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МАЗМҰНЫ

CONTENTS

Preface

Diabetes mellitus is a severe disease, and its prevalence is dramatically increasing world-wide. The complications associated with the disease include cardiovascular disease, blindness, amputations, end-stage renal disease, kidney dialysis, and kidney transplantations and present major public-health problems. Furthermore, diabetes costs are exceeding meanwhile billions of dollars annually and put tremendous burden on national health care systems. Recent years have seen a significant progress in basic knowledge on diabetes due to enormous research efforts, making possible the development of new technology and therapeutics for diabetes management and care.

It was just diabetes research, when Dr. G.A.Meyramov from the Karaganda State University joined me in 1977 in Karlsburg and started out to study how tryptophan metabolites could induce diabetes in animals. Since then we look back on a very fruitful cooperation between the Karaganda State University and the Institute of Diabetes Karlsburg.

It was a landmark decision of the Faculty of Biology of the University and the Publishing House of this journal to issue a volume focusing on problems in diabetes. Of note, this is the first time in the history of Kazakhstan and Central Asia that there is a special issue of this distinguished journal, exclusively devoted to diabetes research. The Editors of this journal, by inviting contributions from international as well as national diabetes experts, made an important step in enhancing and disseminating knowledge about diabetes but, beyond that, lay emphasis on care and management of diabetes in Kazakhstan.

We trust that readers will welcome the present issue and benefit from the contributions herein provided by experts in the field.

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Is early short-term intensive insulin treatment an option to preserve ß-cell function in type 2 diabetes?

Type 2 diabetes mellitus is a complex metabolic disorder characterized by a relative deficiency of insulin in the presence of hepatic, adipose tissue, and skeletal muscle insulin resistance. The pathological process underlying the ß-cell dysfunction occurs already prior to the disease onset. While at the initial stage, ß-cell mass and insulin secretory function are sufficiently well maintained in the majority of individuals with type 2 diabetes, the later stages are characterized by aggravating insulin deficiency. The clinical course of the disease requires escalating therapy with oral drugs over time and eventually consistent application of insulin at the late stage for control of glycemia. Oral therapies are quite effective in improving the short-term insulin secretory capacity, but are incapable of preventing the inexorable decline in ß-cell function during diabetes progression. On the other hand, long-term use of antidiabetic agents is not without various side effects. Since a series of clinical trials have recently shown that implementation of short-term intensive insulin therapy in individuals with newly diagnosed type 2 diabetes can drastically improve and preserve ß-cell function and induce glycemic remission, this treatment strategy has gained considerable interest. However, whether early intensive treatment with insulin can really provide longer-term protection of the pancreatic ß-cells and may be preferable to other therapy modalities is a question that is not yet clearly established and requires appropriate clinical studies.

Key words: ß-cells function, type 2 diabetes, short-term intensive insulin treatment, glycemic remission.

Diabetes mellitus is a complex metabolic disorder that is characterized by absolute (type 1 diabetes) or relative (type 2 diabetes) deficiency of insulin. Autoimmune-mediated processes trigger dysfunction and early destruction of pancreatic ß-cells in type 1 diabetes, whereas gradual reduction in ß-cell mass, defective insulin secretion and sensitivity are the main factors causing initiation and progression of type 2 diabetes. An early indication of the failing ß-cell is the progressive deterioration of glucoregulation with excessive glucose excursion after carbohydrate ingestion and the sequential occurrence of sustained chronic hyperglycemia at postprandial times and during fasting periods. By the time the disease is clinically diagnosed, ß-cell mass and ß-cell function have declined by 25–60 % [1]. Early functional alterations of ß-cells in individuals with type 2 diabetes characteristically include reduced or absent first-phase insulin/C-peptide response to glucose and blunted or delayed insulin/C-peptide release during a mixed-meal test. Chronic sustained hyperglycemia has been shown to exert deleterious effects on the ß-cells via several pathological pathways, among which apoptosis induced by glucotoxicity is the most harmful lesion. ß-Cell function, i.e. glycemic control, declines more rapidly in poorly controlled than in well-controlled diabetes, as has been documented in the Diabetes Control and Complication Trial (DCCT/EDIC) [2] and the United Kingdom Prospective Diabetes Study (UKPDS) [3]. On the other hand, these trials demonstrated that intensive therapy creates a metabolic memory that slows down the development of diabetic complications. The question arises whether strict glycemic control early in the history of dysglycemia is able to normalize or at least preserve the residual ß-cell function over longer times. A wealth of data from numerous experimental and clinical studies has suggested

that reducing any hyperglycemic stress, either induced by sustained chronic hyperglycemia or excessive glucose variability, leads to amelioration of the ß-cell function [4]. These data provide hope that the factors mediating ß-cell preservation can eventually be identified.

ß-Cell failure in type 2 diabetes

The functional and structural alterations of ß-cells in type 2 diabetes are progressive in nature. Mild postprandial hyperglycemia is the earliest metabolic defect observed in individuals with impaired glucose tolerance. It is of note that, at this stage, the decline in ß-cell function is associated with little increase in peripheral insulin resistance, as reported by Kahn [5] and Gastadelli et al. [6]. As disturbance of glucose homeostasis progresses, postprandial glucose excursions become excessive and prolonged, followed by hypersecretion of insulin to compensate for peripheral insulin resistance. These prandial abnormalities in glucose regulation indicate a deficit in first-phase insulin secretion, which gives rise to insufficient suppression of hepatic glucose production. The resulting hyperglycemia launches a pathological process in which both ß-cell mass and function are greatly lost and insulin substitution becomes unavoidable for patients´ survival. Interestingly, the rapidity of the functional decline depends on a variety of environmental as well as genetic factors. A summary of the potentially most important factors to be involved in ß-cell dysfunction is shown in Figure 1.

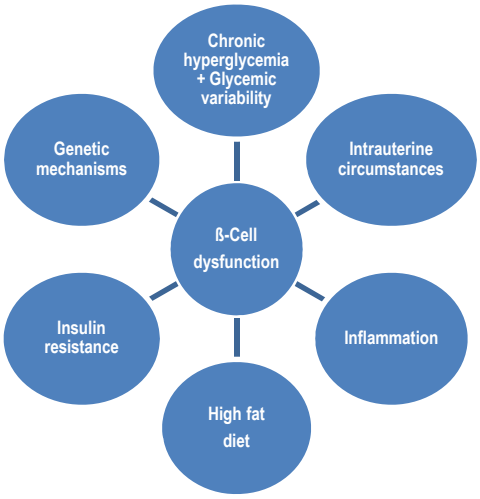

Figure 1. Synopsis of potentially negative factors contributing to ß-cell dysfunction in type 2 diabetes

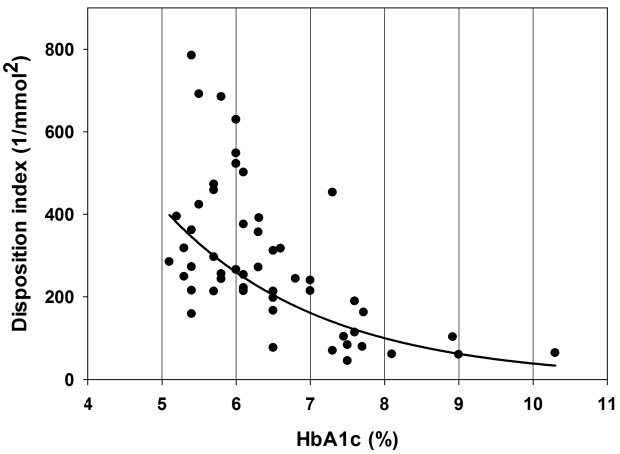

Figure 2. Relationship between ß-cell function measured as disposition index (DI) during withdrawal of ral antihyperglycemic agents and hemoglobin A1c values in a cohort of non-insulin treated type 2 diabetic patients (n = 54). The regression line was obtained by nonlinear regression analysis as DI = 4526.8*exp*(–0. HbA1c); r = 0.683, $P < 0.0001$ (Kohnert et al. unpublished data)

According to current knowledge, there is no doubt that hyperglycemia and excessive fluctuation in glucose levels are main contributors to ß-cell failure [7]. Figure 2 shows that ß-cell function (Disposition index) declines in an exponential manner with worsening long-term glucose control (HbA1c), and patients with HbA1c levels in the range of 5.0–6.5 % maintain better preserved ß-cell function than those above this range, as indicated by higher disposition index values. It should be noted that the disposition index is the most comprehensive measure to express ß-cell function, because it takes whole-body insulin resistance into account.

The pathway from sustained chronic hyperglycemia, increased glycemic variability, and elevated non-esterified fatty acids to ß-cell damage includes glucolipotoxicity, generation of oxidative stress and nitrosative stress through excessive production of reactive oxygen (ROS) and reactive nitrogen species (RNS), respectively [8]. The unbalanced formation of ROS and RNS species promotes lipid peroxidation, protein oxidation, mitochondrial and genomic DNA damage. Furthermore, the interference with signal transduction pathways can lead to ß-cell damage by a various mechanisms [9]. In contrast to animal experiments, evidence for the clinical importance of these processes in humans is so far lacking. Nevertheless, type 2 diabetic islets were shown to contain significantly higher concentrations of stress markers than pancreatic islets obtained from non-diabetic donors, suggesting a causal link between increased oxidative stress and decreased glucose-stimulated insulin secretion. The very low levels of intracellular antioxydant enzymes, such as catalase, glutathione peroxidase, and superoxide dismutase [10] may explain the high vulnerability against ROS and RNS and subsequent changes resulting in a variety of cellular dysfunctions and finally ß-cell apoptosis. Butler et al. [11] ascribed the mechanism responsible for reduced ß-cell volume to a 3- to 10-fold increase in the rate of apoptosis, they observed in obese patients with type 2 diabetes as compared to lean nondiabetic subjects.

Genome-wide association studies identified several risk loci for type 2 diabetes [12], and it has been shown that some genetic risk variants (i.e., TCF7L2, KCNJ11, CDKAL1) act through perturbation of glucose-stimulated insulin secretion. CDKAL1, for example, is strongly expressed in the endoplasmic reticulum and Golgi apparatus in the ß-cell and may affect insulin secretion by causing stress and mitochondrial disruption due to misfolding or defective processing of proteins [13]. BMI-associated variants such as FTO were found to be implicated in regulation of lipid levels [14] and can thus modulate insulin resistance.

Insulin resistance is already established in individuals who are prone to develop type 2 diabetes but still having normal glucose tolerance. High fat diet and its metabolic consequences of increased body mass index are the critical factors in the development of insulin resistance. In order to compensate for the elevated insensitivity of skeletal muscle and liver, ß-cells are forced to hypersecrete insulin owing to chronic sustained hyperglycemia. This *vicious cycle* creates endoplasmic reticulum stress, probably triggering an apoptotic signal with subsequent destruction of ß-cells. It should be noted; however, that insulin resistance can also exist without ß-cell dysfunction.

Of the several mechanisms, which have been proposed to induce ß-cell failure in type 2 diabetes, various components of an inflammatory process are likely to be involved [15, 16]. The observation of amyloid deposits and fibrosis in pancreas section from patients with type 2 diabetes [17] is a strong indication for the occurrence of inflammatory processes in islets. Furthermore, it could be shown by Böni-Schnetzler et al. [18] that hyperglycemia increases interleukin-1ß production at the protein level, a factor that contributes to glucotoxicity. In the presence of elevated glucose concentrations, lipids have shown to exert deleterious effects on ß-cells, and cytokines (i.e., TNFα, interleukin-6, and leptin) secreted by fat cells may act directly or via activation of the innate immune system [19].

ß-Cell dysfunction can already originate *in utero*. Even though there are conflicting reports in the literature, several studies in small-for-gestational-age neonates demonstrated defects in glucose homeostasis [20]; and a study conducted by Nicolini et al. [21] in intrauterine growth restricted fetuses at 26–33 weeks of gestation found a complete absence of the first-phase insulin secretion, whereas Wang et al. [22] reported higher plasma insulin concentrations in small-forestational-age infants at 72 hours *post partal*. Indeed, the prevalence of type 2 diabetes is higher in individuals who had been exposed to intrauterine growth restriction during fetal development. Although the underlying mechanism for disturbances in glucose homeostasis occurring in small-for-gestational-age infants postnatal or later in life are not well understood, fetal under nutrition associated with placental insufficiency appears to be the primary cause.

Antidiabetic therapy and reversibility of ß-cell dysfunction

Although it has been shown that reversal of ß-cell failure and insulin resistance can be achieved without any antidiabetic medication, merely by dietary energy restriction [23], this treatment strategy requires long-

term adherence and does not work in most individuals with type 2 diabetes. Thus pharmacologic intervention is inevitable to control hyperglycemia. Despite growing numbers of antidiabetic agents, the ideal drug that normalizes levels of glycemia throughout day and night is not yet available. In type 2 diabetes, a stepwise approach is routinely used to manage glycemic control. However, this approach has been questioned as it does not ensure consistently good glycemic control in the majority of patients. The «A Diabetes Outcome Progression Trial» (ADOPT) demonstrated, for example, that regardless of the oral drug initially used, monotherapy failed to a remarkable extent. The incidence of secondary therapy failure at five years was 34 %, 21 % and 15 % for glyburide, metformin, and rosiglitazone, respectively [24]. Two main conclusions can be derived from these outc- omes: (1) the therapeutic approach did not address the pathological mechanisms, i.e., progressive declining ß-cell function and (2) monotherapy with oral drugs will surely fail at some time during disease advancement.

Glucagon-like peptide 1 (GLP-1) receptor agonists have shown promising positive effects on ß-cell function and even on ß-cell mass [25]. In a randomized clinical trial, Bunck et al. [26] investigated metabolic effects of exenatide in metformin-treated type 2 diabetic patients. Although these authors observed improvement of ß-cell function and glycemic control during one year of treatment, this effect was not sustained; ß-cell function and glycemia returned to pretreatment levels at 4 weeks after drug discontinuation. Moreover, in a recent, randomized controlled trial, Gudipaty and coauthors [27] evaluated the ß-cell secretory capacity of exenatide with glimepiride as a comparator early in the course of type 2 diabetes and found that the acute insulin response to arginine remained unchanged after 6 months of treatment with exenatide, whereas the sulfonylurea increased the ß-cell secretory capacity.

Analyzing the burden of treatment failure in type 2 diabetes, Brown et al. [28] came to the conclusion that treatment needs to be changed earlier and less likely to fail. As a consequence, antidiabetic therapy both capable of correcting the patho- genetic ß-cell abnormalities, as outlined by Del Prato and colleagues [29], and timely provided may ensure glycemic stability. The proposal by DeFronzo et al. [30] to initiate triple therapy with antidiabetic agents at the earliest stage of the disease is further reaching and in line with the multifactorial nature of type 2 diabetes, as outlined above in Fig 1. A strategy capturing several of the indicated factors at once, e.g., insulin resistance, inflammation, glucotoxicity, and lipotoxicity might be more efficacious than customary stepwise approaches to achieve long-term glycemic control. However, justification for implementation of such an early combination treatment requires large long-term clinical trials.

Initiation of early insulin therapy—potential benefits and negative consequences

The beneficial effect of insulin administration shortly after onset of diabetes has been demonstrated in experimental as well as clinical studies. For example, in pre-hyperglycemic animals and models for type 2 diabetes, insulin administration to normalize glycemia produced improvement of ß-cell secretory function and islet insulin content [31, 32]. In 1996, Kobayashi and colleagues [33] already reported that small doses of subcutaneous insulin prevented progressive ß-cell failure in patients with Latent Autoimmune Diabetes of Adults (LADA) over a follow-up of 30 months. Actually, insulin has been shown to be capable of preventing ß-cell defects [34] by reduction of glucolipotoxicity [35, 36] and inhibition of oxidative stress, as recently shown by Monnier et al. [37].

In the past ten years, several studies have shown that administration of insulin either shortly after onset or early in the course of the disease can improve both ß-cell secretory function and insulin resistance [38–44]. Table summarizes trials that were published from 2003 to 2012 and in which ß-cell function has been assessed, using established methods. It is interesting that in some clinical trials in which initial treatments with insulin and oral hypoglycemic agents were compared head-to-head, both treatment modalities achieved comparable glycemic control. However, the drug-free remission with insulin exceeded by far that attained with oral drugs, for example, 62.5 *vs.* 0.5 %, at 12 months in the study performed by Chandra et al. [45], and similar studies also showed that ß-cell function parameters proved to be better preserved in the insulin than in the oral drug-treated groups [46, 47]. It is also interesting that chronic supplementation of the long-acting insulin glargin produced improvement in the first- and second-phase insulin secretion in type 2 diabetic patients with mean disease duration of 4.6 years, whereas acute insulin injections reduced glucose-induced insulin response [48]. Harrison and colleagues [49] evaluated ß-cell function in drug-naive patients with newly diagnosed type 2 diabetes prior to and 42 months after treatment with insulin and metformin or a combination of metformin, glyburide, and pioglitazone. As the authors did not find any significant change in C-peptide or C-peptide/glucose ratios during the study, they concluded that ß-cell function could be preserved at least for 42 months by either treatment.

Table

Characteristics of recent studies assessing preservation of ß-cell function in type 2 diabetes by short-term insulin treatment

Study	Type of study	n	Mean diabetes duration	Baseline HbA1c (%)	Therapy regimen	Duration of therapy (weeks)	Follow-up of ß-cell function (months)	Euglycemia/ positive response (%)	Assessment of ß-cell function
Alvarsson et al. (2003)	RCT	39	< 2 years	6.9 7.3	OAD Biphasic insulin	24	24	NA	CR (glucagon test), IAPP
Ryan et al. (2004)	Interventional	16	Newly diagnosed	11.8	MDI	2–3	12	44	AUC_I
Li et al. (2004)	Interventional	126	Newly diagnosed	10.0 10.3	CSII	2	24	47	HOMA%B, AIR, AUC_I, AUC_C
Weng et al. (2008	RCT	261	Newly diagnosed	9.8 9.7	CSII MDI	2	12	51	HOMA%B, AIR
Chen et al. (2008)	RCT	50	Newly diagnosed	11.3 11.9	OAD MDI	2	12	NA	HOMA%B
Chandra et al. (2008)	Interventional	60	Newly diagnosed	10.4	Insulin 30/70 OAD	NA	6	80 3	$CR_{Postmeal}$
Chon et al. (2010)	Retrospective	61	Newly diagnosed	10.5 10.8	Biphasic insulin Prandial insulin	6.7 3.7	48	>80	HOMA%B, IGI, ACR
Retnakaran et al. (2010)	Interventional	34	5.9 years	7.0	MDI	4 — 8	4–8	68 FG <7.0 mmol/L	Ratio AUC_C/AUC_G/ HOMA-IR
Pennartz et al. (2011)	Interventional	14	4.6 years	8.4	Basal insulin add-on	8	2	100 FG <7.0 mmol/L	First-phase and second-phase IR
Hu et al. (2011)	RCT	48	Newly diagnosed	10.0	CSII and MDI OAD	2	12	44	HOMA%B, AIR
Chen et al. (2012)	Interventional	118	Newly diagnosed	10.8	CSII	2 — 3	≥12	55	AIR, HOMA%B
Harrison et al. (2012)	RCT	58	Newly diagnosed	6.0	MDI OAD (triple)	12	42	79 81	Ratio AUC_C/AUC_G, $(\Delta C/\Delta G)_{0–30\ min}$

Notes. [a]adapted and modified from Owens [34]. ACR, acute C-peptide response; AIR, acute insulin response; AUC_C, area-under-the-C-peptide-curve; AUC_G, area-under-the-glucose-curve; AUC_I, area-under-the-insulin-curve; CR, C-peptide response; CSII, continuous subcutaneous insulin infusion; FG, fasting glucose; HOMA%B, homeostasis model assessment of ß-cell function; IAPP, islet amyloid pancreatic polypeptide; IGI, insulinogenic index; MDI, multiple daily injections; NA, not available.

A recent meta-analysis [50] has confirmed that early short-term intensified insulin treatment can improve ß-cell function and decrease insulin resistance. Nonetheless, it must also be recognized that the clinical outcomes among the patients revealed a heterogeneous response. Given the phenotypic heterogeneity of type 2 diabetes, it is conceivable that not all participants in the studies listed in Table, but a variable percentage — on the average 58 % after 1 year [34] — did achieve and maintain a drug-free period of euglycemia, i.e. reversibility of ß-cell function after the short intensified insulin treatment. It is thus most important to figure out the key determinants and mechanisms of improvement in ß-cell function. Kramer and colleagues [51] performed a study in type 2 diabetic patients with mean 3-year disease duration and well-controlled glycemia, using intensive insulin treatment consisting of basal and premeal insulin. They found that baseline HbA1c and change in insulin resistance (HOMA-IR) were independent predictors of reversibility of ß-cell dysfunction. This suggests that elevated glucose concentrations *per se* (i.e., HbA1c levels) accelerate decline of ß-cell capacity, as displayed in Fig. 1, and that lowering of insulin resistance importantly contributes to the reversibility of insulin secretory function. The pathophysiological basis for early use of insulin has been presented in reviews by Rolla [52] and Joffe et al. [53].

Considering the study outcomes in favor of early intensive insulin therapy, one might ask whether this treatment modality does have negative consequences.

Because of the phenotypic heterogeneity of type 2 diabetes, it is conceivable that not all patients will benefit from early insulin therapy. As pointed out by Lebovitz [54], patients who initially presenting with severe hyperglycemia will take most advantage of it. Intensive glycemic control has been associated with increased incidence of hypoglycemia [55]. However, patients in the Kramer study [51] had very low rates of hypoglycemia ($\leq$ 3.9 mmol/L), which might reflect the moderating contribution of the endogenous insulin reserve, as the authors emphasized. Initiation of insulin therapy in a later stage of disease progression, following failure of oral antidiabetic drugs, requires usually higher doses than in an early stage to control glycemia and thus increases the risk of hypoglycemia and weight gain. Gain in body weight and insulin treatment go often hand in hand and has been shown to be influenced, for example, by baseline HbA1c, the therapy regimen applied, treatment duration, and oral drugs used in combination with insulin. Use of lower insulin doses has the advantage of hampering weight gain [56]. Thus, the dosage used in early short-term intensive insulin treatment is unlikely to provoke weight gain in patients with type 2 diabetes.

Conclusions

Based on pathophysiological evidence, initiation of insulin therapy is evidently the most effective strategy to control glycemia. Insulin has the unique capability of correcting factors involved in the progressive decline of ß-cell function, such as first-phase insulin secretion, insulin resistance, glucolipotoxicity, and inflammation. A number of recent studies have clearly demonstrated that early short-term insulin therapy may modify the disease progression by protecting and restoring ß-cell function. The benefits of insulin therapy are still offered to late, i.e. when ß-cell mass and function are largely lost. It is of utmost importance to preserve ß-cell function in order to maintain good glycemic control to prevent late diabetes complications and improve patients´ quality of life. As the response to short-term intensive insulin treatment is variable, phenotype-targeted therapy may be required to gain the biggest advantage from this intervention. More studies will certainly be needed to verify the current findings and clarify the question whether early intensive treatment with insulin can really alter disease progression by providing long-term protection of ß-cell function.

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Қысқа мерзімде инсулинмен қарқынды емдеу диабет ауруының 2-түрі жағдайында β-жасушалардың қызметін сақтау мүмкіндігі болып табылады ма?

Диабеттің (сусамырдың) 2-түрі бауырда, май ұлпасында және қаңқа бұлшық еттерінде инсулиннің салыстырмалы жеткіліксіздігімен сипатталатын зат алмасудың күрделі бұзылуы болып табылады. В-жасушалардың дисфункциясының негізіндегі патологиялық процесс аурудың басталуы кезінде байқалады. Дегенмен В-жасушалардың және инсулин бөлуші қызмет диабеттің 2-түрімен ауыратын көптеген адамдарда бастапқы кезеңде сақталып, ал соңғы кезеңдерде инсулиннің жетіспеушілігі арта түседі. Пероральды емдеу инсулин секрециясының қысқа мерзімді мүмкіндіктерін жақсартуда неғұрлым тиімді әдіс болып табылады, бірақ диабеттің қарқынды өршуінде, В-жасушалардың функциясының әлсіреуінің алдын алуда қауқарсыз болады. Клиникалық зерттеулердің бірқатары диабеттің 2-түрі алғаш анықталған науқастарға қысқа мерзімді қарқынды инсулинмен емдеу терапиясы В-жасуалардың функциясын сақтап және көрсеткіштерді жақсарта отырып, ремиссия туындауына себепкер болды. Алайда науқастарға қысқа мерзімді қарқынды инсулинмен емдеу терапиясы панкреатиттік В-жасушалардың ұзақ мерзімді қорғанысын қамтамасыз ете алады ма және емдеудің тиімді түріне жатама деген мәселе алдағы клиникалық зерттеулерді талап етеді.

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Является ли краткосрочная инсулинотерапия способом сохранения функции В-клеток при сахарном диабете 2 типа?

Сахарный диабет 2 типа — сложное метаболическое заболевание, характеризующееся относительным дефицитом инсулина, нарушениями функции печени, жировой ткани и резистентности скелетной мускулатуры к инсулину. Патологический процесс, лежащий в основе дисфункции ß-клеток, развивается еще до начала проявлений болезни. На начальной стадии масса ß-клеток и их инсулинпродуцирующая функция достаточно хорошо поддерживают больного с диабетом 2 типа, однако на более поздних стадиях дефицит инсулина усиливается. В клинической стадии требуется усиление терапии таблетированными препаратами, однако на более поздних стадиях необходимо применение инсулина для контроля гликемии. Пероральная терапия весьма эффективна для краткосрочного улучшения секреции инсулина, но неспособна предотвратить неумолимое ослабление функции клеток в процессе прогрессирования диабета. С другой стороны, длительное использование антидиабетических препаратов не позволяет избежать их побочного действия. Недавние клинические испытания показали, что краткосрочная интенсивная терапия инсулина у лиц с недавно диагностированным типом 2 диабета может значительно улучшить и сохранить функцию ß-клеток и способствовать ремиссии. Однако может ли раннее интенсивное лечение инсулином действительно обеспечить защиту ß-клеток на более длительный период и имеет ли преимущество перед другими видами терапии — это вопрос, который требует соответствующих клинических исследований.

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Assessment of glucose profiles in routine diabetes care

The regular documentation of glucose measurements during insulin and / or oral drug therapy, meal intake, and special events in the daily life are crucial for doctors in the treatment of patients with diabetes mellitus. Therefore, we developed a method, which allows objective, rapid and comprehensive review of glucose profiles for the first time. On the basis of glucose values, either recorded by self-monitoring of blood glucose or by a continuous glucose monitoring with a sensor system, the Q-Score can easily be computed. Classification by Q-Score is simple, time-saving, and useful in terms of daily treatment routine. In addition, it involves analysis of the contribution of individual glycemic components expressed as the Q-Score and enables assessment of therapeutic efficacy. The method for determining the Q-Score, evaluation of glucose readings and self-control data as well as presentation of results and the therapeutic advancement will be implemented into the telemedicine information and communication system TeleDIAB®, which is available via world-wide.

Key words: glucose profile, diabetes care, TeleDIAB, sensor system, insulin therapy.

Introduction

HbA1c reflects glycemic control of the last 8–12 weeks and is the gold standard in diabetes care. However, the advantage of glucose profiles for the assessment of metabolic control is actually intensively discussed [1–3]. Among others, glucose profiles allow evaluation of the variability, show trends for hypoglycemic events, and can be used to find causal relationships between glucose excursions, therapeutic interventions, and meal intake. Glucose profiles representing the actual metabolic control of the patient and are suitable to detect deficiencies in the metabolic management. In order to characterize glucose profiles and find appropriate parameters, a large number of studies have provided a bulk of data. More than 30 metrics for characterization of glucose profiles were published of which each focused only on a single aspect, e.g. the variability within one day, the mean glucose level, risk for hypo- or hyperglycemia and the mean of daily differences in the course of glucose measurements [4–17]. Routine clinical use by calculating up to 30 parameters for complete characterization of glucose profiles seems to be un- realistic. Moreover, an intra- and inter-individual comparison of glucose profiles will be difficult or even impossible. Therefore, we have recently developed a method that allows for the first time objective assessment of the quality of glucose profiles by calculating one single value, which we call Q-Score [18]. The Q-Score integrates 5 specific components of glucose profiles into a single value. Using the Q-score, physicians can obtain both an objective assessment of glucose profiles from their patients and derive therapeutic recommendations to improve glycemic control.

Methods

Using historical data from continuous glucose monitoring (CGM, n = 1562), 15 parameters, which are considered important for assessment of glucose profiles were calculated. The CGM data were obtained during a diabetes care program running from 2006 to 2010 in Germany. In order to identify those factors that determine the characteristics of the glucose profiles, a factor analysis with principal component analysis and varimax rotation was performed. This analysis yielded five primary factors that determined the glucose profile characteristics. As shown in Figure 1, these factors are mean glucose, glucose range, hypoglycemia (thypo), hyperglycemia (thyper), and mean of daily difference (MODD) which were used for constructing the formula for calculation of the Q-Score.

For practical use, the Q-Score was classified into the following categories: no risk, very low risk, moderate risk, high risk, and dangerous (Figure 2). This classification was verified by evaluation of n = 766 CGM profiles by independent diabetes specialists. They allocated the CGM profiles to one of the classifications listed below. The results of the classification were highly correlated with the specialists judgement (Kendall's tau = 0.671, 0.787 and 0.751; $p < 0.001$).

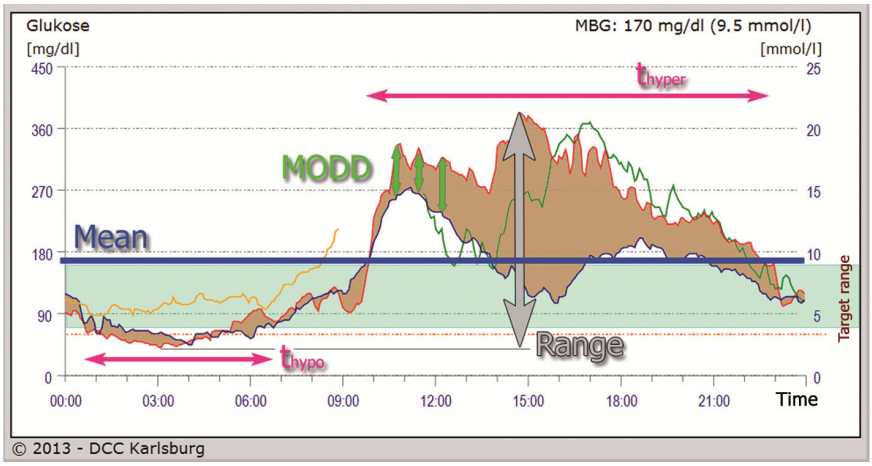

Figure 1. Characteristic metrics of glucose profiles constituting the Q-Score

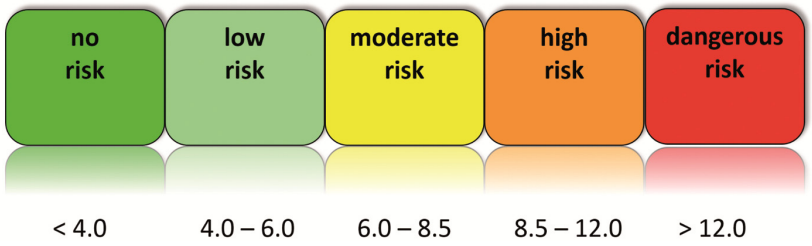

Figure 2. Q-Score classifications for assessment of glucose profile

Results and discussion

To probe the «selectivity» of the Q-Scores, glucose profiles of patients with diabetes were assigned to each of the category indicated in Figure 3. It can be seen that the values of the Q-Score correlated very well with the quality of glycemic control, as reflected by the glucose profiles of diabetic patients. Unlike the HbA1c value, regarded as long-term parameter for glycemic control, the Q-Score provides an objective view of the current metabolic status. For the Q-Score, the following categories were established: «no risk» < 4.0; «very low risk» 4.0 to 6.0; «moderate risk» from 6.0 to 8.5; «high risk» 8.5 to 12.0; «dangerous» > 12.0.

The assessment of glucose profiles using the Q-Scores shows the attending physician immediately the current status of metabolic control of his/her patients. Such a profound and grounded evaluation can not be derived from notebook or diary entries [19]. The calculation of the Q-Score provides an objective assessment of the glucose profiles whereas the assessment of diary data by more than one people may lead to individual differences. In contrast to existing services using notebook or diary data providing statistical methods to assist judgment of glycemic control the Q-Score summarizes important components for estimating the risk. This could be of advantage in routine clinical use.

One might ask what is the advantage of the Q-Score over other evaluation methods have? — Demonstrated by 3 examples of glucose profiles for each category in Figure 3, it can be seen that the individual glucose profile patterns within a specific category, e.g. in the case of «high risk», are different. This is due to the fact that, in individual cases, different factors contribute to the classification as «high risk». The profiles of the middle graph (Q-Score = 8.9) in the category «high risk» are characterized by pronounced hypoglycemic phases, whereas in the left panel (Q-Score 10.2) hyperglycemia is the main problem for poor metabolic control. Thus, the Q-Score addresses pertinent problems in relation to the current metabolic control.

As afore-noted, various components are involved in the evaluation of daily glucose profiles and calculation of the Q-Score, as illustrated in Figure 3. Analysis of the quantitative contribution of each component provides the therapeutic potential for improvement of glycemic control and can thus be used for personalized recommendations to overcome weakpoints (Fig. 4).

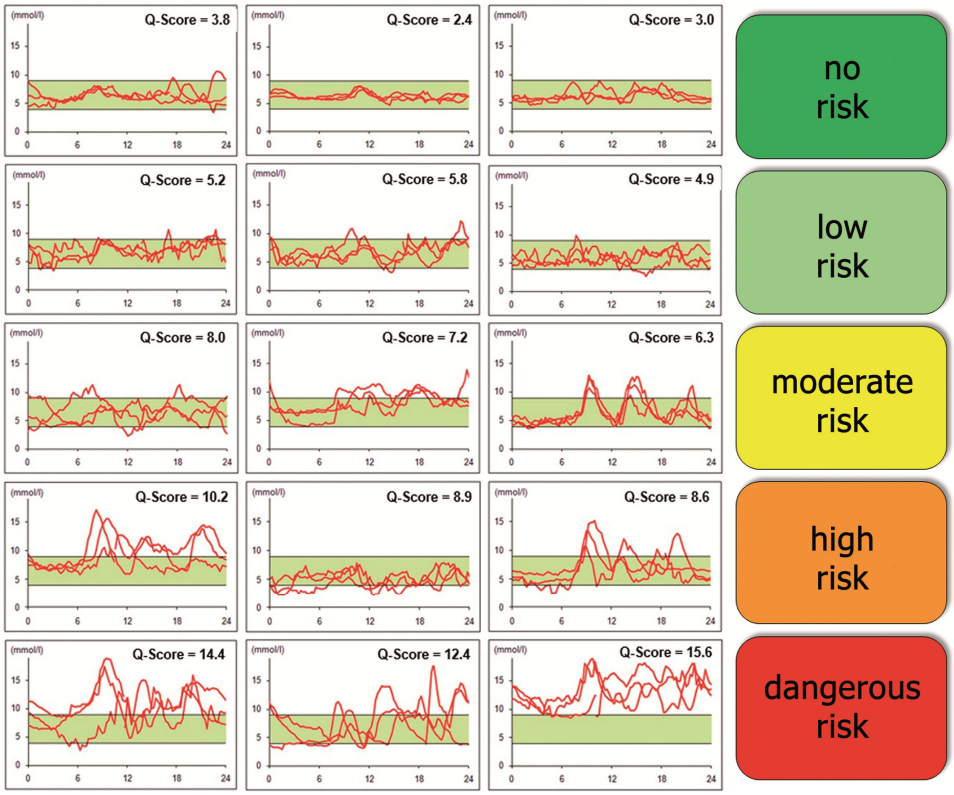

Figure 3. Categories of the Q-Score

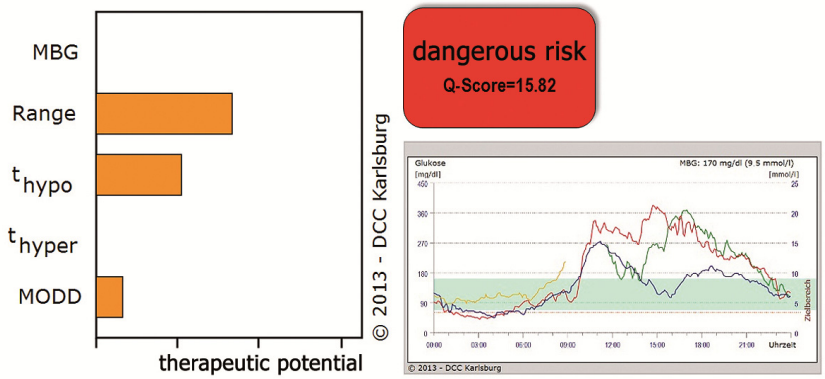

Figure 4. Example demonstrating the metabolic components that constitute the Q-Score category

In the example shown in Fig. 4, both the nocturnal hypoglycemia and the large day-time variation of glucose levels are mainly responsible for the poor metabolic control. The differences between the individual 24-h profiles (MODD) are pronounced only during daytime hours, while at night, almost identical curves with low glucose levels are observed. This leads to the conclusion that in this case changing eating behavior may be beneficial to reduce hyperglycemia. The nocturnal phases of hypoglycemia, however, could indicate that overdosing of antidiabetic medication is the main problem in controlling glycemia. Overall, these examples demonstrate how valuable evaluation and assessment of glucose profiles could be for a family physician, using the Q-Score.

For the case shown in Figure 4, the corresponding results of therapeutic interventions based on the Q-Score analysis are demonstrated in Figure 5. Glucose monitoring after 12 and 24 months was accompanied by subsequent improvement in the Q-Score, illustrating close relationships between scoring and metabolic control.

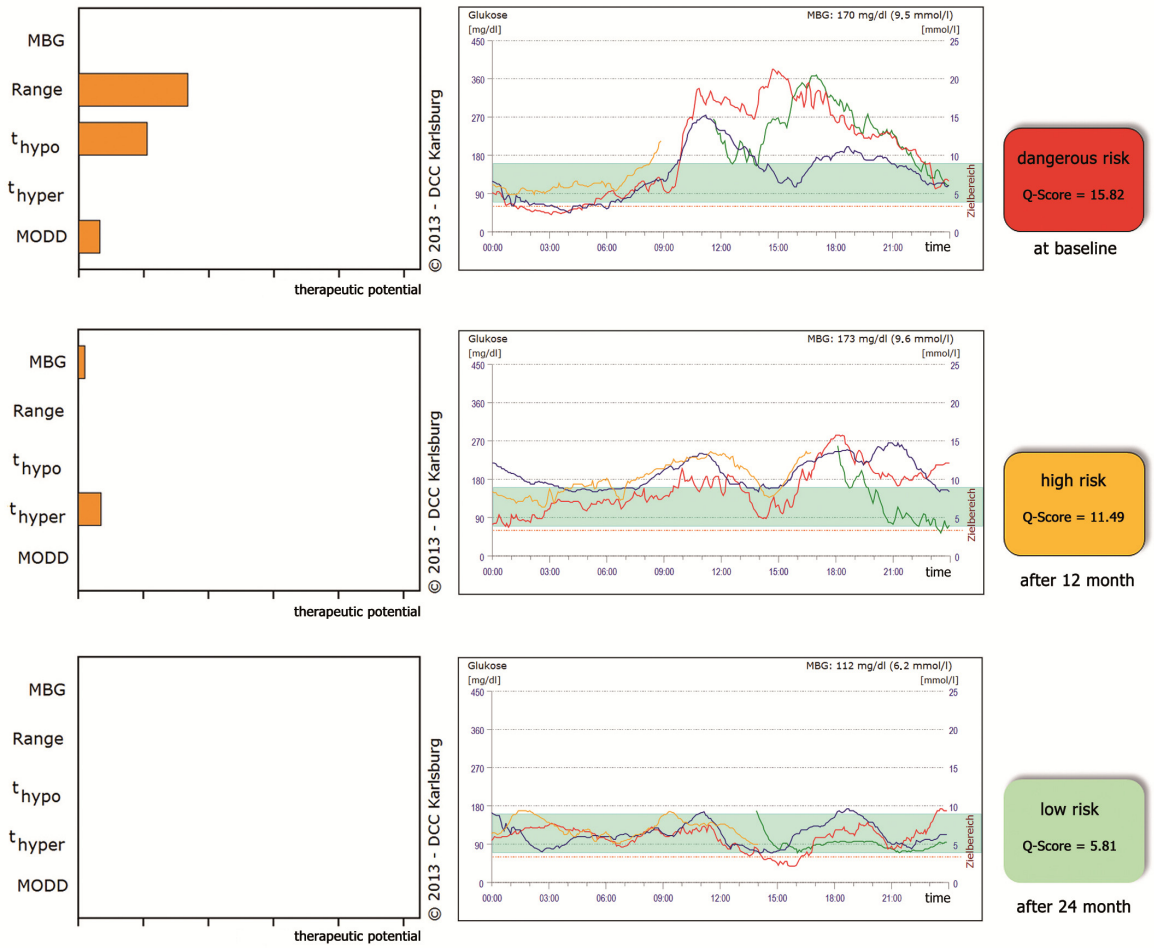

Figure 5. Representative example showing change of Q-Score categories along with improvement in glycemia

Especially when glucose measurement is performed with test strips, as it is usually the case in the majority of patients, objective and comparable assessment of metabolic control could be obtained by using the KADIS®Program [18] and calculation of the Q-Score. Additional laboratory tests are not required for calculation of the Q-Score to get the full picture of the patient´s glycemic status. As there are additional therapeutic recommendations that can be derived, the presented approach could be a useful innovation for doctors and patients. Also the online availability will substantially expand the usefulness of the Q-Scores.

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Диабетті күнделікті емдеу үрдісіндегі қанның глюкоза деңгейін бағалау

Глюкозаны және инсулинді өлшеудің немесе пероральды дәрілік емдеу тәсілдері, тамақты пайдалану және күнделікті өмірдегі шаралардың тұрақты құжаттарының нәтижелері диабетпен ауратындарды емдеуде дәрігерлер үшін шешуші маңызға ие. Біз глюкоза деңгейін объективті, тез және жалпы көлемді бағалаудың жаңа әдісін жасадық. Қандағы глюкоза деңгейін өзіндік бақылау кезінде алынған қандағы глюкоза мөлшері негізінде немесе датчиктер жүйесімен глюкозаға үздіксіз мони- торинг жасау жолымен Q-көрсеткіш жеңіл анықталады. Q-Score бойынша жүйелеу қарапайым, уақыт үнемделеді және күнделікті өмірде емдеуде қолайлы. Сонымен қатар оған жекелеген гликемиялық компоненттер мәндерінің талдауы да кіреді, ол Q-көрсеткіш ретінде сипатталады және емдеу тиімділігіне баға береді. Q-Score бойынша емдеу әдісі глюкоза мен өзіндік тексеру көрсеткіштерінің бағасы және нәтижелерді көрсету ақпараттық телемедицина және интернет желісі арқылы қолжетімді, сондай-ақ TeleDIAB R байланыс жүйесі көмегімен жүзеге асырылатын болады.

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Оценка профилей глюкозы крови в процессе повседневного лечения диабета

Регулярная регистрация показателей контроля уровня глюкозы при терапии инсулином или таблетированными препаратами, использование диеты и специальные мероприятия в процессе лечения больных крайне важны для врачей в лечебном процессе. Нами разработан метод, который позволяет объективно, быстро и всеобъемлюще оценить профили уровней глюкозы в начале лечения. На основе определенных у больного показателей уровня глюкозы либо полученных при использовании методов самоконтроля, непрерывного мониторинга концентрации глюкозы с помощью датчиков можно легко вычислить Q-показатель. Его определение является несложным, экономит время, а использование полезно в процессе ежедневной терапии. Кроме того, он включает анализ значения отдельных гликемических компонентов, выраженных как Q-показатель, и позволяет оценить терапевтическую эффективность процесса лечения. Данные определения Q-показателя, оценка результатов анализа уровня глюкозы и данных самоконтроля будут использоваться в телемедицине и системе связи TeleDIAB®, доступной через интернет.

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Histochemical and immunocytochemical investigation of endocrine tissue of pancreas after administration of B-cytotoxic chemicals

Authors showed that intravenous injection amino acids Cystein and L-Hystidine prevent in majority of animals developing of experimental diabetes caused by chemicals formed in B-cells chelat complexes with Zn^{+2}-ions. It was shown that in all cases administration of these amino acids prior injection of diabetogenic chelator as Diphe nylthiocarbazon (Dithizon) accompanied by almost complete absence of binding of Zn^{+2}-ions in B-cells with formation of toxic complexes which result destruction and death of B-cells within short time. Authors suppose that protective effect is determined by presence in molecules of Cystein and L-Hystidine of SH-groups reacted with reacted with Zn^{+2}-ions in B-cells with forming of not toxic complexes and not able to forming of complexes with a Dithizon. Authors showed also that from the used various histochemical and immunocytohistochemical methods insulin staining in B-cell more precise results was obtained using of immunohistochemical and fluorescent Diethylpseudoisocyanine methods. However, Aldehyde-fuchsine method and method Victoria 4R more suitable for investigation state of histostructure of pancreatic islets.

Key words: pancreas, B-cells, Zn^{+2}-ions, Cystein, L-Hystidine.

Background

Okamoto K. showed that Diphenylthiocarbazone (Dithizon) possess ability for selective destruction of B-cells accompanied by developing of diabetes within a few days [1]. More later it was showed that injection of Alloxan, Derivatives of 8-oxyquinolin and Streptosotozin result selective death of B-cells too. Among methods for investigation result of action of these substances on islets histochemical and immunocytochemical methods have a few advantages as: 1)detail analysis of state of histostructure of islets; 2) analysis of insulin and Zn^{+2}-ions content and disposition of hormone in cytoplasm of B-cells; 3) reveal the early histological and histochemical changes in islets. Pancreatic B-cells contained a large amount of Zn^{+2}ions [2–4] as salivary glands and prostate. In B-cells Zn^{+2}-ions take part in processes of biosynthesis of insulin as in of storage by forming of Zn^{+2}-insulin complex [5, 6]. It is known that Zn^{+2}-ions in B-cells formed with insulin a deposited form of hormone as Zn^{+2}-insulin complex [5]. Proinsulin forms a zinc containing hexamer soon after its synthesis. In addition the zinc ions enhance proinsulin solubility and render insulin insoluble. Zinc ions also appear to play an important role in the microcrystalline character of the precipitated insulin granule [1]. Pancreas of rat, rabbit, dog, cat, some fish, human, birds, mice, hamster, porcine, hoerst, contained a large amount of Zn^{+2}-ions [1]. By electron histochemistry method it was showed that Zn^{+2}-ions concentrated in B-granules only contained deposited form of insulin [7] and that destruction of B-cells caused by Dithizon, which formed in B-cells toxic complexes with Zn^{+2}-ions, started by destruction of B-granules [8, 9].

Aim of work: to investigate influence of 2 groups of diabetogenic chemicals on histostructure and insulin content in B-cells of pancreatic islets: 1) diabetogenic zinc-binding substances as Diphenyldithiocarbazone (Dithizon) [DZ] and 8-para(toluenesulphonylamino)quinolin [8PTSQ]; 2) SH-contains aminoacids as Gluthation Reduced form, Cystein, Hystidine and Na salt of Diethyldithiocarbamic Acid [DDCA]; 3) to compare results of staining of islets by various histochemical and immunocytochemical methods as to compare results of action of diabetogenic substances on B-cells in experiences on animals and on isolated pancreatic islets.

Methods

Animals. 14 Rabbits 2240–3050 g, 22 Rats 158–175 g and 12 white mice 33–42 g were used. 1. Experiences with Dithizon. 2. Experiences with 8PTSQ. 3. Experiences with Na salt of Diethyldithiocarbamic Acid [DDCA]. 4. Experiences with Cystein and Hystidine.

Dithizon [DZ] as 8PTSQ possess a high chemical affinity for Zn^{+2}-ions and in vitro formed color complexes as Zn^{+2}-chelator [1, 2]. 8TSQ formed fluorescent green complexes with Zn^{+2}-ions visible using fluorescent microscopy and Dithizone formed red DZ-Zn^{+2}-ions complex visible using dark microscopy. Maximum of absorbance of Zn^{+2}-DZ complex on spectrum of absorbance correspond for 530 nm [3]. 8PTSQ is very sensitive for revealing of Zn^{+2}-ions in solutions contained minimal concentrations as 10^{-7}–10^{-8} of Zn^{+2}-ions and is used for color revealing of its in solutions. Diabetogenic properties of all these substances were established previously and determined by ability to form complex salt with Zn^{+2}-ions in cytoplasm of B-cells that result necrosis and death of cells within short time [4, 5].

Na salt of Diethyldithiocarbamic Acid [DDCA] possess a high affinity for Zn^{+2}-ions too [17]. DDCA formed not toxic for B-cells complex with Zn^{+2}-ions and not result developing of experimental diabetes [8]. Contrary, binding of Zn^{+2}-ions by DDCA, injected in dose of 1000 mg per kg body weight animals protect B-cells in 95 %-100 % animals of death and of developing of diabetes caused by DZ and diabetogenic derivatives of 8-oxyquinolin for 12–24h [5].

Preparing of solutions

Preparing of 8PTSQ solution: 25 mg. of 8PTSQ (Inst. High Pure Chemicals, Moscow) was dissolved in 65 % Ethanol on +70º Celsium and injected to Rabbits 35,5–38,8 mg/kg [9]. Preparing of DDCA solution: 1000 mg of DDCA (MERCK, Germany) dissolved in 10 ml of bidistillate.

Frozen sections of Rat's Pancreas 4 mcm were investigated 10 min past injection using dark microscopy. Intensity of staining was measured by photometer. 2nd part of pancreas tissue was fixed in Ethanol 70 % contained dissolved H_2S; paraffin sections of tissue were stained by 0,4 % acetone solution of 8PTSQ [6] and were investigated on fluorescent microscope.

Pancreas tissue was fixed in Bouin 24h. Staining technologies. Following methods were used for staining 4–5 mcm paraffin sections of pancreas.

Aldehyde-fucshine method by Gomori G. Violet granules in cytoplasm of B-cells correspond to deposited form of insulin [10–13].Intensity of color of cytoplasm of B-cells directly correspond to insulin content in cytoplasm [12–13]. Insulin content was calculated as parameter K=AB1/AB2 where: AB1 — density of staining of intact B-cells; AB1 — density of staining of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Diethylpseudoisocyanine fluorescent method. Schiebler T. and Schiessler S. showed that A chair of oxidized insulin reacted with Diethylpseudoisocyanine chloride with formation of red fluorescent complex which fluoresces in UV light 360–370 nm. We have used modernized by Coalson R.E. method [14–15].

This method method is used not often, that is why we offer the detailed description of staining procedures. Preparing of staining solution: 0,04 % water solution of Diethylpseudoisocyanine (SERVA, Germany). Staining procedures: 1) deparaffinization of sections in xylol; 2) alcohol 90º, 80º, 70º 1 min in each; 3) washing in cold water; 4) oxidation 0,5–2 min; oxidation solution: 5 ml of 5 % H_2SO_4 + 5 ml 2,5 % solution of $KMnO_4$ + 30 ml bidistillate at +28º Celsius; 5) washing in cold water; 6) 5 % solution of oxalic acid — 5 sec; 6) washing in 2 portions of cold water; 7) 0,04 % cold solution of Diethylpseudoisocyanine — 20 min in refrigerator at +4º Celsius; 8) washing in cold water 5 min; 9) store in refrigerator 1,5–3 h. Insulin content was calculated as parameter K = IF1/IF2 where: IF1 — intensity of fluorescence of intact B-cells; IF2 — intensity of fluorescence of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Victoria Blue 4r method staining of insulin (V4R), Diphenylnaphthylmetane, colour index 42563; MERCK, Germany; FERAK, West Berlin). It was showed by F.Wohlrab (16) that V4R in aqueous solution interacted with oxidized A-chair of insulin that is accompanied by painting of cytoplasm of B-cells in a blue color proportionally to the amount of insulin [16]. V4R paints some peptides hormones but B-cells produce insulin only. This method method is used not often, that is why we offer description of staining procedures. Staining procedures: 1) deparaffinization of sections; 2) washing in cold water a few min; 3) oxidation 3–5 min (oxidation solution: 0,3 % $KMnO_4$ 50 ml + 0,3 % H_2SO_4 50 ml; wash sections; 4) place sections in 2–5 % water solution of natrium bisulphate — 1 min; wash sections; 5) 70º alcohol — 1 min; 6) stain in staining solution (96º alcohol 100 ml + Victoria Blue 4R — 1 g) 15 min — 2h; wash sections; 7) stain in 0,5 %

water solution of Phloxine 30–120 sec.; wash sections; 8) 5 % water solution of phosphortungstic acid 1–2 min; wash section in water; 9) stain in 0,5 % water solution of Light Green 1–2 min; 10) dehydratation in 96 % alcohol. Method was adopted for using of sections of tissue culture of islets [17]. Insulin content was calculated as parameter K = AB1/AB2 where: AB1 — density of staining of intact B-cells; AB1 — density of staining of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Staining by Dithizon. Preparing of Dithizon solution: 30 mg of Dithizon, (SIGMA, USA) + 10 ml bidistillate + 0.2 ml 25 % NH_4OH 10 min. mixing on temperature +70º at Celsium. Solution was injected intravenously to Rabbits and to Mice 46–48,6 mg/kg.

Frozen sections of 4 mcm were investigated 5–10 min past injection on dark microscopy. Density of staining was measured using photometer. Insulin content was calculated as parameter K=AB1/AB2 where: AB1 — density of staining of intact B-cells; AB1 — density of staining of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Staining by 8PTSQ. Zn^{+2}–8PTSQ complex radiates intensive green fluorescence under UV-light 360–370 nm length of wave that was confirmed by spectral analysis [18–20]. Cytoplasm of B-cells not contained Cadmium. Past long time prolonging testing in Institute of High Pure Chemicals (Moscow) 8PTSQ was proposed as fluorescent reagent for identification of very small amounts of Zn^{+2} in solutions and tissues. Later by Y.A.Lasaris and coll. 8PTSQ was tested for revealing Zn^{+2}-ions. 8PTSQ is high specific reagent for staining of Zn^{+2}-ions in pancreatic B-cells. Frozen sections of rat's Pancreas 4 mcm were investigated on fluorescent microscope. Staining procedures: 1) staining by 0,4 % acetone solution of 8PTSQ; 3–4 drop of solution placed on section; wash section by 3 portions of bidistillate. Intensity of fluorescence was measured [21]. Insulin content was calculated as parameter K=AB1/AB2 where: IF1 — intensity of fluorescence of intact B-cells; IF2 — intensity of fluorescence of B-cells past action of diabetogenic substances (calculated as 1,00).

Immunofluorescent staining of insulin. Anticorps for insulin (Institute of Diabetes «Gerhardt Katsch») were used for staining sections of pancreas tissue.

Immunohistochemical method. Standard kits for insulin (DAKO, Denmark) were used for staining. Insulin content was calculated as parameter K=AB1/AB2 where: AB1 — density of staining of intact B-cells; AB1 — density of staining of B-cells past action of diabetogenic chemicals (calculated as 1,00).

Isolation of pancreatic islets by Collagenase

Animals. Pancreas of 14 rats LEWIS 4–5 days old and 8–10 weeks old human embryons were used. Isolation procedures: dissected pancreas tissue were treated 3 times 3 min each by 2 % solution of Collagenase (Boehringer Mannheim, Germany; FLUKA, Switzerland); human embryons pancreas was treated by Collagenase 2 times 1 min each; rinse 3 times in cold Hanks solution and centrifugation; cultivation 12h at +37º Celsius in medium RPMI 1640 (SERVA, Germany) with bovine serum + 5.5 mM of Glucose, pH 7.32–7.41. Fixation in Bouin 15 min – 1 h and embedding in paraffin. Sections 4 mcm were used. Dithizon solution 0,4 ml was added in 10 ml of nutria media 199 contains islets for 20 min that correspond to concentration about 40 mg/kg in experiences on animals. Than media 199 was changed for new fresh portion + 5,5 mmol/l of Glucose + bovine serum; cultivation 5h at pH=7,34–7,41.

Results

Isolated pancreatic islets. Intact islets.

Aldehyde-fuchsine staining. Intact islets. Histostructure of islets without histological changes. Islets have oval form and contains deposited insulin (blue-violet color) (fig. 1.1). Insulin content: AB=1,93±0,06.

Victoria 4R staining. Histoctructure of islets without histological changes. Islets contains a large amount of deposited insulin (fig. 1.2). Insulin content: AB=1,97±0,05.

Immunohistochemistry. Histoctructure of islets without histological changes; B-cells contains a large amount of deposited insulin (fig. 1.3). Insulin content: AB=1,81±0,04.

Diethylpseudoisocyanine method. Histoctructure of islets without histological changes; Red fluorescence of A-chair of insulin; insulin content: IF=2,02±0,05.

Fluorescent staining of Zn^{+2}-ions. Intensive green fluorescence of B-cells (fig. 1.5): IF=2,08±0,05.

Past action of Dithizon

Aldehyde-fuchsine staining. Necrosis, destruction and death of B-cells; marked decreasing of insulin content in majority of B-cells (fig.1.6). Insulin content: AB=1,14±0,04.

Victoria 4R staining. Destruction of islets, destruction and death of B-cells; decreasing of insulin content in majority of B-cells (fig. 1.7): AB=1,38±0,05.

Immunohistochemistry. Deformation of islets; destruction and death of B-cells; decreasing of insulin content in majority of B-cells (fig.1.8): AB=1,18±0,04.

Diethylpseudoisocyanine staining. Destruction of islets; marked decreasing of insulin content in B-cells (fig. 1.9): IF=1,07±0,011.

Fluorescent staining of Zn^{+2}-ions. Almost complete disappearing of Zn^{+2}-ions from B-cells (fig. 1.10): IF=1,01±0,02.

Human embryo islets

Aldehyde-fuchsine staining. Intact embryo islets structure contains not compact or compact groups of polygonal B-cells not completed formation of islet; insulin content visually is reduced comparatively with B-cells of adult rats and rabbits (fig. 1.11); small groups and single B-cells (fig. 1.12) have oval or polygonal form contains deposited insulin.

Victoria 4R staining. A compact groups of 10–15 or single oval form B-cells not completely formed islet; insulin content visually is not reduced comparatively with B-cells of adult rats and rabbits (fig. 1.13).

Diethylpseudoisocyanine staining. Small compact groups of B-cells or disseminated group contains a few B-cells not completed formation of islet; number of cells is 4–5 times less in compared with islets of adult rats and rabbits; insulin content visually is not reduced comparatively with B-cells of adult rats and rabbits (fig. 1.14): IF=1,96±0,09 (intact rat B-cells — 2,02±0,08).

Immunofluorescent method. Compact small groups contains 12±7,6 B-cells visually almost completed forming of small islet; insulin content (green fluorescence) visually is not reduced comparatively with B-cells of adult rats (fig. 1.15). On sections prepared using pancreas tissue, around islets are located exocrine tissue.

State of histostructure and insulin content in islets after action of 8PTSQ and Dithizon

Aldehyde-fuchsine staining. Intact islets: oval form, histostructure without changes, a large amount of deposited insulin (violet color) in cytoplasm of B-cells (fig. 1.16) which maximally are concentrated in B-cells located around blood capillaries; AB=2,01±0,05.

Victoria 4R staining. Intact islets: histostructure of islets without changes; a large amount of deposited insulin (blue color) in cytoplasm of B-cells (fig. 1.17); A-cells on periphery of islets (red color) AB=1,68±0,06.

Immunohistochemistry. Intact islets: histostructure and form of islets without changes; a large amount of deposited insulin (brown color) in cytoplasm of B-cells (fig. 1.18) (AB=1,87±0,07);

Diethylpseudoisocyanine staining. Intact islets: histostructure and form of islets without changes; a large amount of deposited insulin (red fluorescence) in cytoplasm of B-cells (fig. 1.19); IF=2,04±0,06.

Fluorescent staining of Zn^{+2}-ions. Intact islets: intensive green fluorescence of Zn^{+2}-ions B-cells (fig. 1.20): IF=2,08±0,05.

Aldehyde-fuchsine staining. Past action of 8PTSQ: destruction and death of majority of B-cells, marked decreasing of insulin content in B-cells (AB=1,12±0,03) (fig. 1.21).

Victoria 4R staining. Past action of DZ: destruction and death of majority of B-cells, marked decreasing of insulin content in B-cells (AB=1,08±0,09) (fig. 1.22); reduced size of islets; A-cells on periphery of islets without changes.

Immunohistochemistry. DZ: decreasing of insulin content in 90–95 % of B-cells (AB=1,03±0,02) (fig. 1.23); reduced size of islets.

Diethylpseudoisocyanine staining, Past action of TSQ: destruction of B-cells in central part of islet; marked decreasing of insulin content in B-cells (IF=1,11±0,04) (fig. 1.24).

Fluorescent staining of Zn^{+2}-ions, DZ: absence of Zn^{+2}-ions in cytoplasm of B-cells (fig. 1.25): IF=2,08±0,05) (fig. 1.25).

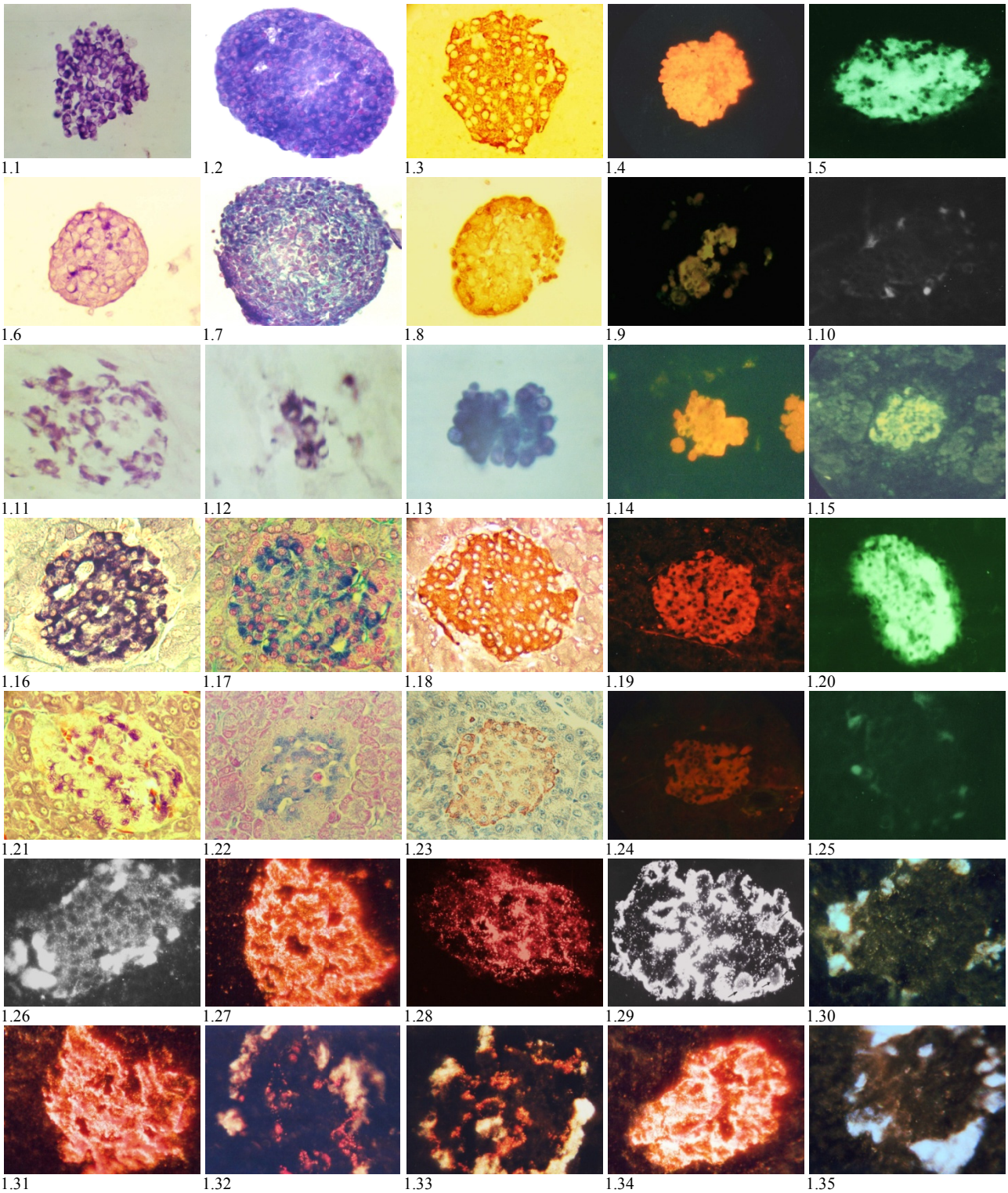

Figure 1

1.1–1.5 Intact isolated pancreatic islets. Histostructure without changes. Normal content of Insulin and Zn^2-ions in B-cells: high density of staining and intensive fluorescence of B-cells; 1.1 — Aldehyde-fuchsine; 1.2 — Victoria 4R; 1.3 — Immunohistochemistry; 1.4 — Diethylpseudoisocyanine; 1.5 — 8PTSQ

1.6–1.10 Pancreatic islets treated by Dithizon. Destruction and death of B-cells; marked decreasing of Insulin and Zn^2-ions in B-cells; 1.6 — Aldehydefuchsine; 1.7 — Victoria 4R; 1.8 — Immunohistochemistry; 1.9 — Diethylpseudoisocyanine; 1.10 — 8PTSQ.

1.11–1.15 Human embryon's small pancreatic islets and not formed groups of B-cells. Almost normal content of Insulin in B-cells; 1.11 — Aldehydefuchsine; 1.12 — Victoria 4R; 1.13 — Immunohistochemistry; 1.14 — Diethylpseudoisocyanine; 1.15 — Immunofluorescent method for Insulin.

1.16–1.20 Pancreatic islets of intact pancreas tissue. Histostructure without changes. Normal content of Insulin and Zn^2-ions in B-cells: high density of staining and intensive fluorescence of B-cells; 1.16 — Aldehyde-fuchsine; 1.17 — Victoria 4R; 1.18 — Immunohistochemistry; 1.19 — Diethylpseudoisocyanine; 1.20 — 8PTSQ.

1.21–1.25 Pancreas of animals with experimental diabetes. Destruction and death of B-cells; marked decreasing of Insulin and Zn^2-ions in B-cells; 1.21 — Aldehyde-fuchsine; 1.22 — Victoria 4R; 1.23 — Immunohistochemistry; 1.24 — Diethylpseudoisocyanine; 1.25 — 8PTSQ.

1.26–1.30 Pancreas of intact animals (1.25), past injection of DZ (1.27–1.29) and past injection of DDCA and DZ (1.30); Staining by Dithizone.

1.31–1.35 Pancreatic islets past injection of DZ (1.31), Cystein + DZ: prevention formation of complex DZ–Zn^2 (1.32), Gluthatione Reduced form + DZ; prevention formation of complex DZ–Zn^2 (1.33), Gluthatione Oxidized form + DZ; not prevention formation of complex DZ–Zn^2 in B-cells (1.34), Histidine + DZ; prevention formation of complex DZ–Zn^2 (1.35); 1.31–1.35 staining by DZ; ×140 for fig. 1.2, 1.10, 1.15, 1.20; ×280 for other preparats.

Influence of Cystein, L-Hystidin and DDCA on interaction of Zn^{+2}-ions of B-cells with Dithizon

Intact pancreas tissue

Investigation of intact frozen sections of rabbit and mice pancreas without staining showed using dark microscopy: histostructure of islet without changes; on periphery lo- cated white color A-cells (fig. 1.26). Injection of Dithizon to animals accompanied a few minutes later by formation of a large amount of red granules of DZ-Zn^{+2} complex in B-cells (fig. 1.27, 1.28).Concentration of complex in islets of mice is low comparatively with rabbits: AB rabbit=2,02±0,04; AB mice=1,62±0,14. This is determined by reduced concentration of Zn^{+2}-ions in B-cells of mice. Investigation of disposition of granules of DZ-Zn^{+2} complex in islets evidently demonstrated that this is maximal concentration of complex around capillaries (fig.1.29). Elimination of Zn^{+2}-ions from B-cells result prevention formation of DZ-Zn^{+2} in islets (fig.1.30) as by prevention developing of diabetes caused by DZ in animals (9,20).

Past action of Cystein, L-Hystidin and DDCA

Obtained results showed that injection of Cystein and L-Hystidine result almost complete binding of Zn^{+2}-ions in cytoplasm of B-cells. Minimal amount of toxic complex as DZ-Zn^{+2} was formed in B-cells contacted with islet arterial capillaries (fig. 1.32,1.33) in compared with a large amount of complex completed all surface of cytoplasm of B-cells not only around capillaries in intact islets (firg. 1.31). Results of measuring of amount of complex in cytoplasm. Intact islets: AB=2,02±0,04; Cystein: 1,21±0,02; L-Hystidine: 1,32±0,02.

Injection of DDCA 500 mg/kg and 1000 mg/kg accompanied by complete binding of Zn^{+2}-ions in cytoplasm of B-cells that result prevention formation of DZ-Zn^{+2} complex (fig. 1.30); AB=1,01±0,02.

Table 1

Comparative analysis of results of measuring of insulin content in B-cells

№	Method	Intact animals	Diabetes	Difference intact/diabetes
	Isolated islets			
1	Aldehyde-fuchsine	1,93±0,06	1,14±0,04	0,79
2	Victoria 4R	1,97±0,05	1,29±0,05•*	0,59
3	Immunohistochemistry	1,81±0,04*	1,18±0,04	0,63
4	Diethylpseudoisocyanine	2,02±0,05*	1,07±0,01•*	0,95
	Pancreas tissue			
1	Aldehyde-fucshine	2,01±0,05•	1,12±0,03	0,89
2	Victoria 4R	1,68±0,06•**	1,08±0,09	0,60
3	Immunohistochemistry	1,95±0,07	1,03±0,02	0,84
4	Diethylpseudoisocyanine	2,04±0,06**	1,11±0,04	0,93

Note. $P < 0,001$.

A comparative analysis of results using of different histochemical methods staining of insulin showed that most precise results of quantitative estimation of insulin content in B-cells were obtained using Immunohistochemical and Diethylpseudoisocyanine methods. We explain it by high specifity of these methods for insulin; there is no staining of other substances or structures in B-cells using these methods that is

why they does not can to change results of photometry. Analysis showed also the presence of higher values of insulin content using Diethylpseudoisocyanine method comparatively with immunohistochemical technique that is determined by evidently more high sensitivity of Diethylpseudoisocyanine method as fluorescent method. Minimal value of parameter K as 1,68±0,06 (Table 1)was obtained using of Victoria 4R method that is explained by high level of absorbance by exocrine tissue.

Results of investigation of staining of B-cells of human embryo showed that majo- rity of investigated small islets not completed forming. There are groups consisting of a few B-cells. They were not yet formed islet as organ: a circulatory system capillaries and capsule were absent.

The insulin content in B-cells is almost same as in B-cells of rats. We observed also a multiple single B-cells or very small groups consisting from 2–3 of cells.

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В-цитотоксикалық заттар әсерінен ұйқы безінің эндокринді ұлпа жағдайын гистохимиялық және иммуногистохимиялық зерттеу

Авторлар анықтағандай, көктамырға цистеин мен L-гистидин аминқышқылын енгізгеннен кейін мырыш байланыстырушы диабетогенді заттар тудыратын жануарларда диабеттің 1-түрі дамуын 90 % алдын алады. Барлық жағдайда бұл аминқышқылдарын енгізгеннен кейін, В-жасушаларды бұзатын токсикалық кешен түзетін мырыш байланыстырушы В-жасушалар диабетогенді заттар толық жойылуымен байқалды. Авторлар цистеин және L-гистидин алдын алу әрекетін, мырыш иондарымен токсикалық емес кешен түзілуі SH-топ молекулаларының дитизон кешенін қалыптасуын болдырмауын жорамалдайды. Авторлар көрсеткендей, В-жасушаларда инсулинді гистохимиялық және иммуноцитогистохимиялық әдістерден басқа, флюоресценттік әдістер, ал Виктория 4R және альдегидфуксинді әдістер панкреатит аралшаларының гистоқұрылымдық күйін бағалауға мүмкіндік береді.

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Гистохимическое и иммуноцитогистохимическое исследование состояния эндокринной ткани поджелудочной железы после воздействия В-цитотоксических веществ

Авторами установлено, что внутривенное введение аминокислоты цистеина и L-гистидина животным в 90 % случаев предотвращает развитие диабета 1 типа, вызываемого цинксвязывающими диабетогенными веществами. Показано, что во всех случаях введение этих аминокислот сопровождается почти полным отсутствием связывания цинка В-клеток с диабетогенными веществами с образованием токсичных комплексов, разрушающих В-клетки. Авторы предполагают, что предупреждающее действие цистеина и L-гистидина обусловлено содержанием в молекуле SH-групп, через которые формируется нетоксичный комплекс с ионами цинка, препятствующий формированию комплексов с дитизоном. Авторами также показано, что из использованных гистохимических и иммуноцитогистохимических методов количественной оценки содержания инсулина в В-клетках наиболее точные результаты обеспечивают иммуногистохимический и диэтилпсевдоизоцианиновый методы, а альдегидфуксиновый метод и метод Виктория 4R наиболее подходят для оценки состояния гистоструктуры панкреатических островков.

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On the protective effect of 2,3-dimercaptopropanol for destructive action of zincbinding chemicals on pancreatic B-cells

2,3-Dimercaptopropanol is a substance able to re-activate sulfhydryl groups of enzymes and has the property to form temporary complexes with metals, including zinc. It is also known that certain amino acids, particularly cysteine and glutathione also contain in its composition mole coli SH-groups. Administration of these amino acids in to animals result prevention developing of experimental diabetes caused zinkbinding diabetogenic chemicals. It is confirmed that this effect is determined by their ability to form non-toxic temporary complexes with zinc in B-cells of pancreatic islets that protect cells of the destruction caused by diabetogenic chelating agents. The authors have shown that 2,3-dimercaptopropanol at doses of 60 and 120 mg/kg is able to prevent the development of diabetes in almost all experimental animals. Authors found that this ability 2,3-dimercaptopropanol is explained by its property through SH-groups included in its composition, to form non-toxic complexes with zinc in pancreatic cells that protect cells of death.

Key words: B-cells, SH-groups, experimental diabetes, zinc, 2,3-dimercaptopropanol.

2,3-Dimercaptopropanol (DMP) is known as re-activator of SH-group of enzymes and possess ability to form stable complexes with metals. However it is known that some aminoacids contains SH-groups in molecule as Cystein and Glutathuone reduced form protect developing of diabetes caused by chelat active chemicals. This effect determined by high affinity of SH-group for zinc and cadmium [1]. 2,3-Dimercaptopropanol is able in added to destroy other complexes, previously formed with zinc by chelators, that accompanied by re-placing atom of chelator from complex [2] and formation of complex DMT-metal via SH-group.

Aim of work: to investigate state of histostructure of pancreatic islets and possible interaction of Zn^{+2}-ions in B-cells with DMP and Dithizon, a diabetogenic chelator.

Material and methods

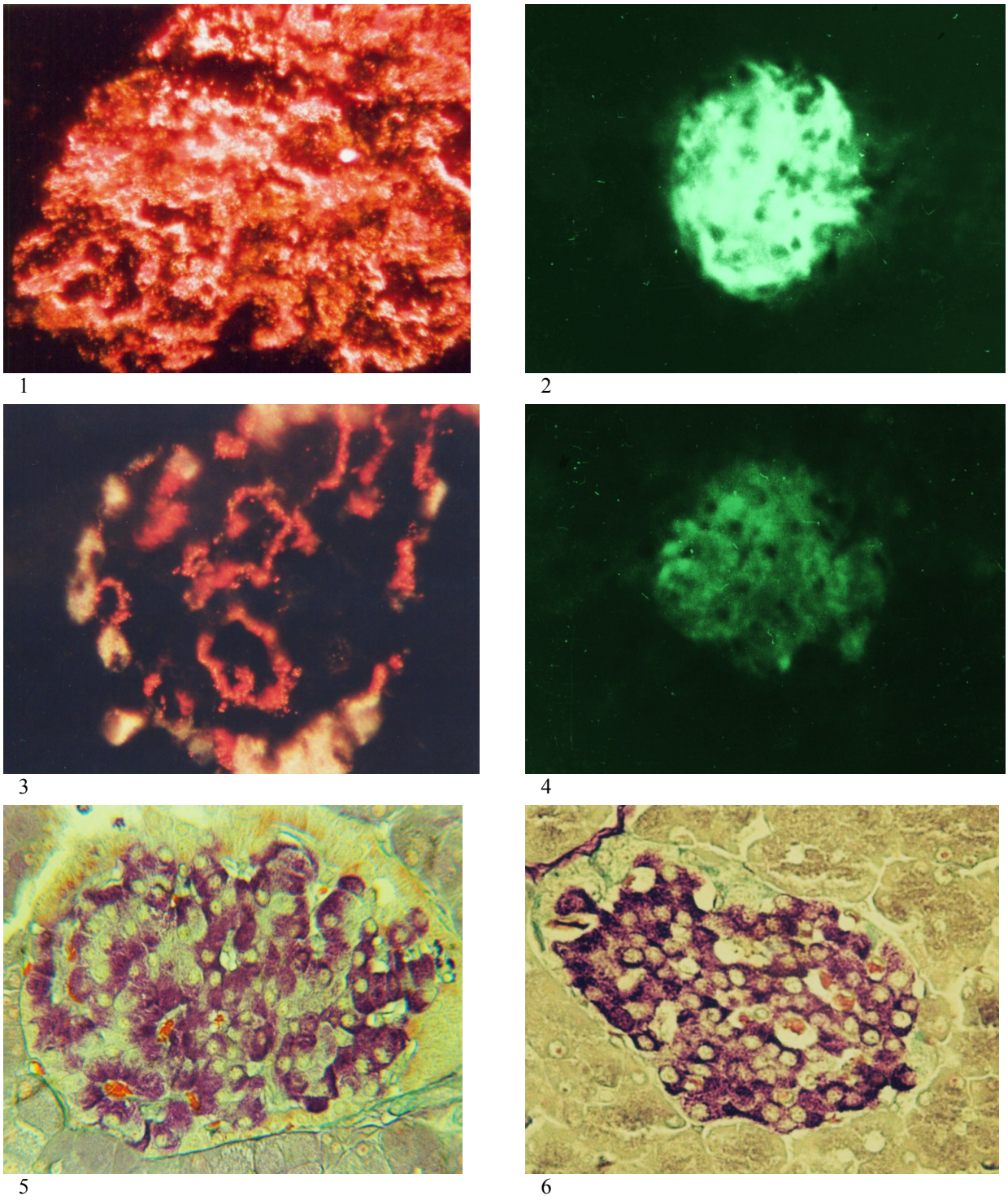
Animals: 10 rabbits 2,200–2,650 g were used. Group 1: control intact animals: A-injection of Dithizon, 48,9 mg/kg; B-intact rabbit; Group 2: injection of DMP («SIGMA») in doses 60 and 120 mg/kg to 8 animals; 30 min later water solution of Dithizon 46,6–49,7 mg/kg was injected to 6 animals; animals killed 10 min past injection of DZ. Group 3: injection of DMP, 60 and 120 mg/kg to 2 animals; animals killed 10 min past injection. Group 4**:** injection of DMP, 60 and 120 mg/kg to 2 animals; 30 min later injection of Dithizon 48 mg/kg; animals killed 5 days past injection of DZ.

Frozen sections of pancreas were investigated using dark microscopy for revealing of DZ-Zn^{+2}-complex in B-cells. The high specific fluorescent method revealing of free Zn^{+2}-ions in B-cells by fluorochrom 8-para(toluenesulphonylamino)quinolin [8PTSQ] was used [3, 4]. 3 animals were killed 5 days past injections of DMP and DZ. Pancreas was fixed in alcohol 70º + H_2S. Staining of sections by aldehyde-fucshine [5–7] and 8PTSQ.

Results

Group 1. Control intact animals section o pancreas. 20 frozen sections of pancreas tissue were investigated using dark microscopy. Cytoplasm of all investigated islets contain a large amount of red chelat complex Zn^{+2}-Dithizon [1] which concentrated on the all surface of cytoplasm, maximally around blood islet's capillaries (fig. 1.1). Reaction for Zn^{+2}-ions: intensive fluorescence of B-cells (fig. 1.2). Group 2. Administration of 2,3-dimercaptopropanol and of Dithizon result prevention of formation in majority of B-cells of Zn^{+2}-Dithizon complex which is partially formed in cells located around blood capillaries (fig. 1.3). Pro-

tective effect determined by not diabetogenic binding of Zn^{+2}-ions by DMT. It is known that DMT possess high affinity for Zn^{+2}-ions.



1 Intact rabbit. Injection of Dithizon, 48,9 mg/kg; dark microscopy; red granules of complex zinc-DZ in B-cells; [×280]
2 Intact rabbit. Positive fluorescent reaction for Zn in B-cells: intensive green fluorescence of Zn in cytoplasm of B-cells; [×140]
3 Injection of DMP 60 mg/kg + DZ; dark microscopy; only B-cells contacted with capillaries contain complex zinc-DZ; [×280]
4 Injection of DMP 60 mg/kg + DZ; negative fluorescent reaction for Zn in B-cells: only a few cells contain a small amount of Zn; [×140]
5 Dithizon, 48 mg/kg. Aldehyde-fucshine staining; destruction of B-cells; degranulation, decreasing of insulin content in B-cells; [×280]
6 Injection of DMP + DZ; Aldehyde-fucshine staining; histostructure of islets and insulin content in B-cells without changes; [×280]

Figure 1. Pancreas tissue

Group 3. Investigation of free Zn^{+2}-ions content in B-cells past injection of DMT showed a negative reaction for Zn^{+2} in islets (fig. 1.4). A few B-cells contains minimal amount of Zn^{+2} in cytoplasm. This result determined by forming by DMT of not visible Zn^{+2}-DMT complex.

Group 4. Investigation of effect of DMT on diabetogenic property of Dithizon showed that administration of it accompanied by absence of any histological changes in pancreatic islets (fig. 1.5; 1.6).

T a b l e

Results of investigation of blood glucose level

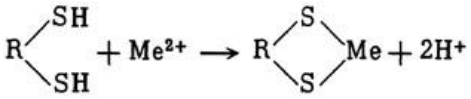
№	Conditions	Blood Glucose (mmol/l), day				
		Before	1	3	6	7
1	Dithizon, 46,8–48 mg/kg 2 animals	5,1±0,3	3,0±0,4	9,6±1,2■	12,6±2,2●	16,2±2,6*
2	DMT, 102–110 mg/kg + Dithizon, 48,8 mg/kg, 4 animals	4,9±0,4	5,2±0,4	5,6±0,5■	5,8±0,7●	5,4±0,6*

Note. * — p< 0,001; ● — p < 0,005; ■ — p < 0,01.

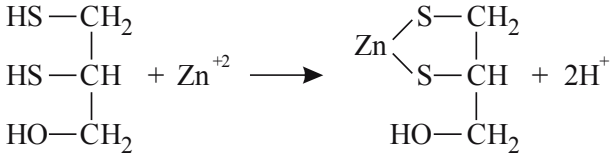
Results of investigation of blood glucose level (table 1) demonstrated that injection of Dithizon accompanied by marked decreasing of blood glucose level that is determined by release of a large amount of insulin as result of destruction within short time of majority B-cells. In other animals, past injections of DMT and followed past 1, 2 and 3 h injections of diabetogenic doses of Dithizon not accompanied by hyperglycemia in animals. We observed only not reliable increasing of blood glucose level until 5,6–5,8 mmol/l (Table).

Discussion

Molecule of 2,3-Dimercaptopropanol ($C_3H_8OS_2$ m.m. 124,22) contains two SH-groups**.** It is known that some metals (Me) as mercury, arsenic, cadmium, lead, zinc interacted with chemicals contains SH-groups and formed stable cyclic mercaptide:



As bivalent metal Zn^{+2}-ions interacts with 2 SH-groups of molecule of 2,3-Dimercaptopropanol with forming of cyclic mercaptide which are more stable in compared with some chelat active chemicals. It is known that 2,3-Dimercaptopropanol is able to destroy complexes previously formed with chelators accompanied by replace atom of chelator from complex [2].



Thus, obtained results showed that 2,3-Dimercaptopropanol protect B-cells of destruction caused by Dithizon and of developing of diabetes. Investigation of interaction in B-cells between Zn^{+2}-ions and 2,3-Dimercaptopropanol evidently showed that DMT protect B-cells of formation of toxic Dithizon-Zn^{+2} complex by interseption of Zn^{+2}-ions and forming new complex DMT-Zn^{+2}.

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Диабетогенді мырыш байланыстырушы қосылыстармен туындайтын панкреатит В-жасушаларының бұзылуын болдырмауда 2,3-димеркаптопропанолдың қабілеті туралы

2,3-Димеркаптопропанол өзінің молекулалық құрамында екі сульфгидрильді топ (SH-топ) болуымен, сульфгидрильді фермент тобы реактиватор ретінде белгілі және оның SH-тобы арқылы байланысатын ауыр металдармен уақытша кешен түзуге бейімді. Сонымен қатар құрамында SH-тобы бар L-гистидин және цистеин аминқышқылдары В-жасушаларының Zn^{+2} ионымен уақытша байланыстыруға бейімді, осылайша диабетогенді мырыш байланыстырушы қосылыстармен өзара әрекетіне және жойылуын болдырмауға әрекет етеді. Авторлар көрсеткендей, жануарларға 2,3-димеркаптопропанол 60 және 120 мг/кг мөлшерде енгізу В-жасушалардағы мырышты толық тоскауылдатады. 2,3-Димеркаптопропанол превентивті әрекеті оның құрамында сульфгидрильді топ молекулаларының бар болуымен сипатталады деген қорытынды жасалды.

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О способности 2,3-димеркаптопропанола предотвращать разрушение панкреатических В-клеток, вызываемое диабетогенными цинксвязывающими соединениями

2,3-Димеркаптопропанол является веществом, способным реактивировать сульфгидрильные группы ферментов и обладает свойством формировать временные комплексы с металлами, включая цинк. Известно также, что некоторые аминокислоты, в частности, цистеин и глютатион также содержат в составе своей молекулы SH-группы. Эти аминокислоты при парентеральном введении предотвращают развитие экспериментального сахарного диабета, вызываемого цинксвязывающими диабетогенными веществами. Доказано, что этот эффект обусловлен их способностью формировать нетоксичные временные комплексы с цинком В-клеток панкреатических островков, защищая их таким образом от разрушающего воздействия диабетогенных хелатообразователей. Авторами показано, что 2,3-димеркаптопропанол в дозах 60 и 120 мг/кг способен предотвращать развитие диабета почти у всех опытных животных. Авторами установлено, что эта способность 2,3-димеркаптопропанола обусловлена его свойством через SH-группы, входящие в его состав, формировать нетоксичные комплексы с цинком панкреатических В-клеток, препятствуя этим повреждающему действию диабетогенных цинксвязывающих веществ.

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Outcomes of clinical diabetes research at the Teaching Hospital and Faculty of Medicine, Palacký University Olomouc (1971–2014)

This paper summarizes results of clinical studies in the period of years 1971–2014 as follows: (1) In persons with type 1 diabetes, effectiveness of conventional therapy with multiple doses of shortacting insulin (MDI) or continuous sucutaneous insulin infusion (CSII) was demonstrated. Dynamic training was shown to enhance insulin sensitivity and plasma HDL cholesterol, and to improve neuropathy, memory and attention. Influence of alcohol and effects of insulin on postprandial alcohol concentrations revealed similar consequences; model experiments were suggested when answering forensic questions. (2) In persons with type 2 diabetes, advantage of complementary therapy with prandial rapid acting insulin was demonstrated. In MDI-persistent hyperglycaemia, insulin pump was shown to reduce HbA1c, to diminish daily insulin dose and to improve quality of life. Benefits of incretin preparations were described. Effects of losartan on renal and cardiovascular outcomes in diabetic nephropathy were documented. (3) Perinatal mortality of newborns of mothers with diabetes decreased due to thorough diabetes and obstetric care. (4) Manual Device for Insulin injections (MADI-pen) was developed and introduced into daily routine. Its accuracy, pharmacological and microbial safety were proved. (5) «Programmed Treatment of persons with diabetes» comprising the principels of Therapeutic Patients Education of the WHO was introduced. (6) Glycaemic Index of foods was calculated using a new method with continuous glucose monitoring. (7) Intensive selfmonitoring. The accuracy and precision of various glucometers were explored. Tenpoint ambulatory glycaemic profile and continuous glucose monitoring system (CGMS) was introduced to practice. In persons on insulin pumps CGMS resulted in reduction of HBA1c.

Key words: diabetes mellitus, dawn phenomenon, selfmonitoring, glycaemic profile, glucometer, insulin pumps, incretins, therapeutic education, Carelink, Diabass 5.

Introduction

This review comprises practice-related outcomes of clinical studies carried out in the period of years 1971–2014 at the University Diabetes Centre in Olomouc, Czech Republic [1]. The problems were solved in cooperation with other centres in Czech and Slovak Republic and abroad, particulatly with the Institute of Diabetes «G.Katsch», Karlsburg, Diabetiker Sanatorium Bergfried, Saalfeld, Dept. of Endocrinology, University Heinrich Heine, Düsseldorf, Germany; Royal Infirmary Edinburgh, St. Thomas Hospital, London, UK; Institute of Diabetes «N.C.Paulesco», Bucharest, Romania; etc. Over 100 professionals, medical students and nurses were step by step involved into the presented clinical research. Thousands of various biochemical and radioimunological analyses were performed in highly specialized laboratories in Olomouc, Karlsburg and Moravský Beroun.

All studies paid attention to the optimum diagnostic and therapeutic procedures in type 1 and type 2 diabetes mellitus including the development of aids for insulin administration and educational programmes.

The most important achievements were presented at national and/or international conferences (ADA, EASD etc.) and published. In addition, their implementation into daily routine has always been considered.

In this paper the achievments are summarized in seven chapters:
(1) Treatment of people with type 1 diabetes (PWD1).
(2) Treatment of people with type 2 diabetes (PWD2).
(3) Diabetes and pregnancy.
(4) Technical prerequisites for the intensive conventional insulin treatment.
(5) Education — Programmed treatment of people with diabetes.
(6) Glycaemic Index of foods.
(7) Intensive selfmonitoring.

1. Treatment of people with type 1 diabetes mellitus

1.1. Conventional insulin substitution. A randomized cross-over study (1987–1990) was performed in Institute of Diabetes Gerhardt Katsch, Karlsburg, with 36 PWD1 (men) admitted to hospital for a period of 6 weeks. Each tested person was assigned to one of six arms differing only by the sequence of three defined insulin Regimens A, B, C. In every men all three Regimens were tested (two weeks each). At the end of each 14-day test period a 16-point B-glucose (BG) and P-free insulin (FIRI) profile was performed (Fig. 1).

This study revealed that the basal and prandial insulin substitution with only shortacting porcine insulin given 7 times a day (Regimen A) is the most effective kind of conventional insulin therapy [2]. This approach led to the best metabolic control in 21/36 (58 %) tested PWD1. An insulin regimen with similar insulin doses over morning and afternoon hours but different regimens over night — either intermediate insulin preparation BS at 10 p.m. (Regimen B) or longacting insulin Ultratard HM at 5.30 p.m.(Regimen C) led to the best control only in 6/36 (17 %) or in 9/36 (25 %) PWD1, respectively.

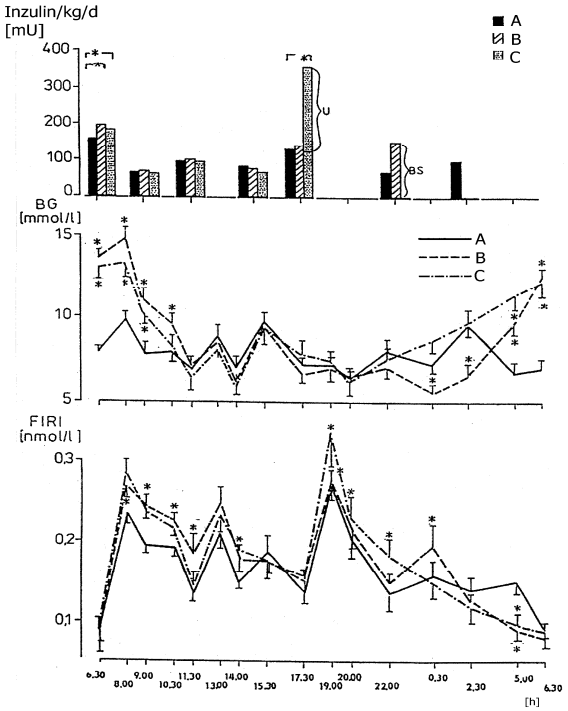

Figure 1. Insulin doses per day and evolution of BG- and FIRI concentrations in 16-point B-glucose profiles at the end of 14-day test periods with respective Regimen A, B, C in 36 PWD1 (men). Mean ± SE

In the last two regimens (B or C), the total daily insulin dose was higher than in Regimen A. The group education and a new insulin pen (MADI) proved to motivate the PWD1 to an intensification of insulin therapy including injections of shortacting insulin at 2.30 a.m. in Regimen A. The acceptance of night injections increased from 2 % at the baseline to 42 % at the end of the study. However, despite of such an intensive treatment, a longlasting euglycaemia could not be reached, even though the plasma insulin concentrations were higher than in persons without diabetes (Fig. 2, Fig. 3). There were no differences in metabolic control when using the needle-pen or catheter-pen [3, 4]. The needle pen was preferred in 54 % of all patient-days [5].

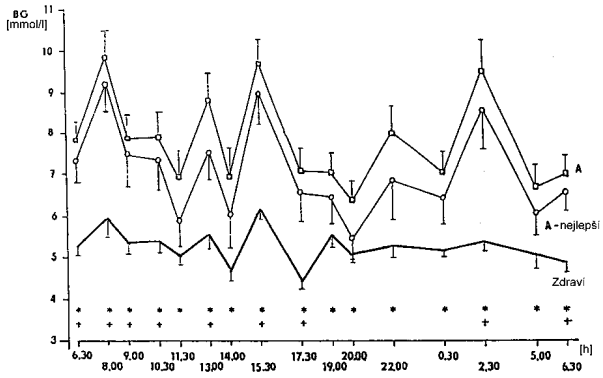

Figure 2. Evolution of BG concentrations in 16-point B-glucose profiles at the end of 14-day test period with Regimen A in all PWD1 (n=36), in the best PWD1 (A — nejlepší, n=21) and in healthy men (Zdraví, n=9). Mean ± SE

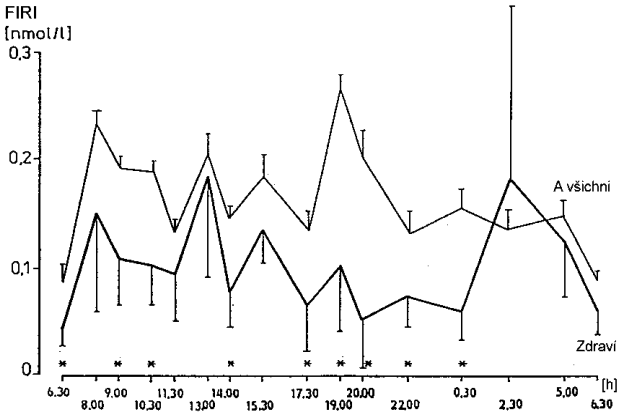

Figure 3. Evolution of FIRI concentrations in 16-point B-glucose profiles at the end of 14-day test period with Regimen A in all 36 PWD1 (upper curve) and in healthy men (n=9, lower curve). Mean ± SE

1.2. Continuous subcutaneous insulin infusion (CSII) in PWD1. A retrospective study (1993–1998) with 13 men and women on an insulin pump (Dahedi, H-Tron, Minimed) demonstrated that CSII resulted within 72 days in a decrease of concentrations of HbA1c in blood (9.3±0.46 vs 7.6±0.28 %, $p<0.05$, DCCT units), reduction of total serum cholesterol (5.47±0.29 vs 4.85±0.19 mmol/l, $p<0.05$) and triacylglycerols TG (1.58±0.24 vs 1.13±0.15 mmol/l, $p<0.05$). The total daily dose of insulin was reduced (47.8±2.75 vs 41.3±2.3 IU/d, $p<0.05$) and the body mass did not change. An improved metabolic control was also found in a check-up 554 days later. There were no serious complications resulting from handling insulin pump [6]. In the year 2004 we have replaced phosphate buffered insulin (Velosulin) with insulin aspart (Novorapid) for all PWD1 on insulin pumps. Reduction of HBA1c without increased frequency of hypoglycaemias appeared within one year (Fig. 4). The daily insulin dose and body mass did not change [7].

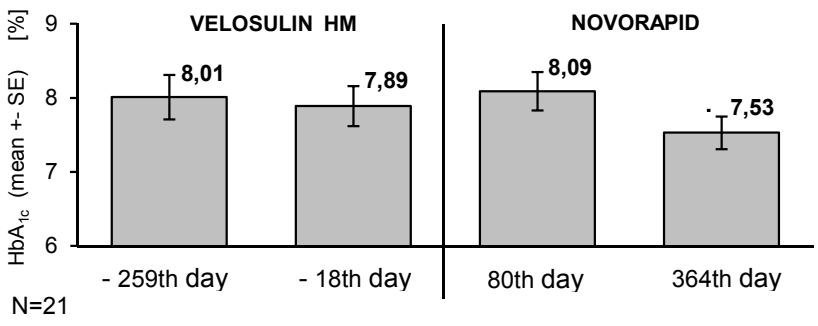

Figure 4. HbA1c concentration (DCCT) in PWD1 on CSII in the course of treatment with phosphate buffered human insulin (VELOSULIN) and after switching to insulin aspart (NOVORAPID). Mean ± SE [7]

1.3. Dynamic physical training. A prospective study (1978–1982) with 19 men improved insights in submaximum physical training (duration 157±43 days). Here are the effects of training in men with type 1 diabetes:

1.3.1. An improvement of carbohydrate metabolism demonstrated by the increased insulin effectiveness without any change in blood glucose control. An approximate relation between insulin effectiveness Q and physical working capacity (PWC 170) was calculated using formula Q [g Carb./IU = 0,03 PWC 170 [W] – 0,5, (where 90 W < PWC 170 < 295 W). This formula was derived from our observations and needs to be confirmed. The insulin effectiveness Q reached values ranging from 2.5 to 22.4 g Carb./IU. The increase of the PWC depends on the amount of energy expended for the submaximal training. Following the training interruption, the insulin effectiveness drops depending on the decrease of PWC 170 (Fig. 5).

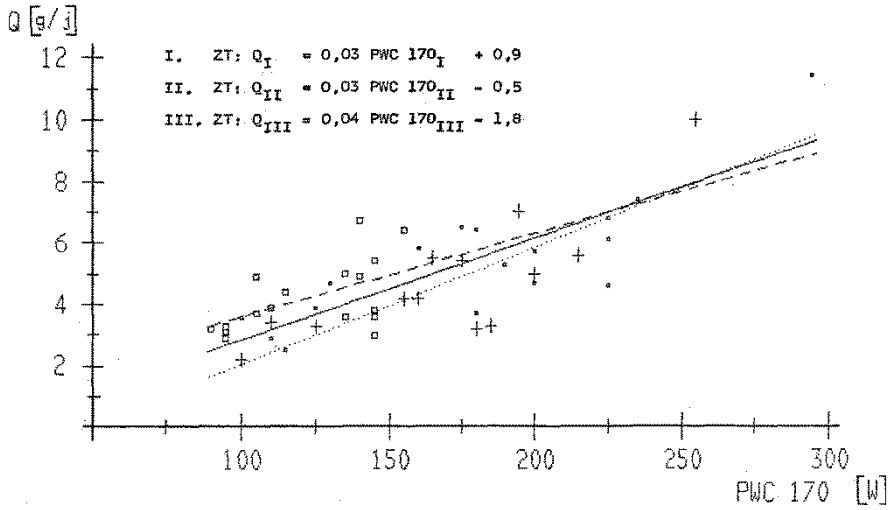

Figure 5. Relation between insulin effectiveness Q and physical working capacity PWC 170 in PWD 1 (n=19) before (ZT Q I) and after (ZT Q II and ZT Q III) submaximal dynamic training

1.3.2. An improvement of lipoprotein metabolism demonstrated by an increase in HDL cholesterol concentration (1.19±0.08 vs 1.86±0.22 mmol/l, $p<0.05$, Fig. 6) and by a decrease in the index of total cholesterol/HDL cholesterol. These significant changes were also found 7 days after the end of the training.

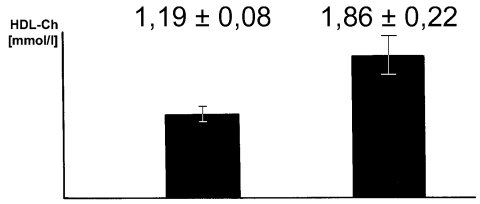

Figure 6. S-HDL cholesterol in PWD1 at baseline (before training) and at the end of physical training

1.3.3. A beneficial influence on some signs of neuropathy, on memory, attention and on the general condition of men with type 1 diabetes was demonstrated as well.

Based on this study, a submaximal dynamic physical training may be recommended as an additive treatment of persons with type 1 diabetes with no signs of catabolism. At the beginning, the insulin should be reduced or the amount of carbohydrates in food increased, according to the change of insulin effectiveness. Even in patients with high physical working capacity it is not possible to replace insulin by physical exercise. Following the training cessation, the insulin should be increased or the amount of carbohydrates in food decreased according to the decrease of insulin effectiveness [8, 9].

1.4. Ethyl alcohol consumption. A controlled prospective study (1986–1988) on influence of ethyl alcohol consumption on B-glucose concentrations and on the influence of B-glucose on ethyl alcohol concentrations was performed. A group of 15 men with DM1 and a group of 15 healthy men consumed alcohol in order to reach the alcohol concentration in blood of 1 g/kg. Following formula was used to calculate the amount of consumed alcohol:

Amount of consumed alcohol [g] = 0.7 × body mass [kg] × alcohol concentration in blood [g/kg]. This trial brought the following conclusions [10]:

1.4.1. Development of alcoholaemia after application of a usual insulin dose and after a fixed breakfast in men with type 1 diabetes is similar to that in healthy males. A reduction of the insulin dose (and omitting of the breakfast) results in a quicker increase of alcoholaemia with a higher maximum concentration.

1.4.2. The maximum alcoholaemia up to 1 g/kg does not influence neither the B-glucose nor the B-acetone within 240 min after drinking the alcohol. The serum concentration of non-esterified fatty acids (NEFA) in men with diabetes (but not in healthy persons) decreases, the concentrations of TG and lactate does not change. Alcohol had no influence on the concentrations of some amino acids (threonin, glutamic acid, prolin, ornithin, arginin) and ambivalent influence on other amino acids.

1.4.3. Due to large inter-individual differences in the development of calculated alcoholaemia (Fig. 7) a model experiment appears to be necessary in order to answer some important forensic questions [11].

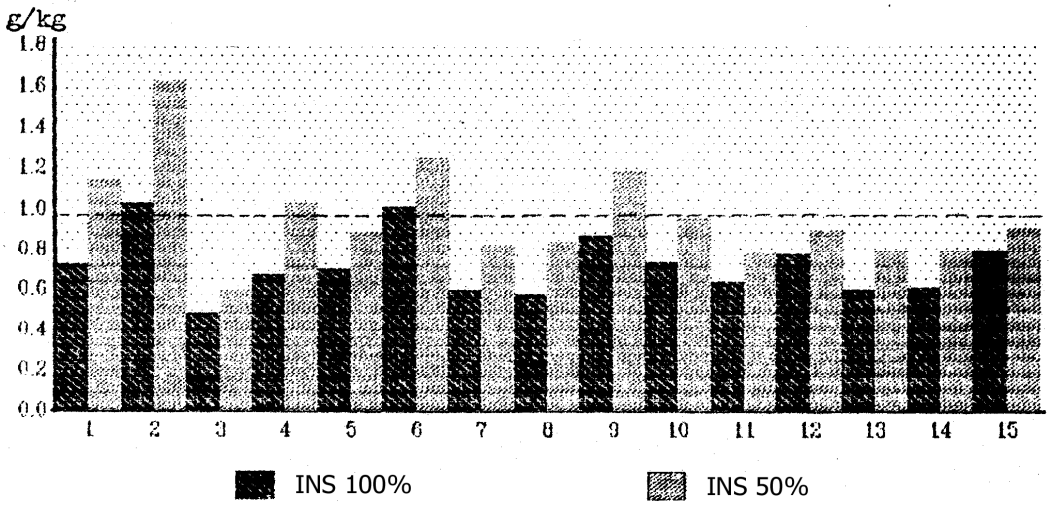

Figure 7. Individual alcohol concentrations in blood reached in PWD 1 after consumption of the calculated amount of alcohol [g] with full dose (100 %) of insulin + full meal, and with half dose of insulin without meal

2. Treatment of persons with type 2 diabetes mellitus

2.1. Start of Complementary insulin therapy [12]. A prospective study (1991–1994) with 251 men and women with type 2 diabetes (PWD2) aged between 20 and 82 years demonstrated benefits of small supplementary insulin doses (1 to 10 IU) before each meal both in a group of 108 PWD2 previously on diet/oral antidiabetic drugs which started a complementary treatment with total insulin 26 IU/d (Fig. 8), and in a group of 143 PWD2 previously treated with longacting insulin in which on complementary treatment the daily insulin dose was reduced from 47 IU/d to 32 IU/d (Fig. 9) [13].

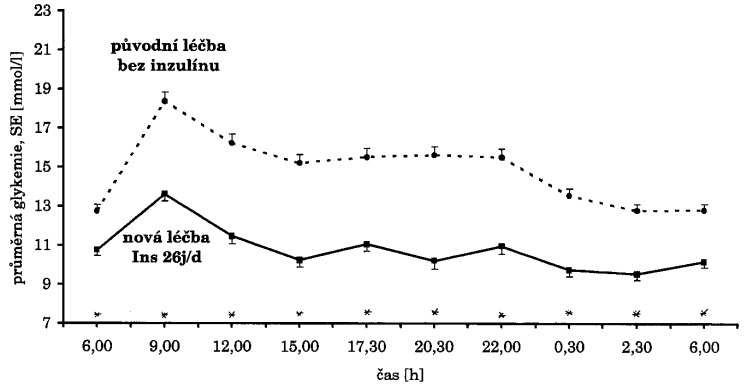

Figure 8. Evolution of BG concentrations in 10-point B-glucose profiles without insulin (upper curve) and with complementary prandial doses of 1 to 10 IU of shortacting insulin (lower curve), the mean total of 26 IU/d. n = 108, mean±SE [13]

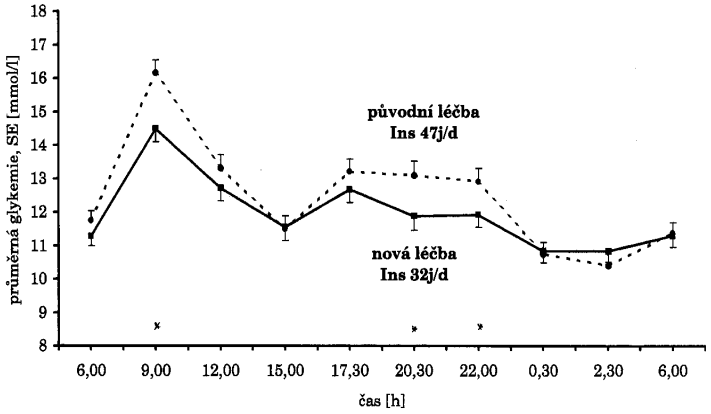

Figure 9. Evolution of BG concentrations in 10-point B-glucose profiles with longacting insulin 1–2 injections/d, the mean dose 47 IU/d (upper curve) and with complementary prandial doses of 1 to 10 IU of shortacting insulin the mean daily dose 32 IU (lower curve). N=143, mean±SE, *P<0.05 [13]

In the course of 10 weeks the BMI decreased from 29.4±0.73 to 28.9±0.73 kg/m2 ($p<0.05$). In addition, this treatment resulted not only in an improvement of B-glucose profile, mean B-glucose, HbA1c but also in a better spectrum of lipoproteins (increase of LpA, decrease of apo B and Lp(a) — Fig. 10). The patients´ satisfaction with the new treatment was good. The increased number of injections has not been an obstacle: 91 % of PWD2 (when using MADI pen) decided to go on the intensive complementary treatment [14].

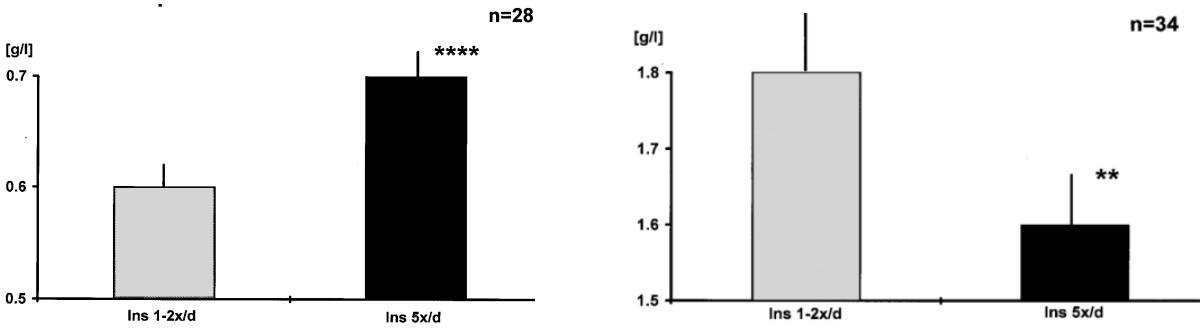

Figure 10. Changes in serum concentrations of Lipoprotein A (upper diagram) and lipoprotein B (lower diagram) in PWD2 between baseline and 10 weeks with complementary treatment [14]

2.2. Continuation of Complementary (supplementary) insulin therapy over 5 years. A retrospective analysis of a group of 70 PWD2 in which a complementary insulin treatment was introduced in our centre in the year 1991 or later on (so that this treatment lasted 5.0±0.12 years), and which were consequently treated in three different diabetes centres, brought the following results: the body mass decreased in 56 %, the concentration of HbA1c decreased in 56 % and the daily insulin dose decreased in 57 % of all 70 PWD2 [15]. Based on these observations the complementary insulin treatment may be recommended for a longlasting treatment of both non-obese and obese PWD2.

2.3. Rapid acting insulin analogs in complementary therapy. In our trial (2002–2007) 57 PWD2 treated with human regular insulin for 5.2 ± 0.44 years were investigated [16]. Following two checkups performed in the course of the 364 ± 17.9-day baseline period, human regular insulin was replaced with aspart in equivalent boluses, and two checkups in the course of 330 ± 11.1-day sequential period were performed (Fig. 11). The control group consisted of 17 PWD2 treated with insulin for 4.2 ± 0.57 years. In the intervention group, following the switch from human regular insulin to aspart, hemoglobin A1c (HbA1c) decreased from 8.4 ± 0.23 % at baseline to 7.9 ± 0.17 % ($P = 0.031$), and thereafter to 7.5 ± 0.20 % ($P < 0.001$) daily insulin dose (37.1 ± 1.39 IU/d), BMI (30.5 ± 0.82 kg/m2), and frequency of hypo- and hyperglycaemic episodes did not change ($P > 0.05$).

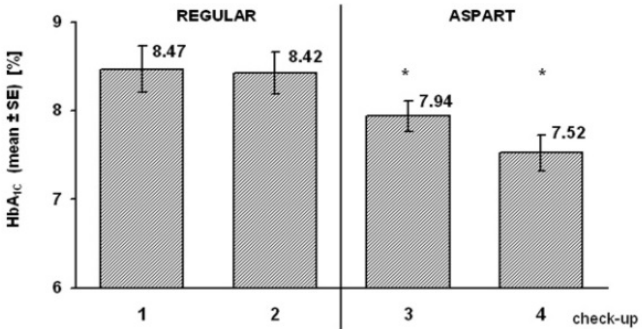

Figure 11. Evolution of HbA1c following the switch from human regular insulin to insulin aspart. N=57, mean ± SE, *P<0,05 [16]

In the control group, no significant change of baseline HbA1c (8.4±0.54 %), insulin dose (33.1±3.17 IU/d), and BMI (32.1±1.12 kg/m2) was found. Aspart appears to be more effective than human regular insulin for supplementary (com- plementary) insulin treatment in individuals with type 2 diabetes. Our conclusions correspond to other studies [17–19]

2.4.Continuous subcutaneous insulin infusion in PWD2. Our pilot monocentric uncontrolled trial PARASEN (2006–2010) demonstrated that CSII in trained PWD2 results in reduction of daily insulin dose without change in HbA1c concentration and body mass [20, 21]. This is in accordance to other studies, in particular to the recent prospective randomized multicentre controlled study OpT2mise (2011–2014) [22, 23].

2.5. Inkretin preparations. In 35 obese metformin-treated PWD2 with persistent hyperglycaemia exenatide twice daily or liraglutide once daily was injected. In the course of 3 to 6 months reduction of body mass up to 1 kg/week (Fig. 12) and HbA1c concentrations about 10 % of baseline (Fig. 13) was registered in most of them. However, following the 6-month successful period, these effects were mostly less pronounced or none. Exenatide QW (once per week) appears to be helpful.

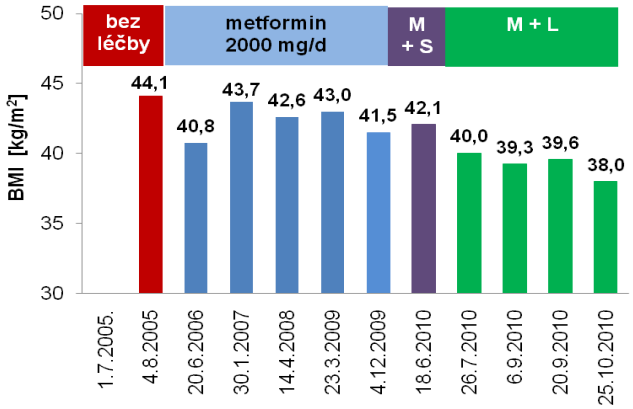

Figure 12. Evolution of BMI since the beginning of DM 2 in the course of metformin (M) therapy, and effects of sitagliptin (S) and liraglutide (L)

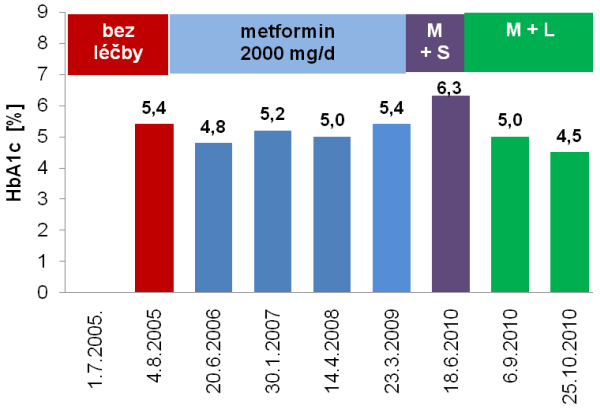

Figure 13. Evolution of HbA1c since the beginning of DM 2 in the course of metformin (M) therapy, and effects of sitagliptin (S) and liraglutide (L)

2.6. Study RENAAL (Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Renal Protective Effects of Losartan in Patients With Noninsulin Dependent Diabetes Mellitus and Nephropathy; 1997–2001, principal coordinator B.M.Brenner, California) [24].

A total of 1513 patients were enroled in this study at 250 specialized centres in 28 countries, 25 of them in Olomouc. There were a total of 327 patients in losartan group and 359 in placebo group. In conclusion, losartan conferred significant renal benefits in PWD2 and nephropathy and it was generally well tolerated.

3. Diabetes mellitus and pregnancy

An open epidemiologic study aiming to the diabetes treatment in pregnancy de- monstrated that the intensive management of diabetes carried out at the University Hospital in Olomouc resulted in a marked decrease of the perinatal mortality of newborns of diabetic mothers. This mortality dropped from 66 % in the year 1965 to 6.5 % in the year 1980 and finally reached the same percentage as in non-diabetic mothers (i.e. less than 1 %). To date, the management of a pregnant diabetic woman is based on the intensive treatment with shortacting insulin or insulin aspart (either conventional by means of a pen or as continuous subcutaneous insulin infusion by means of an insulin pump) aiming to the euglycaemia level (P-glucose between 4.0 and 7,0 mmol/l) and on intensive obstetrical care in a specialized centre. Insulin pump and intensive selfmontoring using CGMS is recommended (Fig. 14). Delivery in 38–40 week of pregnancy; in threatening complications any delay of a caesarean section should be avoided [25, 26].

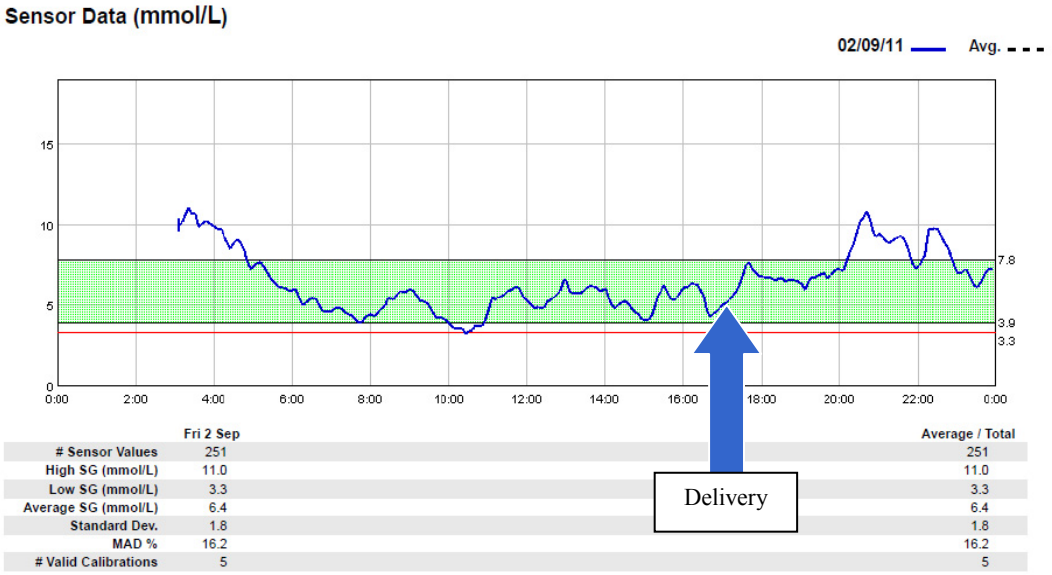

Figure 14. Evolution of P-glucose concentration at the day of delivery. PWD1, Primipara [26]

4.Technical prerequisites for the intensive conventional insulin treatment

4.1. The Pen-like case [5]. In a close cooperation with other institutions a proposal of a simple pen-like case for an insulin syringe filled with insulin (Fig. 15) was submitted and the safety of this case was investigated. The pen case was produced from light metals or plastic materials and used several years before the era of insulin pens.

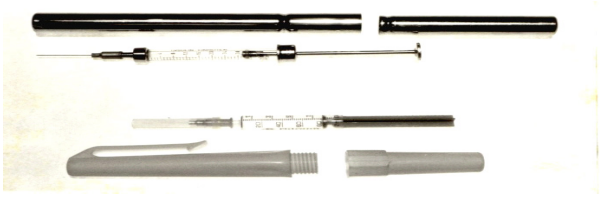

Figure 15. «Disposable» syringe filled with shortacting insulin and the pen-like case

4.2. A new insulin pen MADI (Manual Device for Insulin Injection) was proposed, constructed and developed (Fig. 16). The MADI was one of the first insulin pens worldwide [5, 27–32].

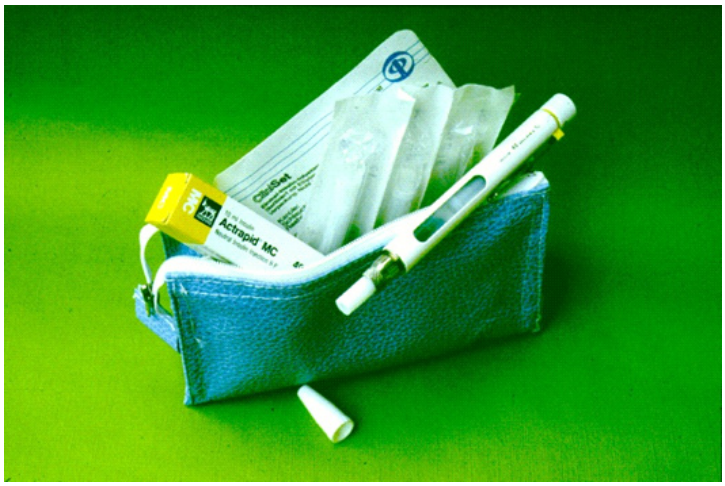

Figure 16. The MADI-pen (year 1991); special case with disposable reservoirs, needles, insulin vial

4.2.1. **The precision of MADI** was tested In a technical study. The variability of individual doses is insignificant. In the type MADI 7/2, the average dose equals 1.9 IU and the coefficient of variation ranges between 1.0 and 4.6 %, exceptionally up to 5.7 %. Neither the speed of application, nor the temperature ranging between 20 and 37oC have any influence on the total dose injected. Even a simulated 5 years usage of the MADI did not lead to any significant change of individual doses.

4.2.2. **Pharmacological assessment.** Under the standard laboratory conditions (Institute of diabetes Karlsburg 1987–1990) it was found out that within one year there is no significant change in the activity of shortacting porcine insulin when stored in a plastic reservoir under 37°C without mechanical stress (Fig. 17). If a heavy mechanical stress is added, the full insulin activity remains three days at least, and then it begans to decrease.

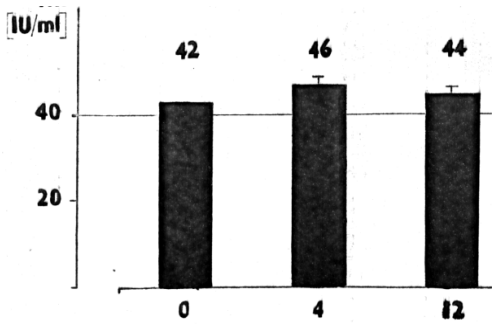

Figure 17. Activity of shortacting porcine insulin (SNC Berlin Chemie)
when stored in a plastic reservoir under 37 ºC without mechanical stress in the course of 12 months

4.2.3. **Microbial safety.** Thorough microbial investigations and clinical observations of over 500 000 injection sites resulted in a conclusion that the repeated use of disposable syringes, reservoirs and needles in one PWD may be recommended as a convenient and safe approach in insulin administration. If no obvious contamination occurs, the period of usage of a needle seems to be limited by its sharpness [33].

4.2.4. **Clinical study**. A prospective multicentre clinical study (1985–1992) confirmed that MADI is a useful aid for the application of all kinds of insulin in all age-groups of PWD1 and PWD2 which are able to handle the pen. No visual control of insulin injection is necessary. The MADI was produced from light metals and plastic materials. The insulin is filled from original vials. The filling and application is performed by twisting the cap. The latest models of MADI from the year 1994 (40–1, 40–2, 100–1, 100–2, 80–1, 80–2) may be distinguished by color. The volume of a reservoir is 3 ml. The needle is protected by a special sliding cover. The MADI was used as a needle-pen or as a catheter-pen. Since 1986, more than 1800 MADI-pens were introduced at the Teaching Hospital Olomouc (Fig. 18).

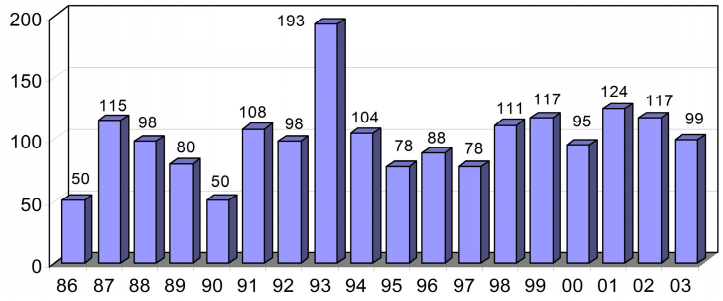

Figure 18. Number of MADI-pens introduced to PWD in Teaching hospital Olomouc at the begining of pen-era (1986–2003)

At present, the MADI production is over. MADI pens have been replaced by disposable flexpens prefilled with insulin aspart or by other pens. Despite of that, the experience and skills with MADI are effectively used on behalf of all PWD.

4.3. Insulin pumps. In the Teaching hospital Olomouc, the first insulin pump (Promedos E 1, Siemens) was introduced in December 1981. However, as late as at the end of the last century the CSII became a sophisticated therapeutic approach in several diabetes centres. To date, over 6000 men and women from more than 100 000 insulin treated PWD are profittig from various types of insulin pumps in the Czech Republic [34, 35].

5. Education — Programmed treatment of people with diabetes

Based on the concommittant clinical studies, principals of education comprising the principels of Therapeutic Patients Education of the WHO was introduced. The proposed educational schedule became a part of the so called «Programmed Treatment of persons with diabetes» which was proposed and checked on hundreds of diabetic patients as a part of the research grant on «The treatment of diabetes by means of an insulin pump and of the pen MADI. The Programmed treatment aims to an introduction of the intensive insulin therapy into the practice and has been composed of 4 parts: (1) diagnostic procedures, (2) introduction of an intensive insulin treatment, (3) patient´s education and supplying him with a pen and a glucose meter, (4) paitent´s self-decision dealing with his forthcoming treatment (acceptance or rejection of intensive insulin therapy). The PWD and the educator are substantial parts of any professional team dealing with diabetes treatment [36, 37].

6. Glycaemic Index of foods (GI)

The glycaemic index (GI) is a measure of the food power to raise plasma glucose (PG) concentration after a meal. For its determination, classical methods register the development of glucose concentration in capillary plasma or whole blood in the course of 120 min [38, 39].

There is no standardized protocol for measuring glycemic index (GI) that takes the time-of-day effects into account. Using the CGMS, software CareLink and MS Excel the enhanced data processing software (Degif XL4 and recently Degif XL5) enabeling the GI calculation at breakfast, lunch, afternoon snack and dinner times has been made possible (Fig. 19).

Using the Degif XL5, the glucose concentrations of 20 volunteers are monitored after they consumed either 50 g of glucose or one of six alternative foodstuffs at breakfasts, lunches, snacks and at dinner times. Within the 9-day test period, 7 such meals were monitored in 5 replicates for each volunteer. Specifically, CGMS (monitor Guardian, Sofsensor and transmitter Minilink) was used to monitor plasma glucose levels at 5 minute-intervals for a period of 120 min following ingestion. At the end of day 9, the data from all volunteers are transferred into Carelink, to MS Excel and processed [40].

Any tests that did not fulfill the evaluation criteria (fasting before meal shorter than 210 min, consumption lasted longer than 30 min, incomplete portion or additional food was eaten or sensor failure appeared) were not processed. Next, GIs exceeding three times the interquartile interval were excluded. Such as 312 out of 350 tests (89 %) were analyzed.

The Shapiro-Wilk test showed a non-normal distribution of GI values. Median and 1st and 3rd quartiles were used to express the value of group-related GI. The evolution of plasma glucose in the course of 210 min after the meal start is demonstrated (Fig. 20–22).

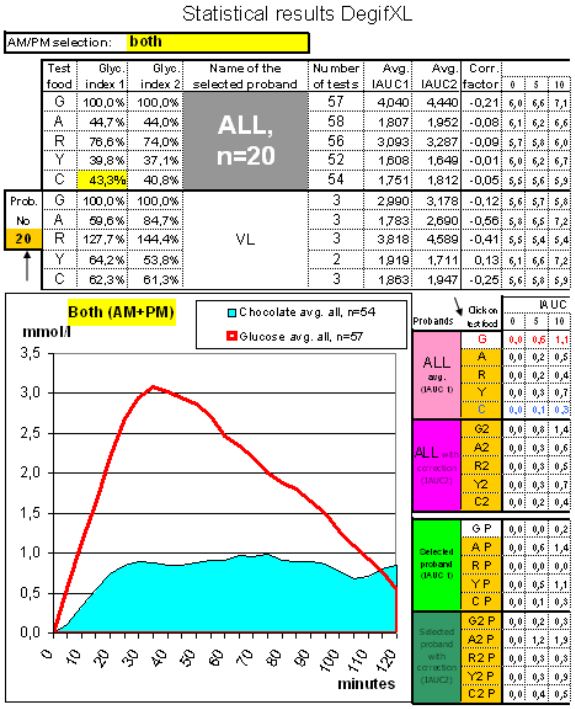

Figure 19. Degif XL — printscreen; evolution of P glucose concentration after consumption of 50 g glucose (red line) and after the tested meal (chocolate) containing 50 g of absorbable carbohydrates (blue); GI values of tested foods (G-A-R-Y-C) as means of the whole group of 20 tested persons or of tests for one selected volunteer (VL, Prob. No 20) [41]

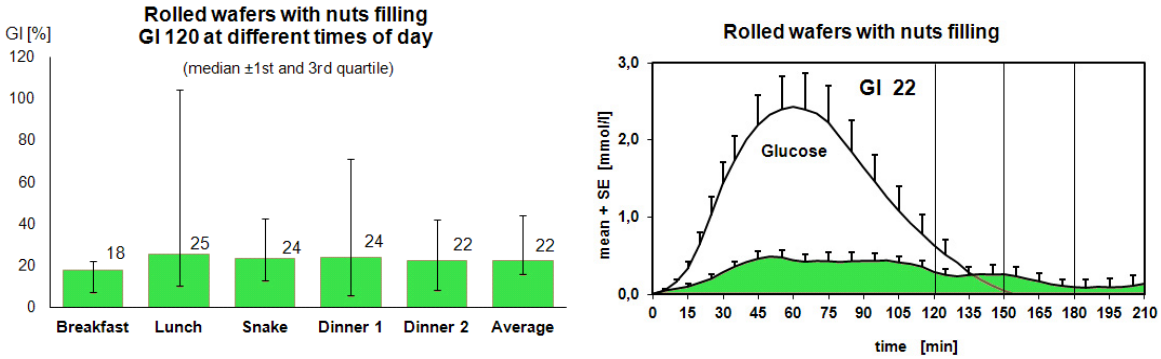

Figure 20. The values of group-related GI of rolled waffers at 5 different times of the day and average value. The evolution of plasma glucose in the course of 210 min after the meal start [40]

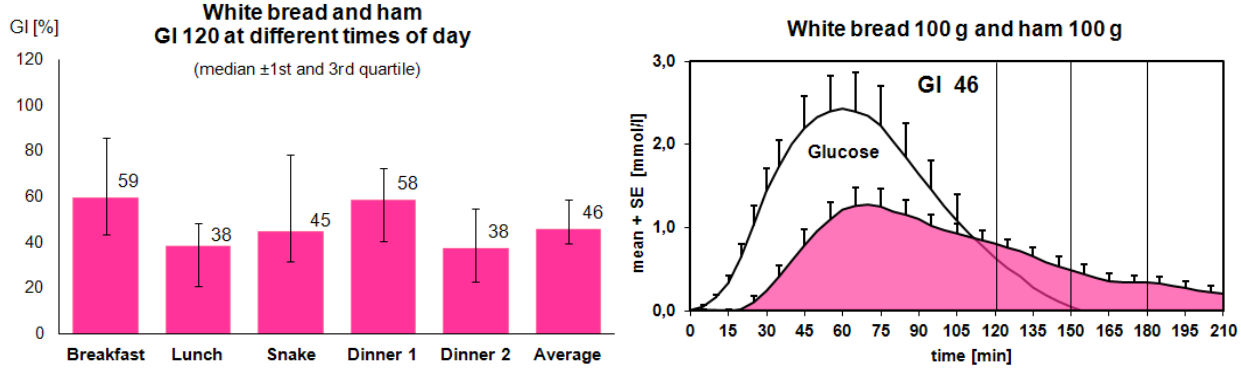

Figure 21. The values of group-related GI of white bread and ham at 5 different times of the day and average value. The evolution of plasma glucose in the course of 210 min after the meal start [40]

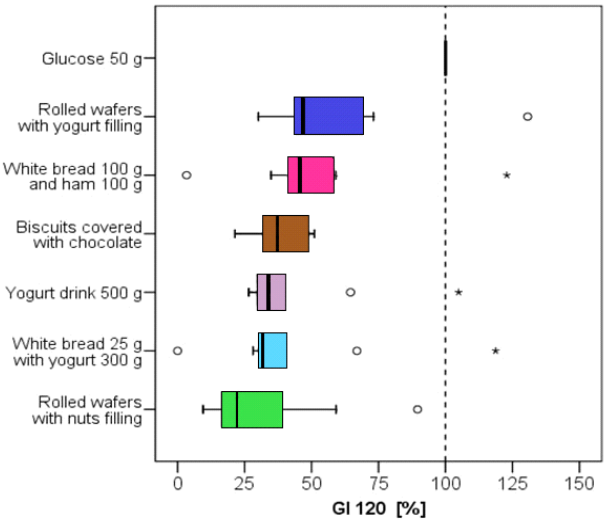

Figure 22. The average values of group-related GIs of 6 different foods [40]

Our findings suggest that tests performed at different times of day using CGMS are an acceptable approach to GI determination. This approach appears to be worthy of consideration as an alternative to present methods according to the standard ISO 26642. This method appears to be useful to the assessment of therapeutic effectiveness of oral antidiabetic drugs etc. [42].

7. Intensive selfmonitoring

The accuracy and precision of various glucometers was estimated [43–48]. Ten-point ambulatory glycaemic profile and continuous glucose monitoring (CGMS) was introduced to practice [49]. In persons on insulin pumps CGMS resulted in reduction of HBA1c [50].

7.1. Accuracy and precision of glucometer-strips systems was evaluated in several studies using different methods. Within the course of 15 years we have tested the glucometer systems Card (Medisense), Optium (Abbott), Advance (Hypoguard, GB) and Linus (Agamatrix, USA) at our diabetes centre considering their accuracy when used in real life. The purpose of our recent trial was to assess the accuracy and precision of the electrochemistry-based glucometers CONTOURLINK, Bayer, Germany, using FAD glucose dehydrogenase strips, and CALLA, Welion, Austria, as well as LINUS, Agamatrix, USA, both using glucose oxidase strips. The tests performed with these glucometers resulted in acceptable results (Fig. 23) [49].

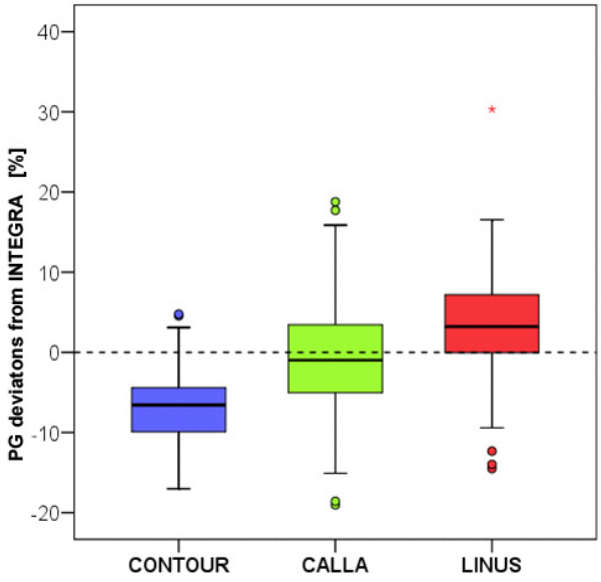

Figure 23. Relative deviations of Plasma glucose concentration estimated on glucometers from laboratory analyzer Cobas Integra [49]

7.2. Ambulatory glycaemic profiles. We also paid attention to the assessment of diabetes control in real life using Ambulatory Glycaemic Profiles (AGP) as markers of therapeutic effectiveness (Fig. 24). In our centre, the ten-points ambulatory glycaemic profiles are performed as a substantial part of regular diabetes check-ups. The PWD´s are trained in AGP including corrections of timing of fingerpricks, meals and insulin application exceeding ± 15 min of the times printed on the AGP sheet. On the evaluation of an AGP is especially recommended:

– to compare the fasting PG values at 6,00 h at the beginning and at the end of the AGP to assess the stability of diabetes control;
– to pay attention to the evolution of PG between midnight and 6,00 h a.m.; the increase ≥ 1.0 mmol/L indicates a dawn phenomenon;
– to explain the postprandial PG variations over the day;
– to identify hypoglycaemias;
– to discuss all items dealing with insulin dosage, meals and exercise;
– to suggest adaptations.

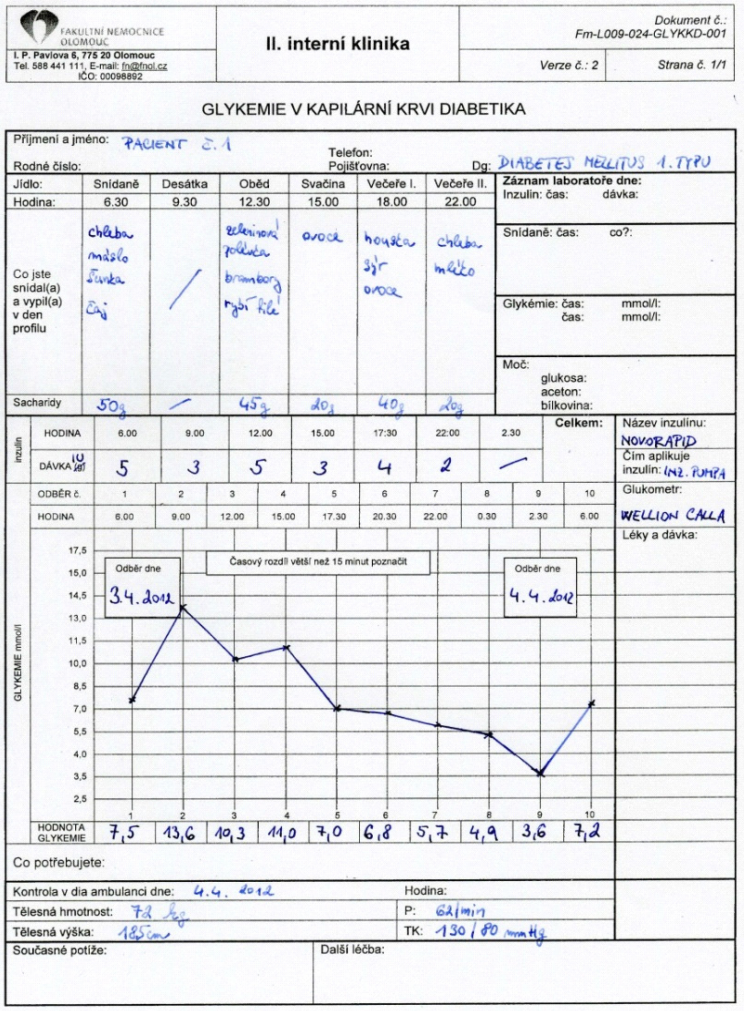

Figure 24. 10-point P-glucose profile carried out by a men with DM 1

7.3. Continuous glucose monitoring. In the course of our study PARASEN (sensor augmented CSII using insulin pump Paradigm x22 or Paradigm x54) following observations were made: Since 1993, insulin pump treatment was started in 167 PWD1 or PWD2. Long-term Continuous Glucose Monitoring (Fig. 25) was shown to reduce HbA1c (Fig. 26). The purpose of a prospective study was to assess the real patient´s interest in routine use of transcutaneous sensors related to the hypothetical optimum,, always on CGM». In the course of 7 years (2006 to 2012) the sensor-augmentation of Continuous Subcutaneous Insulin Infusion (CSII) was repeatedly offered free of charge to all PWD on pumps (n=123) attending the regular check-ups

supported by Carelink Personal software. The CGM was accepted for variable number of days by 63 (51 %) of them. Even after offering sensors and CGM education to all PWD free of charge, there seems to be lack of interest/motivation in PWD to try CGM [51].

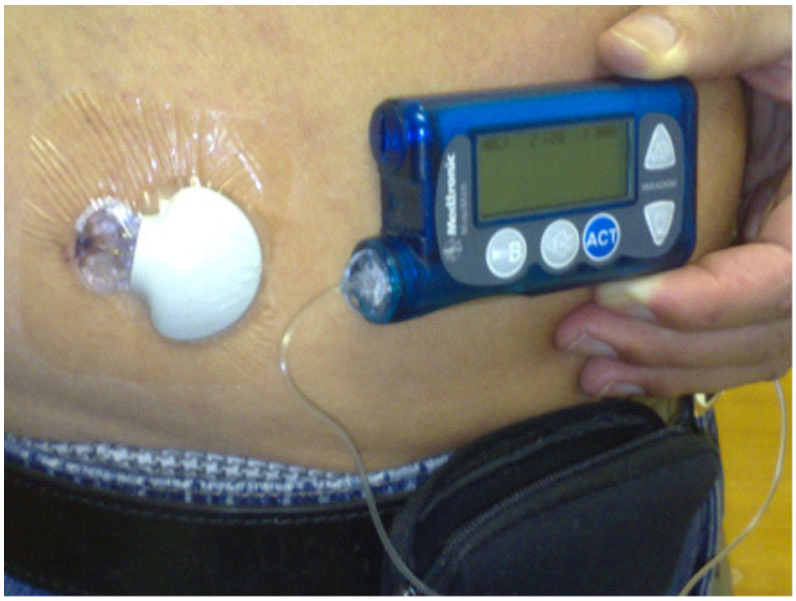

Figure 25. Continuous glucose monitoring: Sofsensor inserted into subcutaneous tissue and connected to transmitter Minilink (left); insulin pump Paradigm x22 (right)

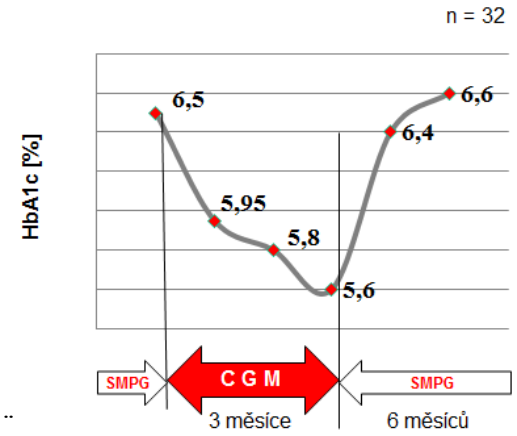

Figure 26. Evolution of HbA1c in PWD1 on CSII (insulin pump Paradigm x22, Medtronic Minimed, Northridge, CA, USA) when performing selfmonitoring of plasma glucose (SMPG) using glucometer-strips system Advance (Hypoguard, GB) and during the 3-month period with continuous glucose monitoring (CGM) using Sofsensor and Minilink (Medtronic Minimed) [50]

The positiv influence of CGMS on glycaemic variability and evolution of HbA1c [52–57], and on the other hand, a week acceptance (around 50 %) of this approach from men and women with diabetes may challenge both professionals in CGMS technology and professionals in patients´education to improve the outcomes of their endeavour. Occurrence of adverse events is very rare [58].

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Р.Хлуп

Чех Республикасы Оломоуц қ. Университетінің Медицина факультетінің клиникасында (1971–2014) диабет ауруына шалдыққан науқастар терапиясының клиникалық зерттеулер нәтижесі

Мақалада 1971–2014 жылдар аралығында клиникалық зерттеулердің нәтижелерінің жалпы саны белгіленген. Диабеттің 1 түрімен ауырған адамдарда қысқа әрекет ететін (MDI) инсулин терапиясы және инсулинді үздіксіз құю (CSII) терапиясы тиімді екені көрсетілген. Инсулинге жоғары сезімталдық, плазмада HDL-холестерин мөлшерінің деңгейінің азаюы, нейропатия терапиясының нәтижелерінің, есіне сақтаушылық пен зейіннің жақсаруы белгіленді. Және де алкоголь қабылдаған жағдайда қан құрамында алкоголь постпрандиальдік мөлшерінің азайғындығы байқалды. Авторлар диабетикалық нефропатиямен ауыратын адамдардың бүйрек пен жүрек тамыр жүйесінің қызметіне Инкретин мен Лозартан препараттарының жағымды әсерін көрсеткен. Жүргізілген емдеу нәтижесінде диабетпен ауратын аналардың перинатальдік өлімі көрсеткіштері төмендегені дәлелденді.

Р.Хлуп

Клинические исследования результатов терапии больных сахарным диабетом в клинике Медицинского факультета Университета г. Оломоуц, Чехия (1971–2014)

В статье суммированы результаты клинических исследований в период с 1971 по 2014 годы. Показано, что у лиц с диабетом 1 типа эффективной оказалась терапия препаратами инсулина короткого действия (MDI), а также терапия путем непрерывного вливания инсулина (CSII). Отмечалось повышение чувствительности к инсулину, снижение уровня содержания в плазме HDL-холестерина, улучшение результатов терапии нейропатии, показателей памяти и внимания. Отмечено также снижение постпрандиального уровня алкоголя в крови в случаях его употребления. Авторами показано положительное влияние препаратов Инкретин и Лозартан на деятельность почек и состояние сердечно-сосудистой системы у больных с диабетической нефропатией. Отмечено снижение перинатальной смертности матерей, больных диабетом, в результате проводимого лечения. Применение инъекторов многоразового пользования (шприц-ручки) показало более высокую точность вводимой дозы инсулина и микробную безопасность; авторами были показаны преимущества определения гликемического индекса пищи в условиях непрерывного контроля уровня гликемии, преимущества использования для самоконтроля глюкометров, а также снижение уровня гликозилированного гемоглобина при использовании инсулиновых помп.

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Histochemical method for fluorescent staining of Zn^{+2}-ions in glands

It is showed by authors that using of 8-para(toluenesulphonylamino)quinoline — a derivative of 8-oxyquinolin result histochemical revealing by using of fluorescent microscopy of Zn^{+2}-ions in cells of tissue of prostate, in pancreatic B-cells and in salivary glands contains a large amounts of ions. This method is high sensitive and high specific for revealing of Zn^{+2}-ions and there are only one step of staining procedures. Meanwhile it is possible to use only fresh frozen sections of tissues for investigation within short time limited by 15–20 min.

Key words: B-cells, Prostate, Salivary glands, 8-para(toluenesulphonylamino)quinolone, Zn^{+2}-ions.

Background. Pancreatic B-cells contains a large amount of Zn^{+2}-ions [1–3] as salivary glands and prostate. In B-cells Zn^{+2}-ions take part in processes of biosynthesis of insulin as in storage by forming of zinc-insulin complex [4, 5]. It is known that Zn^{+2}-ions in B-cells formed with insulin a deposited form of hormone as Zn^{+2}-insulin complex [4]. Proinsulin forms a Zn^{+2}-ions containing hexamer soon after its synthesis. In addition the zinc ions enhance proinsulin solubility and render insulin insoluble. Pancreas of animals as of Human contain Zn^{+2}-ions [6].

There are between insulin and zinc content in B-cells: decreasing of insulin con- tent accompanied by decreasing of amount of Zn^{+2}-ions and in opposite in intact B-cells a large amount of insulin accompanied by a large amount of Zn^{+2}-ions. Meanwhile for estimate ability of B-cells for storage of insulin in cells it is necessary to use method of staining of zinc-ions.

Some diabetogenic derivatives of 8-oxyquinolin [8OXQ] possess high chemical affinity for Zn^{+2}-ions and in vitro formed color complexes as Zn^{+2}-chelator [7]. One of tits, a 8-para(toluenesulphonylamino)-quinoline [TSQ] is used for color revealing of Zn^{+2}-ions in solutions [8].

Aim of work: 1) to investigate Zn^{+2}-ions content in B-cells using staining by TSQ in pancreas tissue, in prostate and salivary glands of intact animals.

*Methods***.** Animals: 8 Rabbits 2,2–2,6 kg. Frozen sections of Pancreas tissue as of Prostate and Salivary glands were used. Group 1: A) Staining of Zn^{+2}-ions in B-cells on sections of intact animal's pancreas tissue, prostate and of salivary gland using of 0,4 % acetone solution of TSQ. B) Injection of TSO, 38,6 mg/kg and fluorescent microscopy of frozen section of pancreas.

Staining procedures

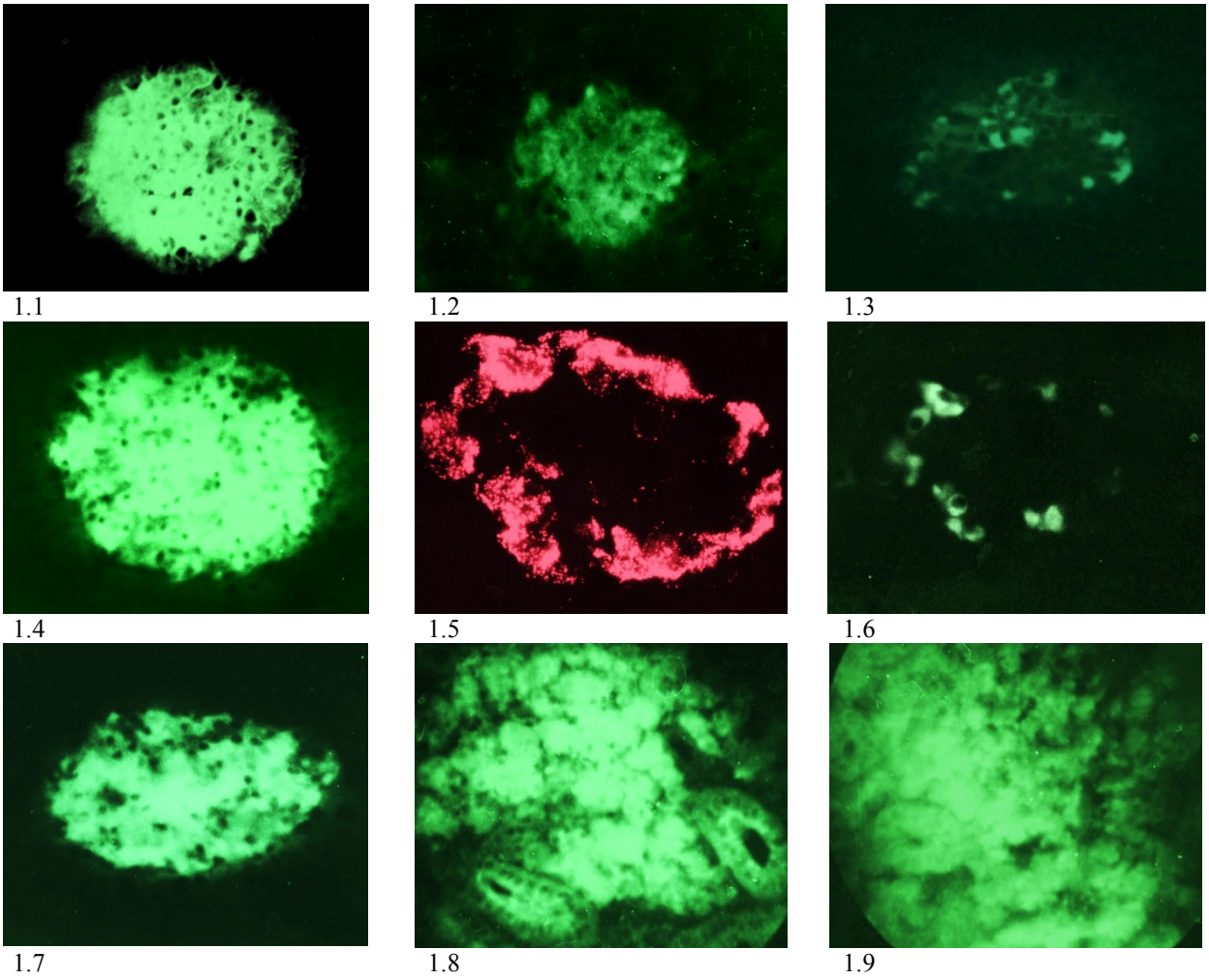
1. Staining procedures for sections of pancreas using fluorescent reagent 8PTSQ: 0,4 % acetone solution of TSQ prepared using NH_4OH 25 %-solution. Staining procedures: a few drops of 8PTSQ solution place on frozen sections for 10 sec.; 3 times washing by distilled water and investigation on UV-light microscope with measuring of intensity of fluorescence (control intensity of fluorescence of exocrine tissue's cells was accepted for 1,00); length of wave of UV-light 360–370 nanometers. For quantitative estimation of results of measuring intensity of fluorescence parameter K was calculated as rate: intensity of fluorescence of B-cells IF1/intensity of fluorescence of exocrine tissue cells IF2 (IF1/IF2);

2. Preparing of TSQ solution for injection (vital staining of Zn^{+2}-ions in islets, prostate and salivary glands): 25 mg of 8PTSQ (Institiute for High Pure Chemicals, Moscow) was dissolved in 70 % Ethanol at $+70^0$ Celsius and injected to Rabbits 36,5–38,8 mg/kg.

3. Staining of insulin and Zn^{+2}-ions content in B-cells of animals with experimental diabetes caused by injection of Dithizone.

Results

1. Intact animals. Intensive fluorescence of complex Zn^{+2}-ions-TSQ as positive reaction for Zn^{+2}-ions in cytoplasm of B-cells was revealed in cytoplasm of B-cells of pancreas past staining by TSQ solution as by using of vital histochemical reaction past intravenous injection of TSQ to animals. We observed decreasing intensity of fluorescence past partial and almost complete removing of Zn^{+2}-ions off B-cells by 3 days prolonged per oral treatment by Glibenclamide, 15–20 mg/kg daily [Fig. 1.2, 1.3].Results of fluorescent microscopy of sections demonstrate partial or almost complete negative reaction for Zn^{+2}-ions — as result of removing of Zn^{+2}-ions from B-cells [Fig. 1.3]. Negative fluorescent reaction for Zn^{+2}-ions in islets of animals with diabetes caused by selective destruction of B-cells by Dithizone (Fig. 1.5, 1.6; Tables 1, 2).

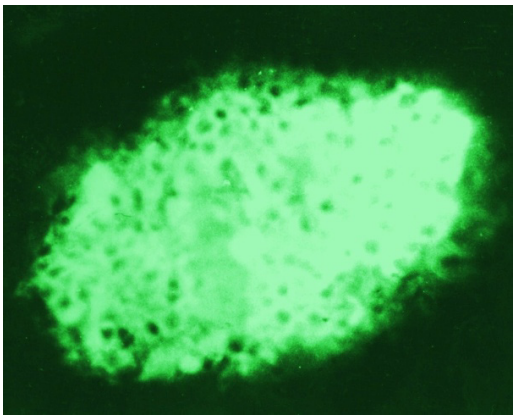


1.1 Intact Rabbit. Fluorescence of Zn^{+2}-ions in B-cells. Frozen section of Pancreas. Staining by 8PTSQ; ×140;
1.2 Injection of DDCA 250 mg/kg. Staining by 8PTSQ; partial binding of Zn^{+2}-ions in B-cells by DDCA; ×140;
1.3 Injection of DDCA 1000 mg/kg. Staining by 8PTSQ; almost complete binding of Zn^{+2}-ions in B-cells by DDCA; negative fluorescent reaction for Zn^{+2}-ions in B-cells; ×140;
1.4 Injection of 8PTSQ 38,8 mg/kg; vital staining of Zn^{+2}-ions in B-cells past injection. Frozen section of pancreas; ×140;
1.5 Rabbit, Destruction of B-cells caused by injection of Dithizone, 48,6 mg/kg; dark microscopy; frozen section of pancreas; ×200;
1.6 Rabbit, Destruction of B-cells caused by injection of Dithizone, 48,6 mg/kg; frozen section of pancreas, staining by 8PTSQ: absence of Zn^{+2}-ions in B-cells;
1.7 Intact Mice. Fluorescence of Zn^{+2}-ions in B-cells. Frozen section of Pancreas. Staining by 8PTSQ; ×140;
1.8 Frozen section of Prostate tissue of Rabbit. Staining by 8PTSQ. Fluorescense of Zn^{+2}-ions; ×140;
1.9 Frozen section of Salivary gland; Staining by 8PTSQ. Fluorescence of Zn^{+2}-ions; ×140

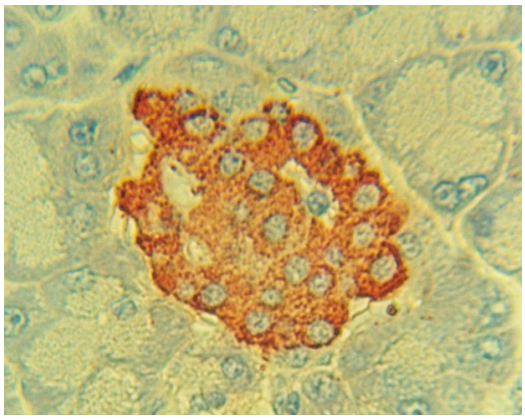
Figure 1

We observed intensive fluorescence of cytoplasm contained a large amount Zn^{+2}-ions cells of Prostate and Salivary gland (Fig. 1.8, 1.9) past intravenous injection of 8PTSQ solution.

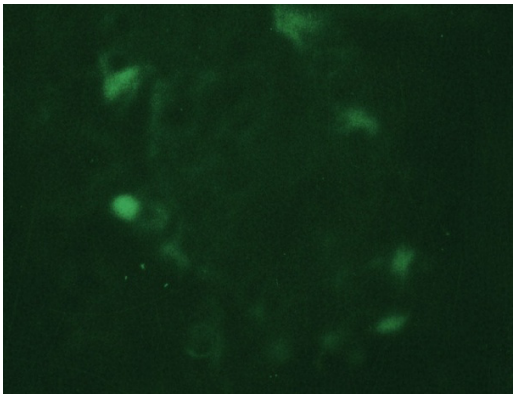
2. Animals with diabetes caused by injection of DZ (50,2 mg/kg). Negative reaction for Zn^{+2}-ions with 8PTSQ as for insulin in B-cells in sections of pancreas tissue (fig. 2.3, 2.4; control 2.1, 2.2; Table 1, 2) that demonstrate absence in cytoplasm of B-cells as of Zn^{+2}-ions as of insulin in result of necrosis and destruction of cells: Rabbits: K(IF1/IF2)=1,03±0,05; control: intact B-cells: K=2,06±0,07 ($p<0,001$); Mice: 1,89 ±0,06 and control (intact)=1,06±0,04.Insulin content in B-cells: K(IG1/IG2)=1,12 ±0,03; intact B-cells IG1/IG2=1,92±0,04 (Table 1, 2).



2.1



2.2



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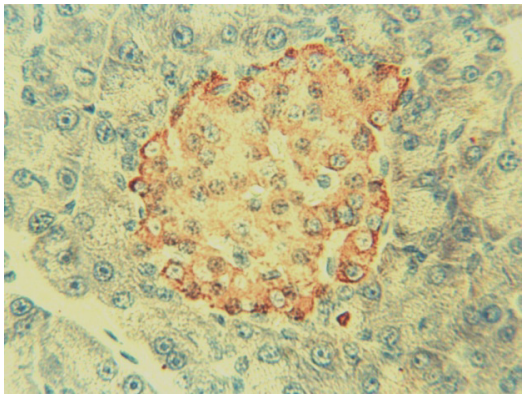

2.4

2.1 Pancreatic islet of intact rabbit. 8PTSQ fluorescent reaction for zinc. Intensive fluorescence (a large amount of zinc in B-cells); UV-light microscopy; ×140;

2.2 Pancreatic islet of intact rat. Immunohistochemical method. Normal content of deposited insulin in B-cells (blue-violet color); ×280;

2.3 Pancreatic islet of rat with dianetes. 8PTSQ fluorescent reaction for zinc. Negative reaction for zinc (absence of fluorescence) determined by destruction of B-cells and by absence of zinc-ions in cytoplasm; UV-light microscopy; ×140;

2.4 Panc reatic islet of rat with diabetes. Immunohistochemical staining method. Decreasing of insulin content in B-cells and of size and number of islets in sections; ×280

Figure 2. Zinc-ions and insulin content in B-cells of intact and experimental rats

Table 1

Zn^{+2}-ions content in B-cells (parameter K: IF1/IF2)

№	Animals	Intact animals (IF1/IF2)	Diabetes caused by Dithizone (IF1/IF2)
1	Rabbits	2,06±0,07•	1,03±0,05•
2	Rats	1,94±0,05	–
3	Mice	1,89±0,06*	1,06±0,04*

Note. • * — $p<0,005$.

Table 2

Insulin and Zinc content in pancreatic B-cells (parameter K)

№	Conditions of experience	Insulin (IG) and Z+2 content (IF) in B-cells (parameter K)	
		insulin (IG)	zinc (IF)
1	5 min. past injection of DZ	1,88±0,05	1,03±0,05
2	Diabetes caused by DZ (48,8–51,6 mg/kg)	1,12±0,03•	1,08±0,03
3	DDCA (987 mg/kg)	1,85±0,04	1,02±0,04*
5	Rabbit (intact)	1,92±0,04•	1,98±0,06*

Note. • * — p<0,001.

Results showed that in 3 cases method demonstrated a full coincidence of Zn^{+2}-ions content with content of insulin in B-cells: 1) in intact animals; 2) in animals with experimental diabetes; 3) in animals after removing of Zn^{+2}-insulin complex from B-cells by drugs.

This method demands following conditions. For fixation of tissue of pancreas to use the 70º alcohol sated with hydrogen sulfide (H_2S) or to use sections of frozen-pancreas tissue. Filters for UV-microscopy: UV-filter between UV-lamp and microscope and yellow filter for ocular of microscope. 8PTSQ is high specific fluorescent reagent for revealing of minimal concentrations of Zn^{+2}-ions in solutions as 10^{-7}–10^{-8}.

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Zn^{+2} иондарын бездерде флюоресцентті анықтаудың гистохимиялық әдісі

Авторлармен 8-пара(толуолсульфониламино)хинолин — 8-оксихинолиннің туындысын қолдану флюоресценттік микроскопияның көмегімен қуықалды безінің ұлпа жасушасында, панкреатикалық В-жасушасында және сілекей бездерінде Zn^{+2} иондарын айқындауға мүмкіндік беретіндігі көрсетілген. Әдістің өзгешілігі — оның жоғары сезімталдығы, жасушаларда Zn^{+2} иондарын айқындау ерекшелігі және боялу тәртібінің бірсатылығы. Салыстырмалы түрдегі кемшілігі — тұрақты гистологиялық препараттарды алу мүмкіндігінің болмауы. Препараттар аз уақыт сақталады — 15–20 мин.

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Гистохимический метод флюоресцентного выявления ионов Zn^{+2} в железах

Авторами показано, что использование 8-пара(толуолсульфониламино)хинолина — производного 8-оксихинолина позволяет с помощью флюоресцентной микроскопии выявлять ионы Zn^{+2} в клетках ткани предстательной железы, в панкреатических В-клетках и в слюнных железах, где он содержится в значительных количествах. Отличительная особенность метода — его высокая чувствительность, абсолютная специфичность в отношении выявления ионов Zn^{+2} в клетках и одноэтапность процедуры окраски. Относительный недостаток состоит в отсутствии возможности получения постоянных гистологических препаратов. Препараты сохраняются относительно недолго — в течение 15–20 мин.

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Interrelations between polymorphism of a gene of a methylenetetrahydrofolatreductase with level of Homocystein in patients with 2 type of diabetes

Purpose of the research — to study the frequency of occurrence of polymorphism ST gene methylenetetrahydrofolate patients with diabetes mellitus type 2 and its correlation with homocysteine levels and the development of ischemic heart diseases. 118 patients with diabetes mellitus type 2 at the age of 45–60 years were surveyed. Statistically significant differences were observed frequencies of alleles of T and S, genotype SS between groups of patients with pancreatic diabetes type 2 with ischemic heart disease and tran- sferred myocardial infarction and healthy persons. It was found that the allele T 677 gene, methylentetrahydrofolatreductase associated with an increased risk of myocardial infarction in patients with diabetes mellitus type 2 (OR=1,879, p=0,029). The combination of genotype T677 gene methylenetetrahydrofolatereductase with hyperhomocysteinemia in 2 type diabetic patients with coronary heart disease is related with other risk factors for cardiovascular diseases and can have a significant impact on the course of ischemic heart disease.

Key words: diabetes mellitus type 2, coronary heart illness, homocysteine, methylenetetrahydrofolate.

Actuality of research. Cardiovascular diseases, in particular the coronary heart disease (CHD), are one of main cause of death of patients with diabetes (DM) [1]. The relative risk of its emergence is increased at men depended from age by 1,5–2,5 times, at women by 1,7–4 times and the increase in risk is always more expressed at women comparatively with men. Existence of DM is related with high risk of emergence of all ishemia forms (ICD), including stenocardy, ischemia of a myocardium, a myocardial infarct and death [2]. ICD in patients with DM in comparison with patients without DM develops at earlier age and characterized by more severe damage of coronary arteries with involvement of distal part [1, 2]. Pathogenesis of cardiovascular complications based on multiple factors and determined by insufficient metabolic control of DM, by immunological and hormonal changes as by changes in system of hemostasis. Previously a special significance in pathogenesis of the cardiovascular diseases (CD) is attached with genetic disorders [3, 4].

Now hard works on studying of one of genetic markers of CHD — polymorphism — of a gene 5,10 — a metylentetragidrofolatreductase (MTGFR) and its role in formation of this pathology are conducted. Polymorphism of a gene of MTGFR characterized by replacement of a cytosin on tymin in the T677th nucleotide (C677T) As result enzyme is transformed in thermolabile and its activity decrease by 30–65 %, that result destroying of processes of transformation of Homocysteine in Methionin and to development of a hypergomocysteinemya (HGC) [3, 5].

Recently value of HGC as important risk factor of atherosclerotic changes of vessels and also of arterial and venous tromboembolia is widely discussed [6–8]. Homocysteine (GC) is the sulfur-containing amino acid which is formed in the course of an exchange of methionine and cysteine. The methionine is metabolized with formation of S-adenosilhomocystein as result of hydrolysis, is transformed into GC. Disturbances of transformation of this amino acid into methionine and cysteine leads to increase in its content in blood. Increased GC level in plasma result activation of processes of oxidation with formation of free radicals toxic for endothelial cells that result proliferation muscle cells and stimulation of platelets and leukocytes [4, 9]. The HGC promotes oxidation of lipids that stimulates processes of atherogenesis [7, 8].

It was established that HGC high level in plasma of blood is independent risk factor for developing of CHD [4, 9]. It was showed that risk of cardiovascular pathology considerably increases when the CHD is combined with DM [6, 10, 11]. However there are MTGFR low studied polymorphism of a gene and its interrelation with the GC level and their influence on development of ICD at 2 type of DM.

Research objective. To study polymorphism of C677T of a gene of MTGFR, its interrelation with the GC level and role in development and progressing of ICD in patients with 2 type of DM.

Materials and methods

Genetic material was obtained from 118 patients with 2 type of DM aged from 45 till 60 years. Duration of disease is 7,8±5,7 years. For investigation influence of polymorphism gene of MTGFR on development of ICD patients were divided for 2 groups. The first group included 69 patients age — 53,6±4,4 years. This

group in 42 patients in the anamnesis had a myocardial infarction (18 men and 24 women) age — 53,7±7,5 years. The second group 49 patients with 2 types of DM without ICD (15 men and 34 women), age — 52,1±4,3 years.

Criteria of exception from 1 and 2 groups: the age of patients till 45 years and over 60 years; existence at patients of chronic renal failure, recurrence of accompanying chronic diseases, of purulent — necrotic complication, B12-and folic acid deficiency induced anemia and also last month treatment by folic acid or vitamins of group B.

Control group included 89 patients (blood donors) aged from 45 till 60 years without disturbances of carbohydrate metabolism and the atherosclerosis and trombosis in the anamnesis.

All patient conducted full clinical and biochemical analysis. For identification of ICD Holterovsky monitoring of electrocardiogram using «Markett HELLIGE» system and a tredmillmetry using «HELLIGE CardioSoft V3.0» was carried out.

For DNA analysis samples of epithelial cells were used. Method phenolchloroformic extraction was used for separation of DNA. Polymorphic parts were amplifycated by Polymerase reaction using amplificator of «Eppendorf». Analysis of GC level was carried out by an immunoenzyme method using the analyzer IMMULITE 2000 type the Homocysteine (AXSIS) sets.

The statistical analysis was carried out using applied programs «Biostat» and «Statistica 6.0». All data are provided in a type of arithmetic averages and their standard deviation (M±SD). Tests for balance of Hardy — Weinberg and identification of associations by method χ^2. Reliability of coefficients of distinctions was accepted at value $p<0,05$. For estimation of associations of polymorphic options with pathological phenotype a relative risk (OR — odds ratio) was counted using formula $OR = ad/bc$, where: *a* — frequency analyzed allele at patients; *b* — frequency analyzed allele in control selection; *c* and *d* — total frequency of other alleles at patients and in control respectively. The size OR = 1 indicated absence of association at OR>1 the positive association allele with a disease («risk factor») were confirmed, at OR<1 — negative association allele with disease, as protective action given allele.

Results and discussion

Results demonstrated prevalence of normal alleles C677 in patients with type 2 of DM which was defined at 75,4 % of patients. Thus an allele of T677 which in some researches proved as a marker of CHD [12, 13] was revealed at 24,6 % of patients and at 21,9 % of persons of control group (OR=1,161, C.I. = [0,731–1,844], $\chi^2 = 0,40$, p=0,52) (Table 1).

Table 1

Frequency of alleles and genotypes of C677T polymorphic locus of MTHFR gene in patients with type 2 diabetes mellitus and in the control group

Selection	Alleles, *n* (%)		Genotypes, *n* (%)			The Hardy-Weinberg law matching (χ^2*, *df* = 1), *p*
	C677	T677	C677C	C677T	T677T	
Patients with type 2 diabetes mellitus (*n*=118)	178 (75,4)	58 (24,6)	68 (57,6)	42 (35,6)	8 (6,8)	0,664651
Control group (*n*=89)	139 (78,1)	39 (21,9)	55 (61,8)	29 (32,6)	5 (5,6)	0,652185

Frequency of occurrence of genotypes corresponded to Hardy's law — Weinberg (table 1). At the surveyed patients with 2 type of DM the heterozygotic genotype of C677T showed in 35,6 % of cases; the homozygous (normal) genotype of C677C — in 57,6 %; at 6,8 % of patients was a homozygous genotype of T677T.

Statistically significant distinction in frequencies of occurrence of genotypes of C677C, C677T and T677T between patients with 2 type of DM and control group is not revealed (table 1). At calculation of stratification risk the DM it was revealed some association of 2 types with T677 alleles (to OR = 1,1) with homozygous genotype of T677T (OR = 1,2) and with heterozygotic genotype of C677T (OR = 1,1) but this association wasn't reliable. There are risk for developing of ICD in patients with a homozygous genotype of T677T [9, 10].

One of problems of this research was to estimate influence of polymorphism of a gene of MTGFR as one of the factors of influence on metabolism of a homocystein on development of ICD. Results of investigation patients with ICB duration of current of DM and of arterial hypertension (AH), glycemia before and

postprandial were authentically higher as more high level of HbA1c. In patients with ICD a higher level of cholesterol (OHS), triglycerides (TG), lipoproteides of the low density (LPNP) and the lowered indicators of lipoproteides of the high density (LPVP) (table 2) are registered. These results correspond to data obtained by other authors [1, 14].

A.Engbersen et al. [15], A.Gardemann et al. [16] demonstrated existence of association of a homozygous genotype of T677T of gene of MTGFR with CHD [13, 11]. Other researchers didn't find relation between this polymorphism and vascular pathology [5, 17].

Results demonstrated that frequency of allele T677 of gene MTGFR in patients with DM 2 types with ICD and without ICD authentically didn't differ in gene MTGFR at patients with DM and made 26,8 % and 21,4 % respectively (table 3).

Analysis of distribution of genotypes of MTGFR at 7,3 % of patients with 2 type DM with ICD and at 6,1 % of patients without ICD are revealed existence of a homozygous (T677T) genotype of MTGFR (table 3). The heterozygot genotype of C677T prevailed in patients of 1 group (2 type DM with IBS) in compared with patients of 2 groups (2 type DM without IBS) (39,1 % and 30,6 %, respectively. Analysis of stratification risk the DM showed some association 2 types in combination with ICD with T677 alleles (to OR = 1,3), a homozygous genotype of T677T is revealed (OR = 1,4) and a heterozygotic genotype of C677T (OR = 1,3). However this association isn't reliable. Similar data are described also by other authors. In groups of the French, Swedish and Australian patients with CHD the association of allele T677 and a genotype of T677T of a gene of MTGFR with atherosclerotic damage of coronary arteries is also not revealed [11, 12, 18].

Table 2

Clinical-laboratory characteristics of patients with type 2 diabetes mellitus and coronary heart disease and without coronary heart disease

Indicator	Type 2 diabetes patients mellitus with coronary heart disease	Type 2 diabetes mellitus patients without coronary heart disease
Duration of diabetes, years	8,7±5,9	6,5±5,2*
Duration of hypertension, years	10,3±5,7	7,5±5,3*
Fasting blood glucose, mmol/L	8,3±1,8	7,2±1,4*
Postprandial blood glucose, mmol/L	11,2±1,9	8,7±2,04*
Average daily blood glucose, mmol/L	9,8±1,7	7,7±1,4*
HbA1c, %	10,2±1,9	8,9±2,2*
Total cholesterol, mmol/L	6,4±1,3	5,4±1,0*
Triglycerides mmol/l	2,8±1,2	1,9±0,9*
HDL cholesterol, mmol/L	1,11±0,22	1,28± 0,14*
LDL cholesterol, mmol/L	4,0±1,3	3,3 ±0,9*
CA, ED	5,4±1,3	4,4±0,9*

Table 3

Frequency of alleles and genotypes of C677T polymorphic locus of MTHFR gene in patients with type 2 diabetes mellitus (DM) with and without coronary heart disease (CHD)

Selection	Alleles, *n* (%)		Genotypes, *n* (%)			The Hardy-Weinberg law matching (χ^2*, *df* = 1), *p*
	C677	T677	C677C	C677T	T677T	
Patients wih type 2 DM and CHD (*n*=69)	101 (73,2)	37 (26,8)	37 (53,6)	27 (39,1)	5 (7,3)	0,980493
Patients wih type 2 DM without CHD (*n*=69)	77 (78,6)	21 (21,4)	31 (63,3)	15 (30,6)	3 (6,1)	0,524539

Note: * — Pirson χ^2 agreement criteria.

It was demonstrated the presence of high frequency of allele by T677 in patients with repeated myocardial infarctions and correlation this allele with the HZ level in serum [10, 19, 20]. A.Gardemann et al., 1999, demonstrated polymorphism of C677T of MTGFR almost at 2500 Europeans with angiographic the verified atherosclerosis of coronary arteries was investigated. It was noted that carriers of a homozygous genotype of T677T have higher rate of atherosclerotic damage of coronary arteries comparatively with patients have as least one allele of C677 [16].

This regard on the analysis of polymorphism of gene MTGFR in patients with 2 type of DM with ICD depending of existence or absence in anamnesis of a myocardial infarction was carried out. Patients with 2 type of DM with a myocardial infarction in the anamnesis differed from control group on the frequency of occurrence of alleles of T677 (OR=1,879, C.I. [1,059–3,333], χ^2= 4,72, p=0,029) and C677 (OR= 0,532, C.I. [0,300–0,944], χ^2 = 4,72, p=0,029). At patients past myocardial infarction less than in control the homozygous genotype of C677C (OR=0,420, C.I was observed. [0,199–0,890], χ^2=5,24, p=0,022). Also high frequency of genotypes of C677T and T677T (50 % and 9,5 %, respectively) in this group of patients is found in comparison with control group (32,6 % and 5,6 %, respectively) but this distinction wasn't reliable (table 4). Carriers of allele T677 had increased risk of development of a myocardial infarction (OR=1,879, C.I. [1,059–3,333], χ^2 = 4,72, r=0,029).

Patients with myocardial infarction in anamnesis differed on frequency of oc- currence of polymorphism of a gene MTGFR not only from healthy people but also from patients with 2 type of DM without ICD and with ICD but without myocardial infarction. Allele of T677 I is found at 34,5 % of patients with myocardial infarction whereas at patients without myocardial infarction and without ICD was revealed at 14,8 % and 21,4 %, respectively (χ^2 =7,78, r=0,02) (table 4). In group of patients with 2 type of DM and myocardial infarction are revealed prevalence of heterozygotic C677T (χ^2=6,43, r=0,04) and homozygous T677T (χ^2=0,93, r=0,62) genotype in compared with patients with 2 type of DM with ICD without myocardial infarction and 2 types DM without IBS (table 4).

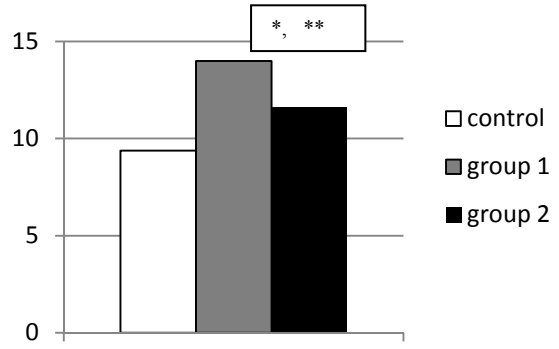
Table 4

The frequency of alleles and genotypes of C677T polymorphic locus of MTHFR gene in patients with type 2 diabetes mellitus, coronary heart disease and myocardial infarction, with coronary heart disease without myocardial infarction and without coronary heart disease

Selection	Alleles, *n* (%)		Genotypes, *n* (%)			The Hardy-Weinberg law matching (χ^2*, *df* = 1), *p*
	C677	T677	C677C	C677T	T677T	
Patients with type 2 DM with CHD and MI (*n*=42)	55 (65,5)	29 (34,5)	17 (40,5)	21 (50,0)	4 (9,5)	0,492288
Patients wih type 2 DM with CHD without MI (*n*=27)	46 (85,2)	8 (14,8)	20 (74,1)	6 (22,2)	1 (3,7)	0,534416
Patients wih type 2 DM without CHD (*n*=49)	77 (78,6)	21 (21,4)	31 (63,3)	15 (30,6)	3 (6,1)	0,524539
Control group (*n*=89)	139 (78,1)	39 (21,9)	55 (61,8)	29 (32,6)	5 (5,6)	0,652185

Note: * — Pirson χ^2 agreement criteria.

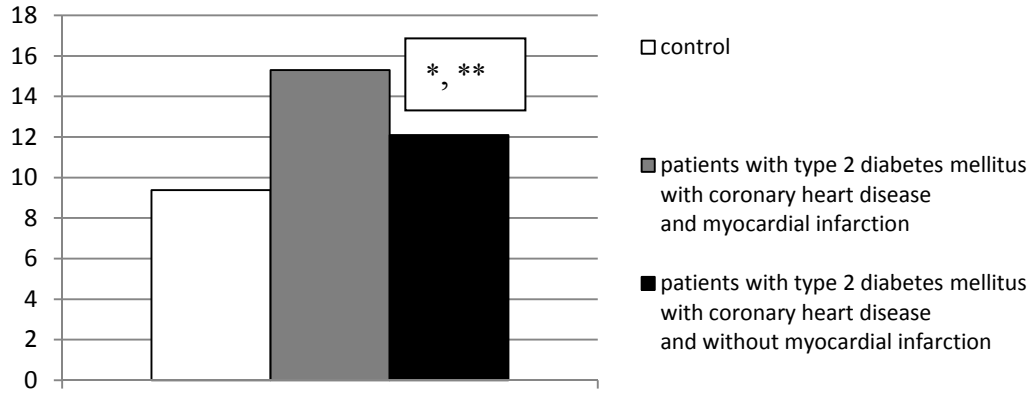
It is known that polymorphism of gene MTGFR can be one of the cause of the HZ causing increase. In patients with 2 type of DM were found higher values of HZ than at persons of control group: 14,0±5,2 umol/l in patients with 2 type of DM and ICD (rk=0,001); 11,6±4,8 umol/l in patients with 2 type of DM without IBS and 9,38±5,4 umol/l in control (Fig.1).



Note. * — significant of differences with the control group, p <0,001; ** — significance of differences with group № 2, p <0.05

Figure 1. Serum homocysteine levels in patients type 2 diabetes mellitus and with coronary heart disease (group 1) and without coronary heart disease (group 2)

The average level of HZ at patients with the post- poned myocardial infarction was authentically higher in comparison with patients without myocardial infarction (15,3±4,3 and 12,1±5,4 of umol/l respectively, p<0,05) (Fig. 2).



Note. * — significant of differences with the control group, p <0,05; ** — significance of differences with group of patients with type 2 diabetes mellitus, coronary heart disease and myocardial infarction, p < 0.05

Figure 2. Homocysteine serum levels in patients with type 2 diabetes mellitus with coronary heart disease and myocardial infarction and without myocardial infarction

Analysis of results of research HZ depending of polymorphism of a gene MTGFR showed that both in control and at patients with 2 type of DM (in 1 and 2 groups) the HZ greatest level is revealed at a homozygous genotype of T677T (Table 5). Especially it was observed in patients with a myocardial infarction in the anamnesis where at a homozygous and heterozygotic genotype of T677T and C677T the greatest values of HZ (Table 6) are revealed. Similar results were showed by K.Arai et al. (1997) that the level of a gomocysteinemiya is higher in patients with 2 type of DM with T677T MTGFR gene genotype than at surveyed with C677C a genotype. Also in group of patients with 2 type of DM with T677T the genotype revealed the high frequency of a myocardial infarction.

Table 5

C677T polymorphism of MTHFR gene and serum homocysteine levels in patients with type 2 diabetes mellitus and in the control group

Parameters	Control homocysteine, umol/l	Type 2 DM with CHD homocysteine, umol/l	Type 2 DM without CHD homocysteine, umol/l
MTHFR C677C	8,8±1,8	13,2±3,9*	11,2±4,6*
MTHFR C677T	10,4±2,3	14,5±6,5	12,1±4,7
MTHFR T677T	12,5	15,8±4,1	13,8±7,5

Note. * — Significant of differences with the control group, p < 0,05.

Thus, authors showed that T677T MTGFR gene genotype indirectly, through moderated by GHZ stimulated developing of atherosclerosis in patients with 2 type of DM and increases risk of a myocardial infarction [6].

Multiple-factor regression analysis of the HZ level in patients with 2 type of DM with ICD with myocardial infarction in the anamnesis and a homozygous genotype of T677T with other risk factors of ICD where in model as independent factor are a variables duration of DM and AG, level of systolic and diastolic the blood pressure, a glycemia before and postprandial, OHS, TG, LPVP, LPNP and as the dependent factor — the level of HZ, showed that HZ level at patients with a homozygous genotype of T677T depended of level of a pre- and postprandial glycemia as of daily glycemia and of HbA1c, LPVP, TG (R^2 = 0,99; r = 0,014).

Table 6

C677T polymorphism of gene MTHFR and homocysteine serum levels in patients with type 2 diabetes mellitus and coronary heart disease and myocardial infarction and without myocardial infarction

Parameters	Type 2 diabetes mellitus patients, coronary heart disease and myocardial infarction Homocysteine, umol/l	Type 2 diabetes mellitus patients, coronary heart disease and myocardial infarction Homocysteine, umol/l
MTHFR C677C	13,7±4,2	12,1±3,3
MTHFR C677T	14,5±4,7	13,8±4,8
MTHFR T677T	16,9±3,8	11,2

Thus, a high HZ level at a homozygous genotype of T677T of a gene of MTGFR can be an important factor in development of a myocardial infarction in patients with 2 type of DM.

Conclusions:

1. Frequency of occurrence of alleles and genotypes of C677T of a gene of MTGFR at patients with 2 type of diabetes without and with Ishemical Cardiac Disease (ICD) but without myocardial infarction in the anamnesis corresponded to control group.

2. Patients with 2 type of diabetes with a myocardial infarction in anamnesis differed from control group on the frequency of occurrence of alleles of T677 and C677 of gene of MTGFR (r=0,029). An allele of T677 of gene of MTGFR have relation with the increased risk of development of myocardial infarction patients with 2 type of diabetes (OR=1,879, r=0,029).

3. In patients with 2 type of diabetes ICD the HZ high level of serum of blood revealed in comparison with patients without ICD and control group.

4. Combination of homozygous genotype of T677T of gene of MTGFR to GHZ in patients with type 2 of DM with ICD and a myocardial infarction in the anamnesis is related with other risk factors of cardiovascular diseases (level of a pre- and post-prandial glycemia, average daily glycemia, HbA1c, LPVP, TG and can have important influense on current of ICD and myocardial infarction.

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Диабеттің 2-түрінде метилентетрагидрофолатредуктаза гені полиморфизмінің гомоцистеин деңгейімен өзара байланысы

Диабеттің 2-түрі бар науқастарда метилентетрагидрофолатредуктазаның С677Т гені полиморфизмнің кездесу жиілігі мен оның гомоцистеин деңгейі және жүректің ишемиялық ауруы дамуымен байланысын зерттеу мақсатында 45–60 жас аралығындағы диабеттің 2-түрі бар 118 науқас тексерілді. Диабеттің 2-түрі мен жүректің ишемиялық ауруы және басынан өткерген миокард инфарктісі бар науқастар мен дені сау адамдарда Т 677, С 677 аллелдері мен С677С генотипі кездесу жиілігінің арасында статистикалық дәлелді айырмашылықтар бар екендігі анықталды. Диабеттің 2-түрі бар науқастарда метилентетрагидрофолатредуктаза ферментінің Т677 аллелі миокард инфарктісі дамуының жоғары қауіпімен (OR=1,879, p=0,029) байланысты екендігі дәлелденді. Диабеттің 2-түрі мен жүректің ишемиялық ауруы бар адамдарда метилентетрагидрофолатредуктазаның Т677Т генотипі жүрек-тамыр ауруларының басқа да қауіп факторларымен тығыз байланысты және жүректің ишемиялық ауруының ағымына айтарлықтай әсер етуі мүмкін.

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Взаимосвязь полиморфизма гена метилентетрагидрофолатредуктазы с уровнем гомоцистеина при сахарном диабете 2 типа

С целью исследования изучения частоты встречаемости полиморфизма С677Т гена метилентетрагидрофолатредуктазы у больных сахарным диабетом 2 типа и его взаимосвязи с уровнем гомоцистеина и развитием ишемической болезни сердца обследованы 118 пациентов сахарным диабетом 2 типа в возрасте 45–60 лет. Выявлены статистически значимые различия частот встречаемости аллелей Т677 и С677, генотипа С677С между группами больных сахарным диабетом 2 типа с ишемической болезнью сердца и перенесенным инфарктом миокарда и здоровых лиц. Установлено, что аллель Т677 гена метилентетрагидрофолатредуктазы связан с повышенным риском развития инфаркта миокарда у пациентов сахарным диабетом 2 типа (OR=1,879, p=0,029). Сочетание генотипа Т677 гена метилентетрагидрофолатредуктазы с гипергомоцистеинемией у больных сахарным диабетом 2 типа с ишемической болезнью сердца тесно взаимосвязано с другими факторами риска сердечно-сосудистых заболеваний и может оказывать существенное влияние на течение ишемической болезни сердца.

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Assessment of risk factors of development of diabetes in respondents of the Karaganda region

We studied the frequency and structure of the risk factors of diabetes mellitus in 1453 respondents Karaganda region in the form of screening among urban and rural population using a scale FINDRISK. To identify factors shaping the development of the high risk of diabetes, was conducted the analysis of the socio-economic conditions, lifestyle factors, presence of chronic diseases, as well as a number of quantitative indicators such as: body mass index, waist circumference, measurement of glucose levels and blood cholesterol. The presence of high frequency (99,5 %) risk of developing diabetes among urban populations (46,3 %) and villages of the Karaganda region (53,6 %) in the age group from 45 to 60 years. It is revealed that a high risk of developing diabetes is higher by a factor of 1.2 in the urban population of Karaganda region than in rural areas. Risk factors that determine the high-risk group, as in the urban population, and in rural areas are lack of employment, presence of hypertension, angina, lack of physical activity, passive smoking, obesity, hyperglycemia, hypercholesterolemia.

Key words: diabetes mellitus, risk factors, the urban population of the village.

Introduction. Number of incidence of diabetes (D) is increased in every countries [1, 2] that is determined by aging of the population, prevalence of obesity and decrease in physical activity [3]. In half of cases diabetes is diagnosed more later real terms of its emergence and very often with developed complications. Primary prophylactic activity and optimization of mode of life of patients with prediabetes — is one of ways for to reduce social and economic consequences and to reduce risk developing of diabetes for 45–63 % [4–6].The main tool of preventive programs and a key to management of health of the population is work with risk factors of a disease. In this regard studying of frequency and structure of risk factors of diabetes among city population and in rural areas of the Karaganda region is undertaken.

Research objective: to estimate the frequency and structure of risk factors of diabetes among inhabitants of city population and rural areas of the Karaganda region.

Material and methods. One-stage cross (cross-section) research in the form of screening among population city of Saran (54,000) and rural people (Osakarovsky area) of the Karaganda region is conducted. 1453 persons, from them 1082 women (74,4 %) and 372 men (25,6 %) are included in research. In the Osakarovsky area 544 women (69,7 %) and 237 men (30,3 %) are examined 781 persons, from them. In city of Saran examined 672 persons, from them 538 women (80,1 %) and 134 men (19,9 %).

The following criteria of respondents are formulated: 1) age of respondents of 18–65 years inclusive; 2) accommodation of families not less than one last year in the studied regions of the country; 3) absence at respondent at the time of research of an acute disease and relapse of chronic diseases; 4) knowledge of Russian and Kazakh languages; consent to participation in research. Criteria of an exception were: pregnant women, persons with a mental, serious neurologic illness.

For assessment of risk of development of diabetes during 10 previous years at adults the scale of FINDRISK (FINnish Diabetes Risk Score) [7] was used. For unification of method, calculation and submission of information results in the questionnaire of FINDRISK are subdivided for 2 classes: with low (< 7–11 points) and high risk of development of a disease (12–20 points). For identification of the factors forming development of high risk of diabetes the analysis of social and economic conditions, factors of a way of life, production and labor and extra work, relationship status, existence of chronic diseases, and also a number of quantitative indices was carried out: as body weight index, waist circle, measurement of level of glucose and level of cholesterol of blood, level of systolic and diastolic blood pressure. Statistical processing was carried out using of STATISTICA package. For comparison of frequency of occurrence of a qualitative sign in various independent sets the criterion a HY2 was used: statistical differences were considered statistically significant at $p<0.05$. In the analysis of quantitative signs for each group were defined a median, the lower and top quartiles, the statistical importance of distinctions between groups was estimated by nonparametric criterion of Mang-Whitney, distinctions were considered statistically significant at $p<0.05$.

*Results and discussion***.** Risk of development of diabetes in Karaganda region is revealed in 1447 persons (99,5 %) and number of persons with high risk of a disease of $n = 802$ (55,4 %) that is 1,2 times higher than with low risk of $n = 645$ (44,6 %) prevailed. The obtained data showed existence of a similar tendency in forecasts of growth of prevalence of DM in the world and expediency of carrying out preventive actions. In Osakarovsky district in 776 people (99,4 %) risk of dia- betes are revealed, and the ratio of low and high risk was approximately identical: with low risk — 387 (49,8 %) and with high risk — 389 (50,1 %). In group with high risk of a disease there are prevalence of women — 290 (74,6 %) at men — 99 (25,4 %). The often highest risk of development of diabetes in group of 45–60 years (54,2 %) and prevalence of female persons (71 %) (Fig. 1).

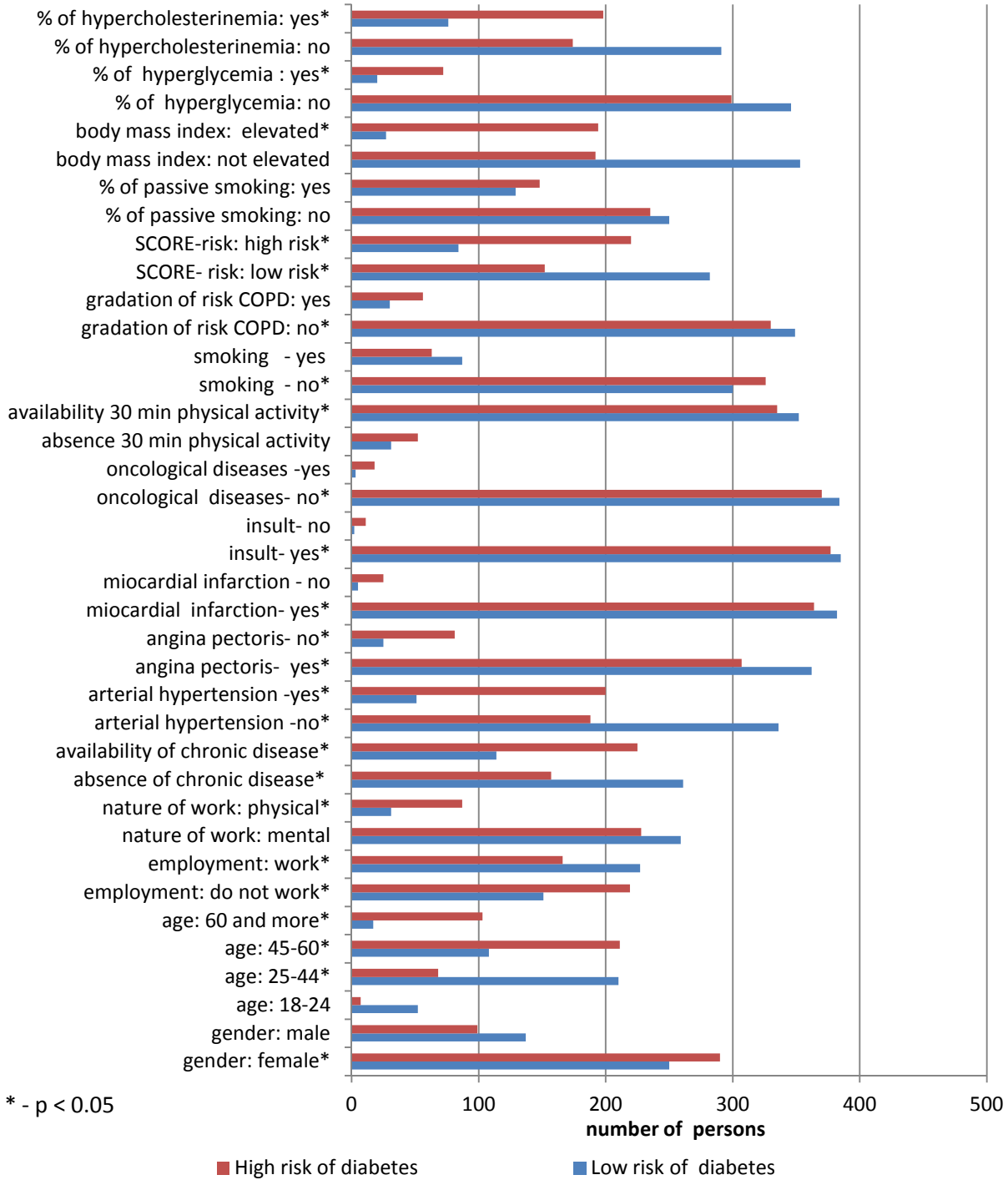

Figure 1. Evaluation of the factors of high and low risk of diabetes among respondents Osakarovsky area

In the city of Saran (population 54,000) the risk of development of diabetes made 671 — (46,3 %) with prevalence of respondents with high risk — 413 (61,5 %) comparatively with low risk — 258 (38,4 %). There are interrelation of high risk of development of diabetes in Saran in age group of 45–60 years (53,2 %) with prevalence of female persons (86,3 %) is traced (Fig. 2).

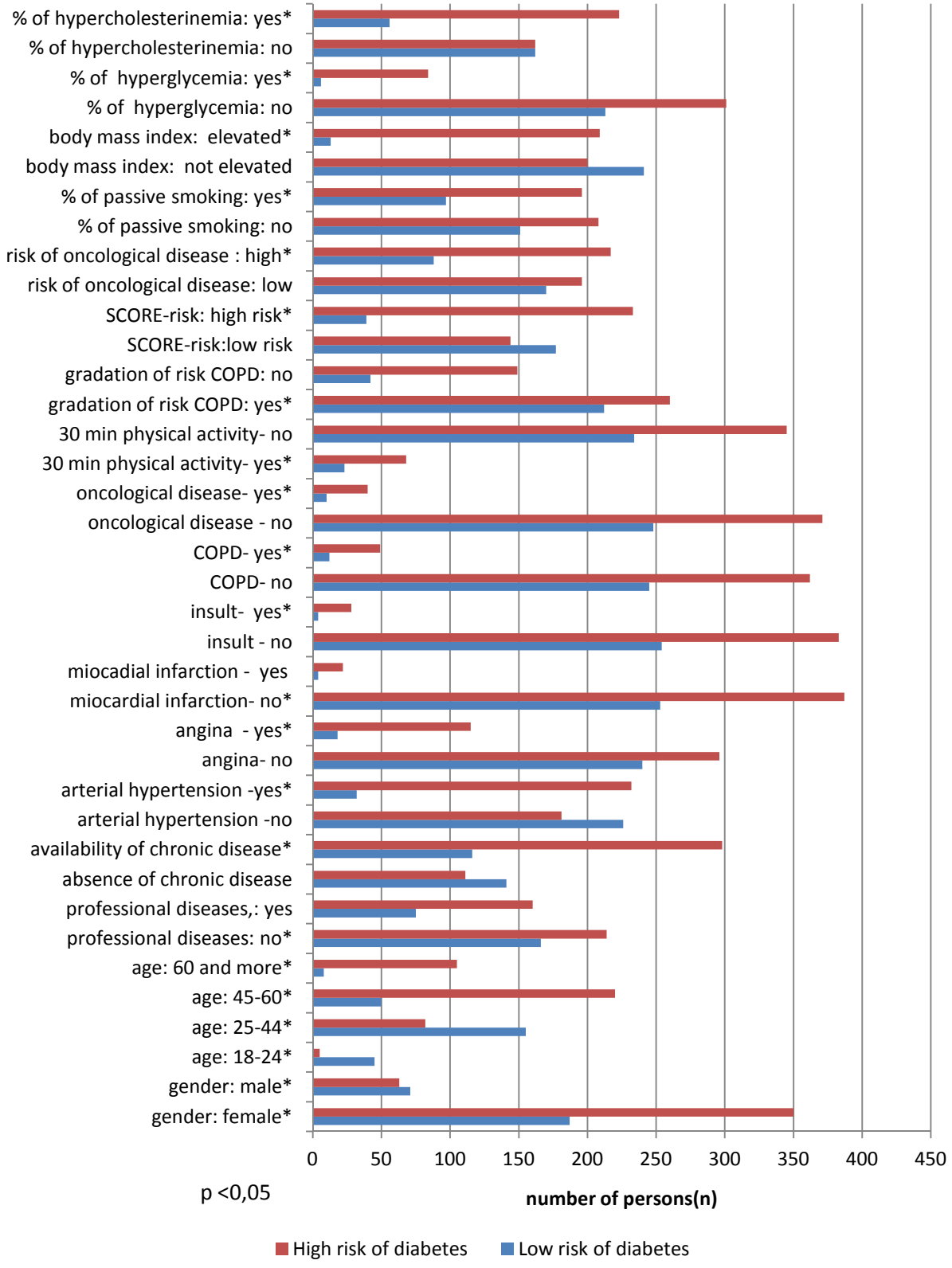

Figure 2. Evaluation of the factors of high and low risk of diabetes among respondents city of Saran

There are reliable prevalence of group with high risk of development of DM as in city as in rural area people it is reliable more high among women in age of 45–60 years that correspond to other author's data on increase of risk of diabetes with increase in age.

Thus, analysis of the obtained data demonstrated a prevalence of general risk of diabetes in rural areas over city however the high risk of development of a disease is higher for city population of the Karaganda region, than in rural areas that will be correspond to literary other author's data on the high frequency of risk of development of diabetes among urban population [8]. It is known that intensive transport traffic in city accompanied by high risk of diabetes [9].

The analysis of influence of social and economic conditions as factors of mode of life, production and work activity, relationship status, existence of chronic diseases in city population and rural areas was carried out. Results of research are shown on figure 1, 2.

In Osakarovsky area, among investigated respondents the unemployed — 219 (56,2 %) (p=0,002) in comparison with group of low risk of a disease — 151 met with high risk of diabetes by 1,4 times more often (38,8 %). In group of high risk of a disease of persons with physical nature of work is 1,1 times less (p=0,01), than in group of low risk of DM.

Results of interviewing evidently confirm increase in risk of development of diabetes in the presence of chronic diseases (57,8 %) especially more often at women. Arterial hypertension in group of respondents with high risk observed in 200 persons that is more often for 38 %, stenocardia at 81 that is for 14,3 % more often (p=0,04), a myocardial infarction at 25 that is 5,2 % more often (p=0,01) comparatively with group of low risk.

Physical activity is important factor for prevention of development of diabetes, In this regard existence 30 minute physical activity was analyzed. It is established that in group with high risk development of disease physical activity (existence of 30 min. physical activity) was available at 328 (84,3 %) the respondents that is 5,4 % lower (p=0,01) in comparison with the persons having low risk of a disease — 349 people (90,1 %).

Among the examined persons with high risk of development of diabetes the percent of smokers made 63 (16,2 %) that is 6,3 % lower than among persons with low risk have diseases 87 (22,5 %). However it is necessary to note that the percent of the passively smoking was 4,6 % higher in group of high risk at 148 respondents.

A gender distinctions in group of respondents with high risk of a disease the factor of active smoking was higher at men (86,8 %) than at women — (47,8 %), however the factor of daily smoking was higher at women, than men have 36,2 % and 6,0 % respectively.

At respondents of the Osacarovsky region with high risk of DM it isn't established to reliable interrelation with professional activity, material level of life, relationship status, disability, existence of heart attack, a stroke, oncological diseases at relatives. Also it isn't revealed reliable interrelations with alcohol intake, level of depression and alarm.

Obesity, hypercholesterolemia, hyperglycemia are belong to main factors of risk of DM, especially obesity. Epidemiological researches testify to higher prevalence of DM among persons with obesity than without it. Among respondents of the Osakarovsky region with high risk of development of diabetes the high percent of persons 194 (49,8 %) with body weight index as 30 kg/sq.m (p=0,0000) is revealed that by 7,1 times exceeds this indicator in group of low risk 27 (6,9 %). High risk of DM was revealed in patients with hyperglycemia-72 prevailed (18,5 %) than-20 without hyperglycemia (5,2 %) and are 3,6 times higher (p=0,0000) than in group of low risk. In 198 (50,9 %) respondents with high risk of DM the percent of a hypercholesterolemia is 2,6 times higher (p=0,0000), than in group of low risk 76 (19,6 %) respondents.

Also in group of high risk it is established that at 100 % of respondents the waist circle was increased as result of obesity. The similar tendency is noted at respondents of both floors, with prevalence of percent of female. More high percent of raised index of body weight as of hyperglycemia and hypercholesterolemia was observed at women and also in the general age group of 44–59 years.

Thus, the risk factors determined group of high risk in rural areas are: unemployment, intensive physical of work, chronic diseases as arterial hypertension, stenocardia, myocardial infarction, insufficient physical activity, passive smoking, obesity, hyperglycemia, hypercholesterolemia.

The analysis of risk factors of diabetes is carried out to city Saran, for establishment of influence of the studied factors in city conditions. In Saran, among the interrogated respondents with high risk of development of diabetes less persons with the higher education (p=0,01) were reliable, (is 1,2 times lower, than persons with low risk). In city population the high risk of development of diabetes 3,1 times more often was

observed at jobless persons comparatively with group of low risk of diabetes. However thus, in group of respondents with high risk of a disease the ratio working and the unemployed had no sharp distinctions (53,7 % and 45,3 % respectively). The analysis of the obtained data established that the group of high risk of diabetes is formed by the persons who are married which number is 1,7 times higher, than in group with low risk of a disease. The number of not married persons was lower in group with high risk of a disease (p=0,0051).

Among the factors influencing on high risk of development of diabetes in Saran existence of chronic diseases (72,3 %) that is 2,5 times higher (p=0,0000), than in group of respondents with low risk of diabetes is established. In the analysis of gender distinctions the percent of the women having chronic diseases was slightly higher than at men. Among chronic diseases in group of high risk of DM, in comparison with group of low risk, 7 times more often arterial hypertension (p=0,0000) was observed, stenocardia (p=0,0043) is 6,3 times more often. In the analysis of indicators, depending on a sex, it isn't established a reliable difference between men and women in the frequency of the specified diseases.

In group with high risk of a disease, though it is doubtful, such diseases as a myocardial infarction, a stroke, a chronic obstructive pulmonary disease, oncological diseases and hereditary predisposition to oncological diseases were more often observed. In group with high risk of diabetes more persons with a high oncology risk (p=0,0019) were observed. Follows from the above that comorbid states increase risk of development of diabetes. It should be noted that the number of the interrogated persons with existence of chronic diseases is higher in the city, than in the rural area that is explains by more higher risk of diabetes among city population.

Unlike of residents of rural areas among respondents of city Saran the number of the persons having existence 30 min. daily physical activities was authentically 7,5 % higher (p=0,01) in group with high risk of a disease. The percent of the persons having the excess body weight and obesity was 46 % higher (p<0,01) in group of high risk (an index of body weight 30), than respondents with low risk have diseases (an index of body weight 23). The analysis showed that the frequency of obesity is higher at women by 7 times, in comparison with men. Results of research indicate higher frequency of obesity (by 1,3 times) among female urban population, in comparison with the village.

At respondents with high risk of development of diabetes in Saran prevalence of smokers is established, and the percent of the passively smoking was authentically 9,4 % higher (p=0,023), than in group with low risk of a disease. It should be noted that among female persons passive smoking (39,3 % higher, than at males), is widespread in age group of 25–44 and 44–59 years. With high degree of reliability in group of high risk persons with a hyperglycemia (14 times higher, than in group of low risk) prevailed (p=0,0000), a hypercholesterolemia (is 3,9 times higher, than in group of low risk.) (p=0,0000) and the increased waist circle (is 1,2 times higher, than in group with low risk). The specified tendency is traced at persons of both floors, however is more often at women at the age of 45–59 years.

Thus, results showed that risk factors of development of diabetes among urban population in Central Kazakhstan is the following: are low level of education of respondents, the highest number of unemployed, the presence of chronic diseases such as hypertension, angina pectoris, passive smoking, obesity, hyperglycemia, and hypercholesterolemia. Urban residents have a higher percentage of chronic diseases than rural residents, but the percentage of persons with 30 minute physical activity was higher among the urban population.

Conclusions

1. The presence of high-frequency risk of diabetes (99.5 %) among residents of the Karaganda region; in the urban population (46.3 %), rural (53.6 %) in the age group 45–60 years.

2. High risk of developing diabetes is 1.2 times higher than in the urban population of the Karaganda region than in the countryside.

3. Risk factors that determine the high-risk group, both in urban populations, and in the village are the lack of employment, the presence of chronic diseases such as hypertension, angina, lack of physical activity, passive smoking, obesity, hyperglycemia, hypercholesterolemia.

4. Urban residents at high risk of diabetes had a higher percentage of chronic diseases, low level of education than rural residents.

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Қарағанды облысының респонденттерінде диабеттің даму қауіпі факторларын бағалау

Қарағанды облысының 1453 респонденттерінде FINDRISK шкаласының көмегімен қала және ауыл тұрғындары арасында скрининг түрінде диабеттің қауіп факторларының жиілігі мен құрылымы зерттелді. Диабеттің жоғары қауіпінің дамуын қалыптастыратын факторларды анықтау үшін әлеуметтік-экономикалық жағдайлардың, өмір салты факторларының, созылмалы аурулар болуының, сонымен қатар бірқатар сандық көрсеткіштердің: дене салмағы индексінің, бел өлшемінің, қандағы глюкоза мен холестерин деңгейлерінің сараптамасы жүргізілді. Қарағанды облысының қала (46,3 %) және ауыл (53,6 %) популяциясы тұрғындарының 45–60 жас аралығындағы тобында диабет дамуының жоғары қауіпінің (99,5 %) болуы дәлелденді. Қарағанды облысының қала популяциясында ауылды жермен салыстырғанда 1,2 есе диабет дамуының жоғары қауіпі анықталды. Сонымен қатар қала, ауыл популяциясында жоғарғы қауіп тобын анықтайтын факторлар: жұмыстың болмауы, артериалды гипертензия, стенокардия тәрізді созылмалы аурулардың болуы, физикалық белсенділіктің жеткіліксіздігі, пассивті темекі шегу, семіздік, гипергликемия, гиперхолестеринемия болып табылады.

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Оценка факторов риска развития сахарного диабета у респондентов Карагандинской области

Изучена частота и структура факторов риска сахарного диабета у 1453 респондентов Карагандинской области в виде скрининга среди городского и сельского населения с помощью шкалы FINDRISK. Для выявления факторов, формирующих развитие высокого риска сахарного диабета, проводился анализ социально-экономических условий, факторов образа жизни, наличия хронических заболеваний, а также ряда количественных показателей, таких как индекс массы тела, окружность талии, уровень глюкозы и холестерина крови. Установлено наличие высокой частоты (99,5 %) риска развития сахарного диабета среди жителей городской популяции (46,3 %) и сел Карагандинской области (53,6 %) в возрастной группе от 45 до 60 лет. Выявлено, что высокий риск развития сахарного диабета выше в 1,2 раза в городской популяции Карагандинской области, чем в сельской местности. Факторами риска, определяющими группу высокого риска, как в городской популяции, так и в селе, являются отсутствие занятости, наличие артериальной гипертензии, стенокардии, недостаточная физическая активность, пассивное курение, ожирение, гипергликемия, гиперхолестеринемия.

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The application of insulin analogues in the treatment of type 2 diabetes mellitus: the focus on the cardio protection

We have investigated 20 patients with type 2 diabetes, with an average age of 58,0 ± 4,32 years, with disease duration of 6,29 ± 1,28 years. The carbohydrate metabolism was estimated: fasting blood glucose and 2 hours later after meal, glycosylated hemoglobin (HbA1C). For the purpose of determine the safety of insulin analogues, have been studied biochemical parameters of blood before and after 3 months of therapy. The basic criterion of the safety of the therapy within 3 months of application study of insulin analogues was the number of daytime and nighttime hypoglycaemia, as well as the dynamics of body weight during the study period. Structural and functional state of the left parts of heart was assessed by echocardiography and dopplerography of transmitral blood flow. For all patients were assigned insulin analogues, the average daily dose was 32,4 ± 2,8 units, particularly rapid-acting insulin aspart (Novorapid) and acting insulin detemir (Levemir). Analogs of human insulin had a favorable effect on carbohydrate metabolism and reduced the incidence of nocturnal hypoglycemia. Also, there was a trend to improvement of the structural parameters and left ventricular diastolic function in patients with type 2 diabetes after treatment with insulin analogues. Application of insulin analogues have proven the safety of the insulin group in terms of prevention of hypoglycemia, which has a positive effect on cardiovascular events in the outcome of the treatment of diabetes.

Key words: diabetes mellitus type 2, fasting blood glucose level, postprandial hyperglycemia, glycosylated haemoglobin, insulin analogues.

The problem of diabetes mellitus (DM) has become «pandemic», and the number of patients as per the IDF Diabetes Atlas (2012) is 366 million (6.6 %). According to the expert forecast, that number of the patients with diabetes can raise up to 552 million (8 %) by 2030. In-growth is going to occur almost exclusively at the expense of patients with the type 2 diabetes mellitus in both developed countries and the developing countries [1, 2]. In 2010, in the Republic of Uzbekistan there were 117,240 (0.45 %) patients with diabetes mellitus registered; however, the results of epidemiological studies showed that in fact the number of patients was 10 times higher [3]. The large social significance of diabetes is that it leads to early disability and mortality at the expense of late vascular complications.

There are two underlying endocrine defects in the pathogenesis of this disease: impaired insulin production and peripheral insulin resistance. It is the insulin resistance which causes the excessive hepatic glucose production manifested, in particular, by morning hyperglycemia [4]. Hyperglycemia is an independent risk factor for late macro- and micro vascular complications in type 2 diabetes mellitus, as well as one of the major risk factors for mortality in cardiovascular diseases [5, 6].

Given the scale of developing epidemic of DM there is an urgent need to develop an effective therapeutic algorithm of blood-glucose-lowering treatment, which allows achieving the compensation of carbohydrate metabolism and preventing the development of severe vascular complications of this disease. At that, the efficacy of blood-glucose-lowering action of drugs concomitantly with their safety (both short-and long-term) for patients should be the priority in the selection of therapeutic agents. The presence of severe complications of DM, especially, of cardiovascular ones, imposes certain restrictions on setting individual goals of glycemic control. The results of studies such as ACCORD, VADT, ADVANCE and UKPDS (which also involved Russian endocrinology and cardiology centers), show that in the presence of cardiovascular complications the achievement of normoglycemia is dangerous due to the development of hypoglycemia resulting in cardio-cerebrovascular outcomes [7].

The chronic, relentlessly progressive nature of the type 2 diabetes course inevitably leads to the fact that sooner or later most patients with diabetes are unable to achieve or maintain an optimal long-term glycemic control with the help of diet therapy and medications (oral blood-glucose-lowering drugs) [8, 9]. The retrospective analysis of the UKPDS shows that about 50 % of B-cells in the pancreas have been destroyed and have lost their function by the time of the diagnosis of the type 2 diabetes [10]. With the development of the disease, the function of remaining B-cells in the pancreas becomes worse and, therefore the majority of patients require an adequate blood-glucose-lowering therapy [11]. However, within six years from the time of the diagnosis, as the disease progresses, most patients need an insulin therapy [12].

Over the past decade, the treatment approaches have changed. The importance of an individualized approach is increasing. Regardless of the age at which the diagnosis is established, DM is a lifelong disease, and the patients should follow the particulars of the treatment. Current data indicate the existence of the effect of «metabolic memory» whereby even short periods of poor glycemic control at the onset of the disease may increase the risk of future complications and long-term adverse outcomes, especially in respect of the cardiovascular system [13, 14]. Other studies emphasize the negative impact of hypoglycemia caused by an intensive insulin therapy on the cardiovascular system and mortality of patients with the type 2 DM. This issue remains unresolved; the available data are contradictory and not clear [7]. The individualized therapeutic approach recommended today in diabetes mellitus implies an optimal balance of the efficacy and risk of adverse events for each patient [15].

The type 2 diabetes is a progressive disease. The stage of insulin hypersecretion is replaced by the stage of «exhaustion»; during this period, an insulin deficiency occurs, and the patient begins to need an insulin therapy. Different types of insulin are used for the initiation of an insulin therapy and its further intensification. More than 80 years have passed since insulin was discovered. The first commercial insulin preparations were far from ideal, however, the rapid improvement in the technology led to the appearance of highly purified animal insulins, long-acting insulins, and finally, the recombinant human insulin. The completion of the DCCT and UKPDS studies, which demonstrated the ability to prevent the development and progression of diabetic complications with the tight glycemic control, was an important event for diabetology. At the same time, the shortcomings of standard insulin preparations, which did not provide the required flexibility of the therapy and often caused hypoglycemia when trying to normalize the level of HbA1c, became apparent. Today, modern genetic engineering technologies allow making changes to the structure of recombinant protein molecules and getting modified insulins (analogues). The first decade of the XXI century was an epoch of the rapid and widespread introduction of insulin analogues, as the most physiologic and effective drugs to replace a relative and absolute insulin deficiency [16, 3].

The reasons of poor compensation of the type 2 diabetes are varied, and not least related to economic factors, the organization of health care, etc. However, the special barriers to an effective therapy of diabetes are caused by the peculiarities of the perception of the treatment process by both patients and medical personnel. There is a «psychological insulin resistance» to a pathogenetically associated insulin therapy in the type 2 diabetes, not only in the patients but also in doctors. This situation often leads to a late prescription of insulin on the background of the progressive course of the disease and formed severe complications of diabetes, when it is impossible to significantly improve the prognosis. The phenomenon of «clinical inertia» (the delayed start and intensification of insulin therapy) is now well-known and experts define it primarily as a medical problem [8]**.** Naturally, for the doctor, there are many quite rational concerns about the prescription and treatment with insulin, especially concerns regarding hypoglycemia and weight gain. However, these adverse events are actually a natural consequence of the effective action of insulin. It is impossible to completely get rid of them, and the minimization of these effects is the most important task. It is not allowed to neglect the achievement of compensation trying to avoid adverse events [8]. The selection of insulin preparations with least side effects, a simple mode of administration and available algorithms of the dose titration is one of the most effective ways to prevent both clinical inertia and psychological insulin resistance. On that score, the insulin analogues that have recently been introduced into the wide clinical practice for the treatment of DM are preferable. Every year, these preparations become widely popular among both doctors and patients. This is due to their high efficacy, good safety profile and physiological insulin secretion. The clinical efficacy of insulin analogues is determined by the following criteria: the binding to insulin receptors in target tissues, the metabolic / mitogenic potency ratio, biochemical and physical activity and immunogenicity. One of the preparations for the treatment of DM is Detemir (the trade name is Levemir), which is classified among acylated long-acting recombinant human insulin analogues. The results of pharmacodynamic studies have shown that detemir has a more predictable blood-glucose-lowering effect than other basal insulins. The results of several international clinical trials have convincingly shown that the use of Levemir is safe and effective in the patients with type 2 diabetes at the initiation of insulin therapy as a single agent and in combination with oral blood-glucose-lowering agents [9].

The combination of basal and rapid-acting insulin analogues creates a more physiologic profile of action than the regular human insulin, and it is used to improve the efficacy and tolerability.

The information on the effect of insulin on the cardiovascular system in type 2 diabetes is very contradictory. Despite the fact that an insulin therapy is considered the standard for glycemic control in high insulin resistance, the use of insulin is not always beneficial for patients with cardiovascular diseases because of the possible development of hypoglycemia [4, 17–20]. However, the results of numerous randomized clinical

trials indicate that a significant improvement of glycemic control under the therapy with insulin analogues is associated with a very low incidence of severe and nocturnal hypoglycemia and almost no risk of severe hypoglycemia in the monotherapy with Levemir® insulin. What is one of the measures to prevent the progression of cardiovascular diseases in people with type 2 diabetes? In light of the facts above, we decided to evaluate the effect of insulin analogues on both carbohydrate metabolism and structural-functional parameters of the cardiovascular system in the patients with type 2 diabetes.

The aim of the study was to investigate the effect of insulin analogues on the diastolic dysfunction and structural-functional parameters of the left ventricular myocardium in the patients with type 2 diabetes.

*Material and methods***.** The study included 20 patients with moderate type 2 diabetes, the average age of 58.0 ± 4.32 years, disease duration of 6.29 ± 1.28 years, who received inpatient treatment in the endocrinology department of clinic No.3 of Tashkent Medical Academy. The degree of compensation of carbohydrate metabolism was evaluated taking into account the fasting blood glucose levels and the blood glucose levels in 2 hours after a meal, as well as by determining the glycosylated hemoglobin level (HbA1C). In order to determine the safety of insulin analogues, the levels of ALT, AST, creatinine, blood urea were investigated before the therapy and after 3 months of the therapy. The number of diurnal and nocturnal hypoglycemic states within 3 months of using the studied insulin analogues, as well as the dynamics of the body weight change during the period of the study was the main criterion of the safety of the therapy conducted. All the patients were assigned to insulin analogues, namely, aspart (NovoRapid) — an ultrashort-acting insulin and detemir (Levemir) — prolonged-acting insulin, at an average daily dose of 32.4 ± 2.8 units.

The structural-functional state of the left parts of the heart was assessed by the echocardiography and Doppler ultrasound of the transmitral blood flow [21–24, 15]. The investigation was conducted at Sonos ultrasonic cardiograph produced in the USA, with the dopplerographic attachment. The structural parameters of the heart were determined during the investigation in the one-dimensional mode. The pulsed Doppler EchoCG was conducted using B-mode EchoCG [25]. The transmitral blood flow was recorded from the apical 5- or 4-chamber view. The left atrial (LA) end-diastolic dimension and left ventricular end-diastolic dimension, the thickness (mm) of the left ventricular posterior wall (LVPW) and interventricular septum (IVS) in diastole, left ventricular end-diastolic volume (LV EDV, ml), left ventricular ejection fraction (LVEF, %) were determined in the echocardiography [5]. The left ventricular mass (LVM, g) was determined by the formula of Devereux R., Reichek N. (1977); the left ventricular mass index (LVMI, g/m^2): the LVM / body surface area ratio (Devereux R., 1984); left ventricular relative wall thickness index (LV RWTI, mm): the sum of the thickness of LVPW and IVS / LV EDD ratio. The transmitral diastolic flow was investigated to assess the left ventricular diastolic function: the peak of the LV rapid diastolic filling (VE, m/sec.) / peak of active atrial filling (VA, m/sec.) ratio — VE/VA or E/A.This study of the patients with type 2 diabetes was carried out before the treatment and after 3 months of the treatment with the insulin analogues.

Results. The analysis of changes in the measures of carbohydrate metabolism, body mass index (BMI), and a daily dose of insulin after 3 months of insulin therapy, was performed (Table 1).

Table 1

The analysis of changes in the measures of carbohydrate metabolism, body mass index (BMI), and a daily dose of insulin after 3 months of insulin therapy

Measures	Before the treatment, n=20	After the treatment, n=20
Fasting blood glucose, mmol/L	8.87±0.82	6.5±0.53*
Postprandial blood glucose, mmol/L	10.68±1.01	8.61±1.48
Glycosylated hemoglobin, %	9.72±3.2	7.2±0.9*
BMI, kg/m^2	30.7±0.96	30.1±0.95
Daily dose of insulin, units/day	32.4±2.8	34.4±4.3

Note. The significance between the groups before and after the treatment: * $p < 0.05$.

The measures of carbohydrate metabolism, such as, fasting blood glucose, postprandial blood glucose, glycosylated hemoglobin after the insulin therapy decreased by 26.7 %, 19.4 %, 25.9 % respectively. Under the treatment with insulin analogues, the body mass index (BMI) remained unaltered. The daily dose increased by 6.2 % taking into account the titration of insulin.

The levels of ALT, AST, creatinine, and blood urea were measured before and after the administration to determine the effect of the insulin analogues on the liver and kidney functions (Table 2).

Table 2

Measures of liver and kidney functions under the therapy with the insulin analogues

Measures	Before the treatment, n=20	After the treatment, n=20
ALT, U/L	21.3±3.43	23.56±1.76
AST, U/L	17±2.47	18±2.03
Creatinine, mcmol/l	70.68±5.75	69.28±7.17
Urea, mmol /l	6.06±0.79	6.38±0.79

According to the findings of the study, no significant effect on the liver and kidney functions was found.

The incidence of hypoglycemic states is an independent measure of the safety of the replacement insulin therapy conducted (Table 3).

Table 3

The incidence of hypoglycemic states

Diurnal, %		Nocturnal, %	
Baseline	After 3 months	Baseline	After 3 months
25.2	23.4	29.3	13.5*

Note. The significance between the groups at baseline and after 3 months. * p <0.05.

Based on the anamnestic data it was found that after 3 months, under the intensive metabolic control of the disease, there is a slight decrease in the incidence of diurnal hypoglycemia. Meanwhile, it was a very significant fact that the incidence of the nocturnal hypoglycemia decreased by almost 2 times; it was 13.5 % versus 29.3 %, which was primarily due to the positive pharmacodynamic properties of the insulin analogues.

There was a trend towards the improvement of the left ventricular structural parameters and diastolic function in the patients with type 2 diabetes after the treatment with the insulin analogues. The following changes were observed: the left atrial diameter decreased by 1.9 % and the left ventricular end-diastolic volume (LV EDV) — by 4.4 %; the thickness of the interventricular septum (IVS) was reduced by 2.9 % and the left ventricular posterior wall (LVPW) — by 3.24 % and the relative wall thickness (RWT) — by 1.8 %. The reduction of the wall thickness and internal volume of the cavity of the left ventricle resulted in the decrease in the left ventricular mass index (LVMI) by 6.1 %. In investigating the LV diastolic function, the significant increase in the PE by16.4 % and the decrease in the PA by 5.7 % were found, as well as the increase in the E / A ratio by 24 %; that is the evidence of the improved diastolic function.

The LV structure and diastolic function changed under the treatment with the insulin analogues (Table 4).

Table 4

The structural and functional state of the cardio-vascular system in the patients with type 2 diabetes under the treatment with the insulin analogues

Parameters	Before the treatment, n=20	After the treatment, n=20
LA, cm	3.81±0.16	3.74±0.3
IVS, cm	1.04±0.02	1.01±0.02
LVPW, cm	0.96±0.03	0.93±0.01
LV EDV, ml	129.0±6.41	123.4±5.12
LVMI	132.7±2.51	124.7±3.51*
RWT, rel.u.	0.40±0.01	0.39±0.01
EDV/LVM	0.57±0.03	0.59±0.04
LV EF,%	58.3±1.65	60.1±1.62
LV PE, msec	0.42±0.02	0.49±0.01*
LV PA, msec	0.53±0.02	0.50±0.01*
PE/PA, s.u.	0.79±0.03	0.98±0.03*

Note. The significance of differences before and after the treatment: *p < 0.05.

Discussion. Thus, according to our study, the efficacy and safety of human insulin analogues when used in the patients with the type 2 diabetes, were proved, as well as the trend towards the improvement of the left ventricular structural parameters and diastolic function was observed.

According to Veterans Affairs Diabetes Trial (VADT), it was found that pronounced hypoglycemic reactions in the type 2 DM were the main predictors of myocardial infarction, stroke and death from all causes [26]. The maintenance of the compensation of DM providing a HbA1c level of less than 6.5 % was the starting point for planning the large-scale multicenter randomized two-factor study — ACCORD (Action to Control Cardiovascular Risk in Diabetes), as the primary role of hyperglycemia in the pathogenesis of angiopathy proceeding with the affection of both small and large vessels was proved. When analyzing the overall mortality rate it was observed that in the total group of patients with type 2 diabetes, in patients without hypoglycemic episodes it was 1.2 % per year, and in the presence of hypoglycemia — 3.3 % per year. Moreover, in both branches of the study, the mortality was higher in subgroups with recorded hypoglycemic episodes (2.8 vs. 1.3 % per year in the intensive-control group and 4.9 % versus 1.1 % per year in the standard –control group). Thus, the mortality was higher in the patients with recorded hypoglycemic episodes regardless of a therapeutic strategy. In the risk assessment it was determined that the risk of death was higher in the group of intensive hypoglycemic therapy, in individuals without recorded hypoglycemic episodes, whereas in the group of standard therapy, the mortality was highest in the patients with recorded hypoglycemia.

It was concluded that it is hypoglycemia that causes an increase in the risk of negative cardiac outcomes.

The relative risk of myocardial infarction (MI) associated with undergone episodes of severe hypoglycemia 1 year before MI is 12 %, 5.5 months before MI is 20 %, 2 weeks before MI is 65 % [27].

The consensus the ADA and the scientific analysis of the American College of Cardiology and the American Heart Association: severe hypoglycemia is considered as the most likely reason for the increase in the cardiovascular mortality in the group of intensive glycemic control [27].

The death from hypoglycemia may be mistaken for the death from acute coronary syndrome, as it is generally not preceded by the measurement of the blood glucose level. Anatomical and morphological postmortem signs of hypoglycemia are absent.

Despite the fact that the results may indicate that the decrease in the blood glucose levels to the average HbA1c level of 6.5 % s as a result of the treatment does not reduce the risk of macrovascular events, these data do not detract from the benefit which can be derived as a result of the obtained significant difference between the groups of intensive and standard controls in reducing the risk of total events — serious macro — and microvascular events in the group of intensive therapy.

Thus, hypoglycemic episodes have a negative impact on the course of DM, not only from the standpoint of the difficulties in achieving the compensation, but cause an increased risk of acute cardiovascular events. Cardiovascular events increase the probability of death, cause difficulties in providing the compensation for diabetes and initiate the development of microvascular complications also resulting in a negative prognosis [28].

Our findings in respect of the effect of the insulin analogues on the structural-functional parameters once again proved the safety of this insulin group in terms of the prevention of hypoglycemia, and that has a positive effect on the cardiovascular events in the treatment outcome of diabetes.

Conclusions:

1. The human insulin analogues had a beneficial effect on the carbohydrate metabolism: significantly reduced the fasting glucose, postprandial blood glucose, led to the reduction of glycosylated hemoglobin compared to the baseline values by 25.9 %, which certainly indicates a high clinical efficacy of insulin analogues in the treatment of diabetes.

2. No effect on the liver and kidney functions in the application of the insulin analogues was found.

3. The application of insulin analogues in the treatment of type 2 diabetes mellitus helped to reduce the incidence of nocturnal hypoglycemia by 15.3 %.

4. There was a trend towards the improvement of the left ventricular structural parameters and diastolic function in the patients with the type 2 diabetes after the treatment with the insulin analogues: the left atrial diameter decreased by 1.9 %, the LV EDV — by 4.4 %; the IVS was reduced by 2.9 %, the LVPW — by 3.24 %, the RWT — by 1.8 %, the LVMI — by 6.1 %; the increase in the PE was by16.4 %, the decrease in the PA — by 5.7 %, the increase in the E / A ratio — by 24 %; that is the evidence of the trend towards the improved diastolic function.

5. The application of the insulin analogues proved the safety of this group of insulins in terms of the prevention of hypoglycemia, and that has a positive effect on cardiovascular events in the treatment outcome of diabetes.

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Диабеттің 2-түрін емдеуде инсулин баламаларын қолдану: кардиопротекцияға назар аудару

Біздің зерттеуімізге диабеттің 2-түрімен ауыратын орташа жастағы 58,0±4,32 жас, ауру ұзақтығы 6,29±1,28 20 науқас зерттелінді. Көмірсу алмасуы: ашқарында қандағы глюкоза және тамақтан кейін 2 сағаттан соң гликозирленген гемоглобин (HbA1C) бағаланды. Инсулин аналогы қауіпсіздігін анықтау мақсатында терапияға дейін және 3 айдан соң қанның биохимиялық көрсеткіштері зерттелді. Жүргізілген терапия негізгі қауіпсіздік критерийі ретінде инсулин аналогын 3 ай мерзімде қолданғаннан кейін, күндізгі және түнгі гипогликемиялық күй саны және зерттеу кезеңіндегі дене салмағының динамикасы есепке алынды. Жүректің сол жақ бөлімінің құрылымдық-функционалдық күйі Допплерография трансмитралды қанағысы және ЭхоКГ көмегімен бағаланды. Барлық ауруларға инсулин аналогы жекелей алғанда ультрақысқа әсерлі аспарт (Новорапид) және ұзартылған әсерлі детемир инсулині (Левемир) орташа тәуліктік мөлшерде 32,4±2,8 ЕД бекітілді. Адам инсулині аналогы көмірсу алмасуына жағымды әсер етіп, түнгі гипогликемия жиілігінің төмендеуіне әсер етті. Сонымен қатар инсулин аналогымен емдеуден соң, диабеттің 2-түрі ауруларының сол қарыншаның диастолалық функциясы мен құрылымдық параметрлерінің жақсару тенденциясы байқалды. Инсулин аналогын қолдану диабетті емдеу барысында жүрек қантамырға жағымды әсер етіп, гипогликемияның алдын алуда инсулин қауіпсіздігі дәлелденді.

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Применение аналогов инсулина в лечении сахарного диабета типа 2: фокус на кардиопротекцию

Нами были обследованы 20 больных СД типа 2, со средним возрастом 58,0±4,32 лет, с длительностью заболевания 6,29±1,28 года. Был оценен углеводный обмен: глюкозы крови натощак и после еды через 2 часа, гликозилированного гемоглобина (HbA1C). С целью определения безопасности аналогов инсулина были исследованы биохимические показатели крови до и через 3 месяца терапии. Основным критерием безопасности проводимой терапии было количество дневных и ночных гипогликемических состояний в течение 3 месяцев применения исследуемых аналогов инсулина, а также отслежена динамика массы тела за период исследования. Структурно-функциональное состояние левых отделов сердца оценивалось с помощью ЭхоКГ и Допплерографии трансмитрального кровотока. Всем больным были назначены аналоги инсулина в средней суточной дозе 32,4±2,8 ЕД, в частности, инсулин ультракороткого действия аспарт (Новорапид) и инсулин продленного действия детемир (Левемир). Аналоги человеческого инсулина оказывали благоприятное влияние на углеводный обмен, способствовали снижению частоты ночных гипогликемий. Также была отмечена тенденция к улучшению параметров структуры и диастолической функции левого желудочка у больных СД типа 2 после лечения аналогами инсулина. Применение аналогов инсулина доказало безопасность данной группы инсулинов в плане профилактики гипогликемии, что благоприятно влияет на сердечно-сосудистые события в исходе лечения диабета.

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Assessment of indicators of a kidney blood-groove at chronic pyelonephritis at patients with diabetes 2 types

There was conducted investigation of the nephritic blood circulations in different levels of the nephritic arteries of 90 patients with the 2nd type of diabetes in combination with the chronic pyelo-nephritis in the phase of exacerbation and remission. There was determined disorder of the nephritic blood circulations in the form of the nephritic blood flow velocity lowering and the increasing of the nephritic vascular resistance in different levels of the nephritic arteries, the expressiveness of which is determined by the activity of the inflammatory process in kidneys.

Key words: diabetes mellitus, pyelonephritis, nephritic blood circulations, renal arteries, diabetic nephropathy.

Background. Chronic pyelo-nephritis is important complication of diabetes (DM), taking into consideration its prevalence, difficulty of achievement of full treatment, high frequency of a re-infektion and development of complications as cortico-medullar abscess, the kidney anthrax, emphysematic pyelo-nephritis and pyelitis [1, 2]. Chronic pyelo-nephritis aggravate development of diabetes and result leading to decompensation, resistance to insulin, keto-acidosis and to diabetic coma [3]. The long time prolonged treatment, relapses and unsatisfactory results of antibacterial therapy of chronic pyelo-nephritis in patients with DM suggest about a role of violations of kidney hemodynamic in pathogenesis of aggravation of DM

The ultrasonic Doppler sonography (UZDG) of renal arteries (RA) is important method for investigation of blood circulation in diabetic nephropathy [4].

Research objective: to study of features of kidney haemodynamic at various levels of a renal artery using data obtained by UZDG depending on activity of chronic pyelo-nephritis at patients with diabetes 2 types.

Material and methods: 90 patients with chronic pyelo-nephritis (PN) with DM 2 types which were hospitalized in Regional Clinical Hospital of downtown Karaganda were examined. 2 groups of patients: Group 1 — relapse of chronic pyelo-nephritis (45 patients; 9 men, 36 women), the Group 2 — with remission of chronic pyelo-nephritis (45 patients; 18 men, 27 women). Average age of the Group 1 is 53,4±0,90 and average age of Group 2 is 50,9±0,75. Both Groups were comparable according to the main characteristics of DM and chronic pyelo-nephritis.

Patients with accompanying urological pathology, with chronic renal failure which were expressed by a diabetic nefro-angiopathy, malignant hypertensia were excluded from research. Control group: 20 patients with DM 2 types without chronic pyelo-nephritis.

All patient carried out UZDG on the ultrasonic Doppler system «Aloka-5000» (Japan), in real time with the convex sensor of 3,5 Megahertz. Analyzed data of power Doppler sonography with allocation of 4 degrees: 0 — kidney blood circulation without changes and is observed to the capsule of a kidney; 1 -easy decreasing of a blood cirsulation in a subcapsular space, 2 — moderate violation of kidney haemo dynamic; 3 — expressed decreasing of blood-cirsulation in cortical layer of kidneys. High-speed indicators of a circulation were investigated: peak of systolic speed of a blood cirsulation (V max), final diastolic speed of (V min), average speed of a blood-groove during complete cycle (V mean) and indicators of a vascular resistance, an index of resistance (RI), the pulsation index (PI), a sistola-diastola ratio of speeds (Ratio) in distal parts of main of renal arteries, segmentar, intershare and arc arteries. The obtained data are processed using of program «Statistica-6,0.»

Results and discussion. Patients with chronic pyelo-nephritis in a phase have aggravations and remissions of DM in the mode of power Doppler sonography the color impoverishment of drawing of intra kidney vessels determined by circulator frustration in intra renal arteries was observed. In aggravation of inflammatory process at DM decreasing of blood circulation in subcapsular part was observed at 28 (52,9 %) patients, moderate violation of kidney haemo dynamics (the blood-groove was observed to a cortical layer, vascular drawing of a brain layer is not changed) was observed at 25 (47,1 %) patients. The blood circulation in-

creased in kidneys was asymmetrical, with prevalence of changes in the left kidney. The qualitative analysis Doppler analysis data showed a low systolic and diastolic blood circulation that points to increase of peripheral resistance in kidney vessels. At remission of chronic pyelonephritis in DM the decreasing of circulation in subcapsular part was observed in 29 (64,5 %) patients, moderate violation of kidney haemo dynamics (circulation traced to cortical layer, vascular drawing of a brain layer is not changed) was observed in 11 (24,4 %) patients, the expressed violation of kidney haemo dynamics (circulation in cortical layer is not defined, vascular drawing of a brain layer is impoverished in the form of a reduction arc and «strippings» of intershare vessels in 5 (11,1 %) patients. The blood circulation in kidneys was symmetric.

Quantitative analysis of the Doppler data showed a presence of violations of kidney haemo dynamics in aggravation phase as in phase of remission of chronic pyelo-nephritis. Regardless of activity of inflammatory process in kidneys expressed violations of kidney haemo dynamics were observed characterized by considerable decrease of speeds of kidney blood circulation (Vmax, Vmin, Vmean) with distinct increase of indexes of vascular resistance (Ratio PI, RI) at the level of the main trunk, segmentary, intershare and arc arteries.

Thus, the analysis of indicators of kidney haemodynamics at chronic pyelonephritis in patients with DM 2 type, demonstrated revealed hypoperfusion of kidneys with increasing of vascular resistance at all levels of renal arteries that showed about essential haemodynamic changes in the microcirculation in comparison with control. In turn, decrease in a kidney blood-circulation creates a favorable conditions for maintenance and further progressing of inflammatory process in kidneys.

We carried out the analysis of Doppler parameters of a kidney blood-groove depending on a phase of chronic pyelonephritis at SD 2 types (Table 1). Apparently, reliable distinctions between groups are revealed at the level of the main trunk, intershare, arc arteries. Lower values Vmax, Vmean at the level of the main trunk of PAS in a phase of an exacerbation of chronic pyelonephritis in comparison with a remission phase at SD were noted 2 types. Thus it should be noted that reliable changes are revealed at the level of the main trunk left by PAS ($p<0,001$). Authentically higher PI values in the main trunk left by PAS in a phase of an exacerbation of chronic pyelonephritis, than in a remission phase were noted at SD 2 types.

Table 1

Indicators of kidney blood circulation at an aggravation and remission of chronic pyelo-nephritis at patients with diabetes 2 types

Indicators	Control DM without PM		DM+PM relapse		DM+PM remission	
	right	left	right	left	right	left
Main trunk						
V max см/с	69,8±2,34	71,9±2,54	52,4±2,53***	46,8±1,68***	53,04±2,29***	56,3±2,07***###
V min см/с	28,8±1,22	29,0±1,32	18,8±1,04***	16,9±0,75***	17,4±1,06***	19,2±1,15***
V mean см/с	37,5±1,17	38,6±1,56	27,5±1,74***	22,7±0,99***	29,7±1,60***	31,2±1,36***###
Ratio	2,46±0,07	2,53±0,08	2,93±0,13**	2,87±0,10*	3,43±0,24***	3,38±0,24 **
PI	1,07±0,02	1,11±0,04	1,28±0,05**	1,31±0,04**	1,21±0,05*	1,18±0,03 #
RI	0,58±0,01	0,59±0,01	0,63±0,01**	0,63±0,01*	0,65±0,01**	0,65±0,01**
Segmental VP						
V max см/с	51,2±2,25	56,6±2,80	44,8±1,95*	38,4±1,90***	40,7±1,90***	40,2±1,74***
V min см/с	22,2±1,41	24,07±1,24	17,4±0,79**	14,7±0,79***	15,02±0,95***	14,7±0,72***
V mean см/с	28,6±1,48	30,4±1,54	23,4±1,14**	20,7±1,23***	24,3±1,54*	23,2±1,27***
Ratio	2,36±0,06	2,35±0,03	2,67±0,09**	2,64±0,07**	3,01±0,18**	2,88±0,15**
PI	1,01±0,02	1,06±0,02	1,15±0,02***	1,16±0,04*	1,07±0,03	1,08±0,02
RI	0,57±0,01	0,57±0,006	0,60±0,01	0,60±0,01**	0,61±0,01*	0,61±0,01**
Interglobular VP						
V max см/с	44,3±2,43	45,9±2,34	35,2±1,88**	34,05±2,01***	30,1±1,68***#	28,3±1,74***#
V min см/с	21,7±1,56	19,8±0,99	14,4±0,82***	13,2±0,68***	11,6±0,76 ***#	11,02±0,69*** #

Notes. Significance of differences compared with patients without diabetes chronic pyelonephritis * — $p < 0,05$; ** — $p < 0,01$; *** — $p < 0,001$. Significant of difference between the DM + HP exacerbation and remission * — $p < 0,05$; ** — $p < 0,01$; *** — $p < 0,001$.

At an assessment of change of a kidney blood-groove at the level of intershare renal arteries the tendency to increase in high-speed indicators of a blood-groove (Vmax, Vmin) in a phase of an exacerbation of chronic pyelonephritis in comparison with a remission phase ($p < 0,05$) at sick SD 2 types is noted. Reliable

distinctions in indicators of average speed of a blood-groove and indexes of vascular resistance it isn't revealed. The tendency to higher PI values in right arc PA in a phase of an exacerbation of chronic pyelonephritis in comparison with a remission phase was traced ($p < 0,05$) whereas left arc PA of reliable distinctions it isn't revealed. The analysis of indicators of kidney haemo dynamics in the studied groups at the level of segmentary PASES allows to note lack of reliable distinctions on key parameters of a kidney blood-groove.

Thus, the phase of an exacerbation of pyelonephritis is characterized by lower blood-groove in the main arteries and higher in intershare arteries and increase of kidney vascular resistance in the main trunk and arc arteries.

The obtained data testify to informational content of indicators of a blood-groove at the level of the main, intershare, arc renal arteries in definition of an active phase of chronic pyelonephritis at patients with diabetes 2 types.

Conclusions:

1. Violations of kidney haemo dynamics at chronic pyelonephritis at patients with diabetes 2 types are characterized by decrease in a kidney blood-groove with the simultaneous growth of indexes of vascular resistance at various levels of renal arteries.

2. In an exacerbation of chronic pyelonephritis at patients with diabetes 2 types in comparison with a phase of remission are revealed decrease of a blood-groove in the main arteries and increase in intershare arteries, and also increase of vascular resistance at the level of the main trunk and arc renal arteries.

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Диабеттің 2-түрімен ауратын науқастарда созылмалы пиелонефритте бүйрек қан ағысының көрсеткіштерін бағалау

Диабеттің 2-түрімен ауыратын 90 науқастың созылмалы пиелонефрит қабынуы және уақытша оңалуы кезінде бүйректің әр түрлі деңгейдегі артерияларындағы бүйрек гемодинамикасына зерттеу жүргізілді. Бүйрек гемодинамикасының бұзылуы бүйрек қан айналысының жылдамдығының төмендеуімен және әр түрлі деңгейдегі бүйрек артериясында бүйрек тамырларындағы кедергінің жоғарлауымен байқалып, айқындылығы бүйректегі қабынудың белсенділігімен байланысы анықталды.

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Оценка показателей почечного кровотока при хроническом пиелонефрите у больных сахарным диабетом 2 типа

Авторами исследовалось состояние функции почек при сахарном диабете. У 90 пациентов, больных сахарным диабетом 2 типа в сочетании с хроническим пиелонефритом, в фазах обострения и ремиссии изучено состояние почечного кровообращения. Выявлено достоверное снижение скорости кровотока, а также понижение и повышение сосудистого сопротивления на различных уровнях артерий почек, которые обусловлены активностью воспалительного процесса в почках.

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