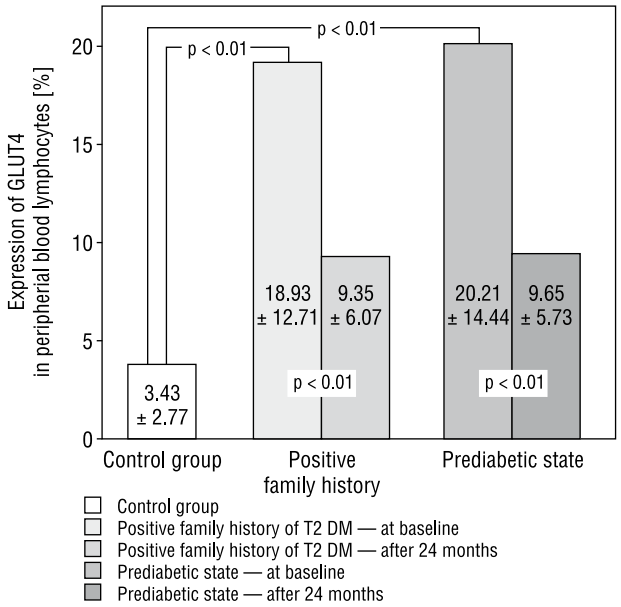

Figure 10. *Comparison of the quantitative expression of GLUT-4 on the surface of peripheral blood lymphocytes in the studied groups at baseline and after 24 months with the control group*

Rycina 10. *Porównanie ilościowej ekspresji GLUT-4 na limfocytach krwi obwodowej wyjściowo i po 24 miesiącach w odniesieniu do grupy kontrolnej*

fasting insulin (13.05 ± 7.51 v. 13.02 ± 8.86 mU/L), serum C-peptide (2.81 ± 1.09 v. 3.28 ± 1.7 ng/mL), HOMA-IR (3.52 ± 1.94 v. 3.93 ± 3.37) (Figs. 3–5), BMI (30.14 ± 5.11 v. 30.8 ± 5.61 kg/m^2) or WHR (0.87 ± 0.06 v. 0.9 ± 0.07) (Figs. 6 and 7) compared to baseline values.

During the two-year follow-up, no significant differences in expression of GLUT-1 (33.42 ± 13.38 v. 31.53 ± 14.10%) or GLUT-3 protein (8.22 ± 3.84 v. 7.48 ± 3.75%) were shown (Figs. 8 and 9). The study revealed a significant decrease in the expression of

GLUT-4 protein (20.21 ± 14.44 v. 9.65 ± 5.73%, $p < 0.01$) on the surface of prediabetic subjects' lymphocytes. The same phenomenon was observed in the positive family history group (Fig. 10).

In the control group, the expression of particular glucose transporter isoforms on the surface of peripheral blood lymphocytes was as follows: GLUT-1 23.62 ± 13.87%, GLUT-3 7.44 ± 4.33%, and GLUT-4 3.43 ± 2.77%.

Discussion

The vast majority of research on the intracellular transport of glucose in carbohydrate metabolism disorders has focused on patients with diabetes, and in the available literature most articles focus on risk groups of type 2 diabetes, including people with pre-diabetic state and individuals burdened with a positive family history of type 2 diabetes [22, 23].

Many years ago, it was shown that people with type 2 diabetes are characterised by a higher expression of glucose transporters on the surface of peripheral blood lymphocytes compared to people with normoglycaemia [18, 19]. It was found that not only in the case of chronic hyperglycaemia, but also in conditions of prolonged hypoglycaemia, there is an increased expression of glucose transporters on the surface of leukocytes. It has been shown that hypoglycaemia induces a significant increase in the expression of GLUT-3 proteins on the surface of monocytes, as well as in GLUT-4 and GLUT-3 proteins on the surface of granulocytes [24]. In this experiment, there was no detection of glucose transporters on the surface of lymphocytes, which contradicts the results of subsequent studies [18, 19, 25].

We demonstrated in this study a decrease in the expression of the main insulin-dependent glucose transporter, GLUT-4, during the 24-month follow-up. This could attest to the positive impact of lifestyle modification on the tissue redistribution of the protein. It is assumed that increased physical activity leads to increased expression of GLUT-4 on the surface of cells that are most sensitive to the action of insulin (muscle cells, adipocytes), while in the remaining cells, the reverse process is observed. Increased expression of GLUT-4 mRNA and/or protein in the cells of skeletal muscle following exercise has been confirmed in several studies [26–30]. Similar results were obtained in the case of experiments on adipocyte models [31]. The authors of the latter work have also shown that an increase in the expression of GLUT-4 on the surface of adipocytes is accompanied by improved insulin sensitivity and decreased plasma concentrations of retinol binding protein (RBP4).

At the beginning of our study, the observation of the GLUT-4 protein expression of lymphocytes in the group of prediabetic people was almost 6-fold higher than in healthy subjects, which corresponds with the results of the experiments mentioned above [18, 19, 25]. However, there are reports that diabetes is characterised not by increased, but by reduced, expression of glucose transporters on leukocyte cell surface [32]. The cited study, comparing the expression of GLUT proteins in specific leukocyte subpopulations in patients with type 2 diabetes and healthy subjects, showed lower expression of GLUT-3 in granulocytes, monocytes and lymphocytes in patients with diabetes, while a lower expression of GLUT-4 was demonstrated only for monocytes.

Our study revealed that disturbances of the cellular glucose transport exist in subjects who are normoglycaemic but burdened with a family history of type 2 diabetes, which means that these abnormalities precede possible glucose metabolism disorders. Our findings are in line with the study concerning glucose transport in skeletal muscle obtained from first-degree relatives of type 2 diabetic patients, which found a 38% decrease of insulin-stimulated glucose transport in these subjects [33]. The authors of the cited study suggest that the impairment of glucose transport should be recognised as an early event in the pathogenesis of type 2 diabetes.

The observed alterations in the expression of GLUT-4 were not accompanied by body weight reduction or a decrease of insulin resistance indicators. Similar results were found in *The Oslo Diet and Exercise Study* (ODES), in which the authors did not find any correlation between lifestyle modification and insulin resistance indicators [34]. The decrease of GLUT-4 protein levels during 24 months of observation may reflect the positive influence of lifestyle modification. However, it cannot be unambiguously stated, because of the following limitations of our study: the lack of strict supervision over putting into practice the recommendations concerning the physical activity and low-calorie diet, the relatively short time of the observation, and the limited number of participants.

Therefore, it cannot be unequivocally stated that the decrease in GLUT-4 expression in patients in the pre-diabetic state observed over the 24 month period is not the result of lifestyle modification, but constitutes a part of the natural history of the disease, which would acknowledge the fact that changes in the expression of GLUT-4 were not accompanied by weight reduction or a decrease in the indicators of insulin resistance.

The current state of medical knowledge does not permit overcoming these concerns, because intracellular glucose transport issues remain largely unclear. Further discoveries in this area are likely to contribute to a better understanding of the pathophysiology of diabetes, and

may allow the development of more sensitive diagnostic tests, enabling early identification of those at the highest risk of diabetes. Such people would gain greater benefits from preventive actions.

Conclusions

The two-year observation did not reveal any significant differences either in studied insulin resistance indicators or GLUT-1 and GLUT-3 expression on lymphocytes in the prediabetic subjects as well as in subjects with a positive family history of type 2 diabetes.

The assessment of the expression of glucose transporters isoforms typical on peripheral blood cells, such as GLUT-1 and GLUT-3, and also an examination of insulin resistance indicators, are insufficient to monitor the progression of metabolic disorders in individuals at high risk of type 2 diabetes.

The determination of GLUT-4 on the surface of peripheral blood lymphocytes can be a useful tool to evaluate the efficacy of therapeutic actions in prediabetic subjects as well as in subjects with a positive family history of type 2 diabetes.

The decrease in expression of the main insulin-dependent glucose transporter, GLUT-4, on the surface of lymphocytes in the examined subjects is probably a reflection of the positive impact of lifestyle modification on the tissue redistribution of that glucose transporter.

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