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Ultrastructural features of pancreatic islets in rats with experimental pathology

T. V. Ivanenko[A,B,C,D,F], **Yu. M. Kolesnyk**[A,E,F], **A. V. Abramov**[A,E,F]

Zaporizhzhia State Medical and Pharmaceutical University, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

In type 1 diabetes mellitus, the primary pathological alterations in the endocrine apparatus arise from the progressive loss of beta-cells, whereas beta-cell dysfunction is the key pathogenic factor in type 2 diabetes mellitus.

The aim of this study was to perform a quantitative assessment of the pancreatic islet architecture in normotensive Wistar rats under conditions of streptozotocin-induced diabetes mellitus, during hypoxic training, and in spontaneously hypertensive rats (SHRs).

Materials and methods. The experiment was conducted on 35 albino Wistar rats and 10 SHRs aged 5–6 months. The animals were divided into four groups: Group 1 (n = 10) – control Wistar rats; Group 2 (n = 15) – Wistar rats with streptozotocin-induced diabetes mellitus by a single intraperitoneal injection of streptozotocin (Sigma-Chemical, USA) at a dose of 50 mg/kg dissolved in 0.5 mL of 0.2 M citrate buffer (pH = 4.5). Only animals with fasting blood glucose exceeded 10.0 mmol/L at week 4 after streptozotocin administration were included in the study. Group 3 (n = 10) – SHR rats with hereditary arterial hypertension; Group 4 (n = 10) – Wistar rats subjected to a 15-day hypoxic training regimen according to the protocol: 15 consecutive days, 6 hours daily during days 1–5 at simulated altitudes ranging from 1 to 5 kilometers above sea level followed by 10 days at 6 km above sea level in a barochamber. Insulin and glucagon in pancreatic islet cells were detected using the immunofluorescence method with Insulin Antibody, clone 2D11-H5 (sc-8033 AF546), and Glucagon Antibody, clone K79bB10 (sc-57171 FITC) (Santa Cruz Biotechnology, USA). Immunofluorescent imaging was performed using an AxioImager-M2 fluorescence microscope (Carl Zeiss, Germany) equipped with an AxioCam-HRm digital camera (Carl Zeiss, Germany).

Results. In control Wistar rats, the specific density of pancreatic islets in the pancreas was 230 ± 3 cm^{-2}. The islet structure was predominantly composed of small islets, while other types of islets, including solitary endocrinocytes, exhibited approximately equal distribution densities. Induction of experimental diabetes in Wistar rats by a single streptozotocin injection led to a reduction in the total number of islets to 117 ± 5 cm^{-2}, i. e., a twofold decrease compared to the control group. Within the structure of the endocrine apparatus, nearly 50 % were small islets, the number of which exceeded that of control rats by 17 %, whereas the number of larger islets was significantly reduced. The genetic factor leading to hereditary hypertension in SHRs caused a 40 % decline in islet density (140 ± 3 cm^{-2}) compared to controls, which nevertheless remained 10 % higher than in diabetic animals ($p < 0.05$), partially explaining the maintenance of normoglycemia in SHRs. Prolonged hypoxic training in Wistar rats did not significantly alter the total number of islets (241 ± 5 cm^{-2}) but increased the number of both β^+/α^- and β^-/α^+ islets, as well as solitary endocrinocytes by 31 % ($p < 0.05$) compared to controls.

Conclusions. The endocrine pancreas is composed predominantly of "classical" islets containing beta- and alpha-cells. Small islets (≤1500 μm^2) constituted approximately one-third of all islets. The total specific density of islets in control and hypoxia-trained Wistar rats was nearly twice that of diabetic or hypertensive SHRs. Clusters of solitary alpha-endocrinocytes were fourfold more numerous than solitary beta-endocrinocytes. In the pancreas of normal Wistar rats, an equal number of small islets with β^+/α^- and β^-/α^+ phenotypes were found. Pancreatic islet β^-/α^+ phenotype predominated in diabetic rats, whereas in hypoxia-trained Wistar rats, the population of β^+/α^- islets was sixfold higher than that of islets with β^-/α^+ phenotype, and this phenotype was absent in diabetic rats.

Keywords: pancreas, pancreatic islets, β-cells, α-cells, arterial hypertension, SHR rats, diabetes mellitus, hypoxic training, insulin, glucagon, automated cell counting.

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Особливості організації острівців підшлункової залози у щурів з експериментальною патологією

Т. В. Іваненко, Ю. М. Колесник, А. В. Абрамов

При цукровому діабеті 1 типу основні патологічні зміни ендокринного апарату підшлункової залози зумовлені втратою бета-клітин, що прогресує, а їхня дисфункція відіграє провідну роль у розвитку цукрового діабету 2 типу.

Мета роботи – дати кількісну оцінку архітектури панкреатичних острівців у нормотензивних щурів лінії Вістар, за умов стрептозотоцин-індукованого цукрового діабету, при гіпоксичних тренуваннях, а також у гіпертензивних щурів лінії SHR.

Матеріали і методи. Дослідження здійснили на 35 білих щурах лінії Вістар і 10 щурах лінії SHR віком 5–6 місяців. Тварин поділили на 4 експериментальні групи. Щури лінії Вістар контрольної (інтактної) групи становили 1 групу (n = 10). Щурам лінії Вістар – 2 група (n = 15) – моделювали цукровий діабет шляхом однократного внутрішньоочеревинного введення стрептозотоцину (Sigma-Chemical, США) в дозі 50 мг/кг, розчиненого в 0,5 мл 0,2 М цитратного буфера pH = 4,5. До дослідження залучали тварин, у яких на четвертий тиждень після введення стрептозотоцину рівень глікемії натще перевищував 10,0 ммоль/л. Щури лінії SHR зі спадковою артеріальною гіпертензією становили 3 групу (n = 10). Щури лінії Вістар – 4 група (n = 10) – зазнавали гіпоксичних тренувань (протягом 15 днів по 6 годин щоденно: на 1–5 день в умовах барокамери імітували підйом на висоту 1–5 км над рівнем моря, а останні 10 днів – 6 км над рівнем моря).

Ключові слова: підшлункова залоза, панкреатичні острівці, бета-клітини, альфа-клітини, артеріальна гіпертензія, SHR, цукровий діабет, гіпоксичні тренування, інсулін, глюкагон, автоматизований обрахунок клітин.

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Інсулін, глюкагон і соматостатин у клітинах виявляли імунофлуоресцентним методом за допомогою антитіл Insulin Antibody клон 2D11-H5 (sc-8033 AF546), Glucagon Antibody клон K79bB10 (sc-57171 FITC) (Santa Cruz Biotecnology, США). Імунофлуоресцентну реакцію знімали на флуоресцентному мікроскопі AxioImager-M2 (Carl Zeiss, Німеччина), що обладнаний цифровою камерою AxioCam-HRm (Carl Zeiss, Німеччина).

Результати. В інтактних щурів лінії Вістар питома щільність панкреатичних острівців у підшлунковій залозі становила 230 ± 3 см$^{-2}$. У структурі острівців домінували малі острівці, а острівці інших типів, зокрема й поодинокі ендокриноцити, мали майже однакову щільність розподілу. Формування експериментального діабету в щурів лінії Вістар шляхом одноразового введення стрептозотоцину спричинило зменшення загальної кількості острівців до 117 ± 5 см$^{-2}$, тобто вдвічі менше порівняно з контрольною групою. У структурі ендокринного апарату майже 50 % – малі острівці, їхня кількість на 17 % перевищувала показники контрольних тварин, зафіксовано достовірне зменшення кількості острівців більшої площі. Вплив генетичного фактора, що зумовлює розвиток спадкової гіпертензії в щурів лінії SHR, спричинив зниження щільності панкреатичних острівців у залозі на 40 % порівняно з контролем до 140 ± 3 см$^{-2}$. Порівняно з діабетичними тваринами питома щільність острівців у щурів лінії SHR на 10 % вища ($p < 0,05$); це почасти пояснює нормоглікемію в цих тварин. Багатоденні гіпоксичні тренування щурів лінії Вістар не спричиняли значних змін загальної кількості панкреатичних острівців (241 ± 5 см$^{-2}$), проте призводили до збільшення кількості і β^+/α^--, і β^-/α^+-острівців. Кількість популяцій поодиноких ендокриноцитів у залозі також збільшилася на 31 % ($p < 0,05$) порівняно з контролем.

Висновки. В ендокринній частині підшлункової залози виявлено острівці «класичного» типу, що містять β- та α-клітини, малі острівці площею до 1500 мкм^2 становлять майже третину від загальної кількості острівців. Загальна питома щільність панкреатичних острівців в інтактних щурів лінії Вістар і тварин із гіпоксичними тренуваннями майже вдвічі більша, ніж у діабетичних і гіпертензивних щурів лінії SHR. У підшлунковій залозі виявлено скупчення поодиноких α-ендокриноцитів, кількість яких учетверо перевищує кількість поодиноких β-ендокриноцитів. У підшлунковій залозі нормальних щурів лінії Вістар виявлено майже однакову кількість малих острівців площею до 1500 мкм^2 із фенотипами β^+/α^- та β^-/α^+. У діабетичних щурів домінують панкреатичні острівці з фенотипом β^-/α^+. У щурів лінії Вістар, які зазнавали гіпоксичних тренувань, кількість панкреатичних острівців із фенотипом β^+/α^- вшестеро перевищує щільність острівців із фенотипом β^-/α^+, а в діабетичних тварин таких острівців не було.

Pancreatic islets (islets of Langerhans) are integral components of the endocrine system in both humans and animals. They comprise five main types of endocrinocytes: beta-cells producing insulin, alpha-cells producing glucagon, delta-cells secreting somatostatin, gamma- or PP-cells releasing pancreatic polypeptide, and epsilon-cells producing ghrelin [1]. The cellular composition and architecture of pancreatic islets exhibit both interspecies and intraspecies differences [2]. In humans, the cellular composition of pancreatic islets is approximately 60 % of beta-cells, 30 % – alpha-cells, and 5–7 % – delta -cells, with gamma- and epsilon-cells distributed randomly throughout the islet [3]. In rodents, which are widely used in biomedical research, the islet architecture is characterized by a central core comprising roughly 70 % of beta-cells, surrounded by a peripheral zone containing about 20 % of alpha-cells, and delta-, gamma-, and epsilon-endocrinocytes distributed throughout the islet, collectively accounting for less than 10 % [1].

Despite interspecies variations in pancreatic islet architecture, which to some extent determine their functional diversity [4], laboratory rodents remain the principal experimental model for studying pancreatic disorders. This is particularly relevant to type 1 and type 2 diabetes mellitus, the prevalence of which in the human population continues to rise [5]. In type 1 diabetes mellitus, the main pathological changes in the pancreatic endocrine apparatus are caused by the progressive loss of beta-cell [6], whereas in type 2 diabetes mellitus pathogenesis, beta-cell dysfunction plays a pivotal role [7].

Previous studies of the pancreas in both humans and laboratory rodents have demonstrated that the pancreatic islet population is heterogeneous in size, changes throughout life, and displays distinct architectural patterns both in healthy individuals and under conditions of clinical diabetes in humans or experimentally induced diabetes in laboratory rats [1]. However, most studies have been limited to a descriptive approach in the examination of different islet types, with few providing comprehensive quantitative analyses of islet architecture [8].

Aim

The aim of the study was to perform a quantitative assessment of pancreatic islet architecture in normotensive Wistar rats under conditions of streptozotocin-induced diabetes mellitus, during hypoxic training, and in spontaneously hypertensive rats (SHRs).

Materials and methods

The study included 35 albino Wistar rats and 10 SHRs aged 5–6 months. All animals were weighed, fasting blood glucose was measured using a GlucoCard-II glucometer (Japan), and systolic blood pressure was determined with a non-invasive Blood Pressure Analysis System (BP-2000 Series II, Visitech Systems, USA).

The animals were divided into four experimental groups. The animals were divided into four groups: Group 1 (n = 10) – control Wistar rats; Group 2 (n = 15) – Wistar rats with streptozotocin-induced diabetes mellitus by a single intraperitoneal injection of streptozotocin (Sigma-Chemical, USA) at a dose of 50 mg/kg dissolved in 0.5 mL of 0.2 M citrate buffer (pH = 4.5). Only animals with fasting blood glucose exceeded 10.0 mmol/L at week 4 after streptozotocin administration were included in the study. Group 3 (n = 10) – SHR rats with hereditary arterial hypertension; Group 4 (n = 10) – Wistar rats subjected to a 15-day hypoxic training regimen according to the protocol: 15 consecutive days, 6 hours daily during days 1–5 at simulated altitudes ranging from 1 to 5 kilometers above sea level followed by 10 days at 6 km above sea level in a barochamber.

Characteristics of the biometric parameters of rats in the experimental groups are summarized in *Table 1*.

Table 1. Biometric parameters of experimental rats, M ± m

Parameter, units of measurement	Group 1 (Control)	Group 2 (Diabetes)	Group 3 (Hypertension)	Group 4 (Hypoxic training)
Body mass, g	232 ± 7	201 ± 7	305 ± 6	249 ± 5
Blood glucose, mmol/L	3.94 ± 0.09	17.68 ± 1.11	4.73 ± 0.10	2.92 ± 0.18
Systolic blood pressure, mmHg	105.0 ± 1.1	108.0 ± 1.5	155.7 ± 0.9	123.4 ± 2.1

Table 2. Percentage distribution of different islet types, M ± m

Groups	Solitary endocrinocytes	Small islets	Medium islets	Large islet	Giant islets
Control	16.89 ± 1.90	31.23 ± 2.60	18.59 ± 2.03	14.83 ± 1.91	18.47 ± 2.02
Diabetes	17.84 ± 1.79	48.23 ± 2.98	12.18 ± 1.54	11.64 ± 1.53	10.11 ± 1.33
Hypertension	30.92 ± 1.98	39.69 ± 1.11	8.40 ± 0.51	6.49 ± 0.45	14.50 ± 0.67
Hypoxic training	21.46 ± 0.99	41.68 ± 1.37	13.02 ± 0.77	9.90 ± 0.67	13.94 ± 0.79

Euthanasia of the experimental animals was performed under thiopental anesthesia (50 mg/kg), after which the pancreas was excised, fixed in Bouin's solution for 20 hours, and embedded in Paraplast (McCormick, USA) following standard histological processing. Serial 5-μm histological sections of the pancreas were deparaffinized and processed for antigen retrieval in citrate buffer (pH 9.0) using a PT Module (Thermo Scientific, USA). Insulin and glucagon in pancreatic islet cells were detected using the immunofluorescence method with Insulin Antibody, clone 2D11-H5 (sc-8033 AF546), and Glucagon Antibody, clone K79bB10 (sc-57171 FITC) (Santa Cruz Biotechnology, USA). For this purpose, a mixture of antibodies against the hormone pair insulin/glucagon was prepared at 1:200 dilution each. After applying the antibody mixture onto the pancreatic sections, the slides were incubated for 24 hours in a humid chamber (t = +4 °C), washed in phosphate buffer (pH = 7.4), mounted with UltraCruz™ Mounting Medium (Santa Cruz Biotechnology, USA), and covered with cover slips (Menzel-Gläser, Germany).

Imaging of the immunofluorescent reaction was performed using an AxioImager-M2 fluorescence microscope (Carl Zeiss, Germany) equipped with an AxioCam-HRm digital camera (Carl Zeiss, Germany) and high-emission filters 38HE and 43HE (Carl Zeiss, Germany). Image analysis was conducted using the ImageJ digital image analysis system, version 2.1.0/1.53c (public open-source license). A minimum of 200 pancreatic islets were analyzed in each experimental group. The area of immunoreactive tissue to the studied biomarkers was measured in an automated mode, the total islet area was calculated, and the islets were classified according to their size as follows: solitary endocrine cells (SC), with an area <120 μm^2; small islets (SML), with an area of 120–1500 μm^2; medium islets (MED), with an area of 1500–3500 μm^2; large islets (LRG), with an area of 3500–7500 μm^2; and giant islets (GIG), with an area >7500 μm^2.

Planimetric analysis of histological sections was performed using a Stemi-305 stereomicroscope equipped with an Axiocam-105 color digital camera (Carl Zeiss, Germany). The total area of the histological sections was measured to assess the specific density of pancreatic islets per 1 cm^2 of glandular tissue. In total, no less than 5 cm^2 of pancreatic tissue sections was examined in each experimental group.

Statistical processing of the obtained data was performed in Microsoft Excel (Office 365). Differences between the compared parameters were considered statistically significant at $p < 0.05$ based on Student's t-test. Data in the tables are presented as mean values with standard error (M ± m). Data in the figures are presented as mean values with confidence intervals.

Results

Double immunofluorescent staining of the pancreas with antibodies against insulin and glucagon has revealed that in all groups of experimental animals, the majority of pancreatic islets were of the classical type, containing both beta- and alpha-endocrinocytes (β^+/α^+). In addition, clusters of endocrine cells composed exclusively of beta-cells (β^+/α^-) or exclusively of alpha-cells (β^-/α^+) were also identified in the pancreas. These clusters formed either solitary endocrinocytes or small islets with an area of up to 1500 μm^2 *(Fig. 1)*. It has been found that in the pancreas of animals across all experimental groups, the number of alpha-cell-only clusters (β^-/α^+) significantly exceeded the number of beta-cell-only clusters (β^+/α^-) *(Fig. 2)*, while small islets represented the dominant islet type *(Fig. 3)*. At the same time, the specific modulatory factors acting on the organism were clearly reflected in the pancreatic islet ultrastructure and in the index of their specific density within the pancreas of the experimental animals.

In control Wistar rats, the specific density of pancreatic islets was 230 ± 3 cm^{-2} *(Fig. 2A)*. Small islets predominated, whereas medium, large, and giant islets, as well as solitary endocrinocytes, showed comparable distribution densities *(Table 2, Fig. 3)*.

The induction of experimental diabetes in Wistar rats by a single streptozotocin injection resulted in a twofold decrease in total islet density (117 ± 5 cm^{-2}) compared with the control group *(Fig. 2A)*. Within the endocrine apparatus, nearly 50 % of the islets were small, exceeding control values by 17 %, while the proportion of larger islets was significantly reduced *(Fig. 3)*.

In SHRs, hereditary hypertension caused a 40 % decline in islet density (140 ± 3 cm^{-2}) compared with controls *(Fig. 2A)*, which nevertheless remained 10 % higher than in diabetic animals ($p < 0.05$), partially explaining the maintenance of normoglycemia in SHRs. A characteristic feature of the endocrine apparatus ultrastructure in SHRs was a marked increase in the number of solitary endocrinocytes, which was nearly twofold higher than in control animals *(Table 2, Fig. 3)*, as well as an increase in the number of β^-/α^+ islets, accounting for about 1/3 of the total population *(Fig. 2B)*.

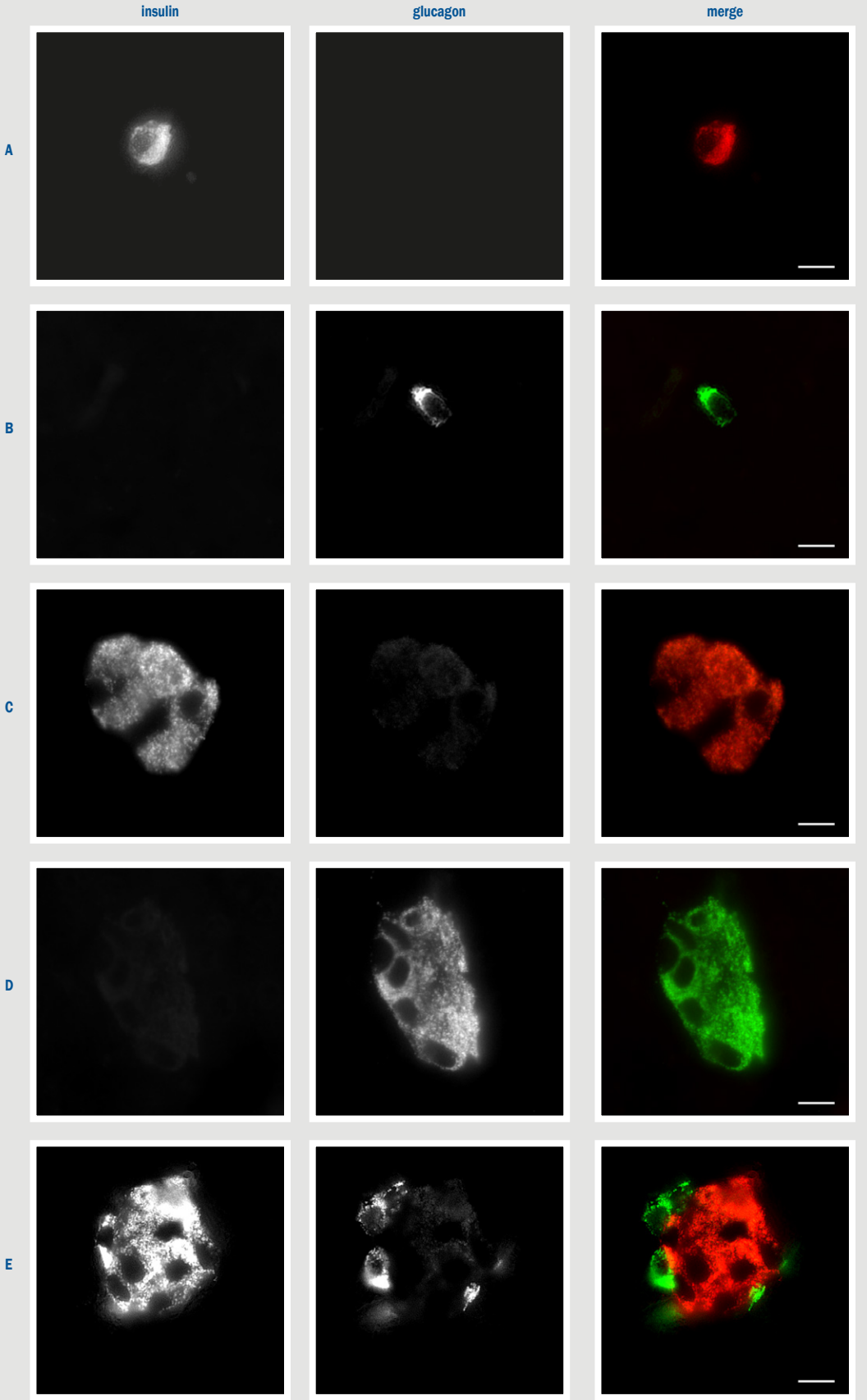

Fig. 1. Solitary beta-endocrinocytes (**A**), alpha-endocrinocytes (**B**), small islets composed exclusively of beta-endocrinocytes (**C**) or alpha-endocrinocytes (**D**), and classical islets consisting of both cell types (**E**). Pancreatic sections were simultaneously stained with antibodies against insulin and glucagon. Scale bar = 10 μm.

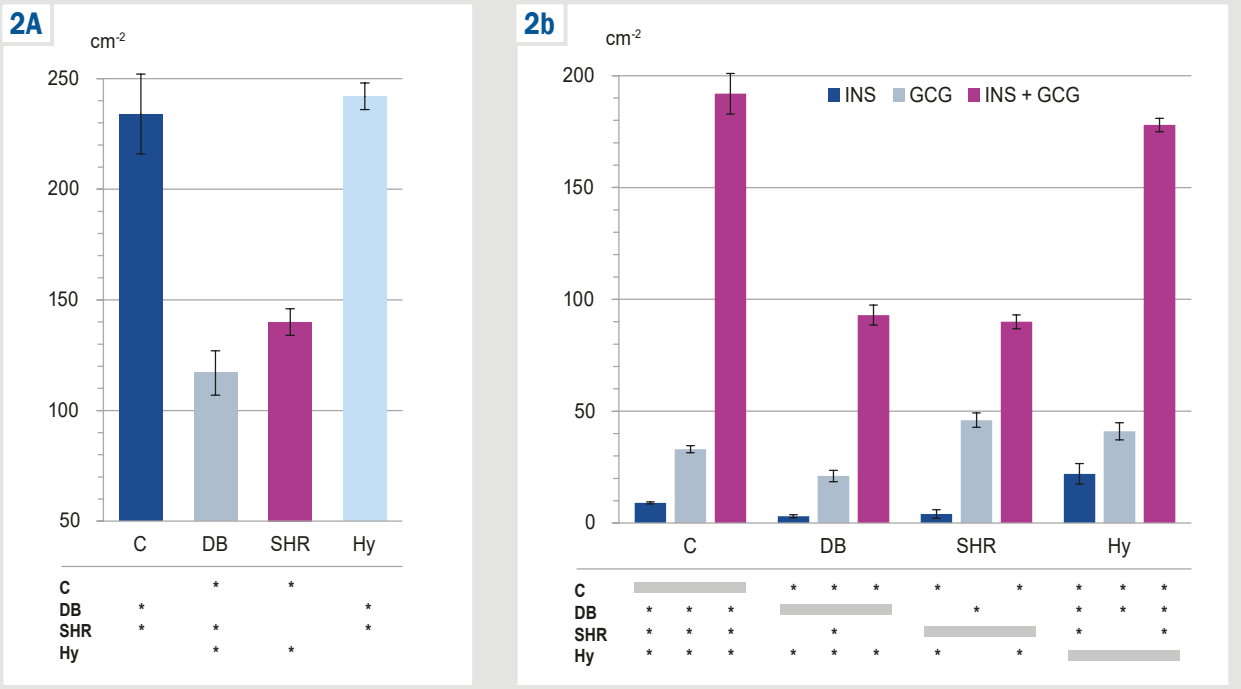

Fig. 2. Specific density (cm^{-2}) of pancreatic islets. **A:** total; **B:** by endocrine cell composition; ***:** statistically significant differences in values ($p < 0.05$).

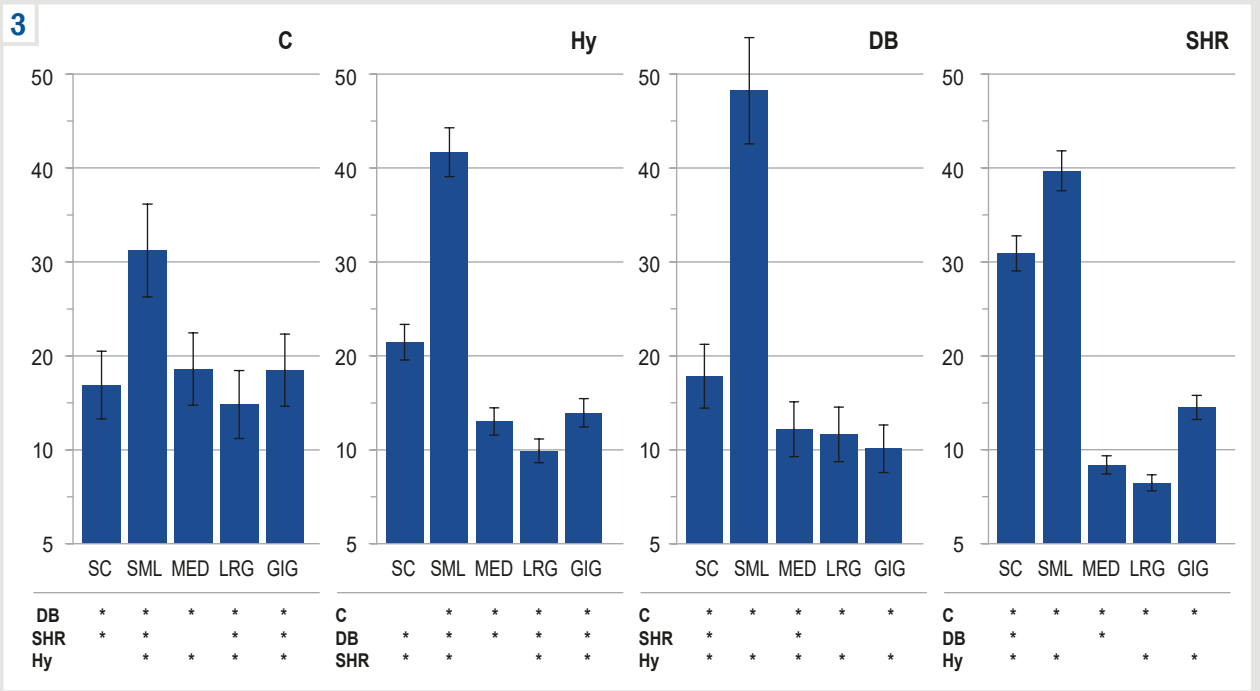

Fig. 3. The percentage distribution of pancreatic islet morphological types, categorized by size, demonstrates the relative frequency of solitary endocrinocytes (**SC**), small (**SML**), medium (**MED**), large (**LRG**), and giant islets (**GIG**). ***:** statistically significant differences ($p < 0.05$).

Multiday hypoxic training did not significantly alter the total number of islets (241 ± 5 cm^{-2}) but increased the number of both β^+/α^- and β^-/α^+ islets, as well as solitary endocrinocytes by 31 % ($p < 0.05$) *(Fig. 2)* compared with controls *(Fig. 3)*.

The conducted study has demonstrated that the modulating factor action on the organism can induce alterations in the pancreatic endocrine apparatus ultrastructure, manifested not exclusively in the total islet number but, most notably, in the populations of solitary endocrinocytes and small islets. Another important aspect of the modulatory impact is the change in the number of endocrine clusters composed entirely of a solitary endocrinocytes type – either β^+/α^- or β^-/α^+.

Consistent with previous findings, the pancreatic endocrine apparatus is structurally characterized by a higher ratio of solitary alpha-endocrinocytes over solitary beta-en-

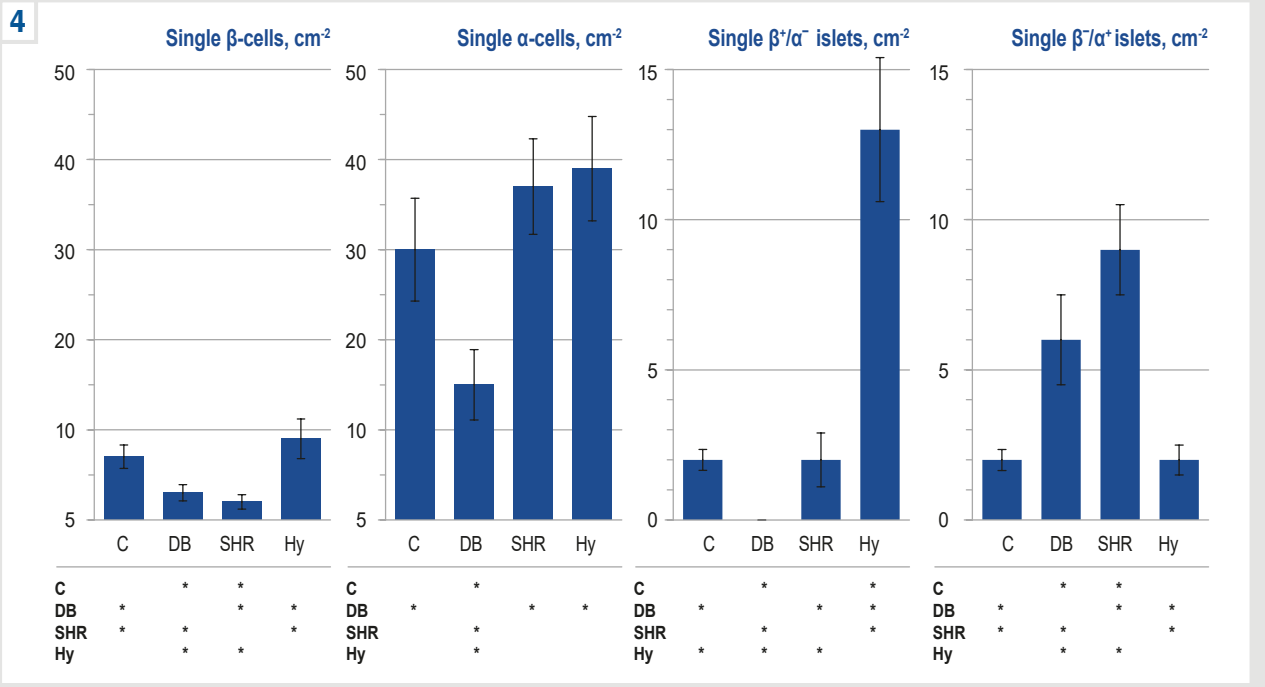

Fig. 4. Specific density (cm^{-2}) of solitary beta- and alpha-endocrinocytes, and small islets with β^+/α^- and β^-/α^+ phenotype. *: statistically significant differences ($p < 0.05$).

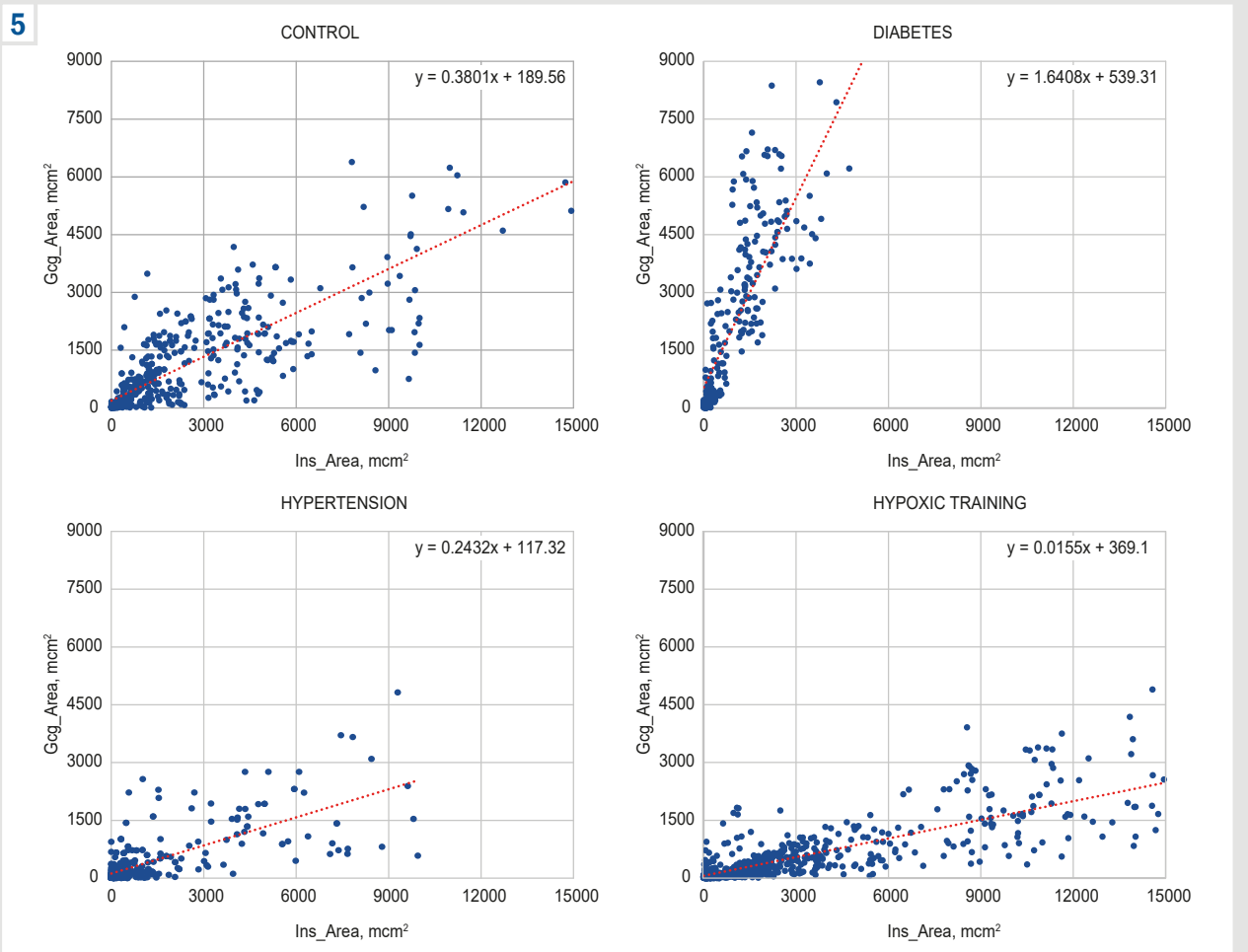

Fig. 5. Scatter plot illustrating the relationship between the insulin-immunopositive area (Ins_Area) and the glucagon-immunopositive area (Gcg_Area) within pancreatic islets. The linear regression line (red) and its corresponding equation are provided.

docrinocytes. This phenomenon, observed across diverse modulatory factors, specifically impacted the numerical density of solitary endocrine cells. Specifically, the induction of diabetes in Wistar rats and the presence of hereditary hypertension in SHRs both resulted in a pronounced reduction in the count of solitary pancreatic beta-endocrinocytes. Conversely, exposure to hypoxic conditioning stimulated a quantitative increase in these cells *(Fig. 4)*. Notably, solitary alpha-endocrinocytes demonstrated a significant decrease exclusively in the diabetic Wistar rats.

A significant finding was that the modulatory intervention (referring to hypoxic training) predominantly affected the population of β^+/α^- and β^-/α^+ endocrinocyte clusters, which were categorized as small islets (area <1500 μm^2). Specifically, while the development of diabetes in Wistar rats resulted in the complete absence of pancreatic β^+/α^- islets, subsequent hypoxic training induced a six-fold increase in their number compared to the control group *(Fig. 4)*. Concurrently, the number of β^-/α^+ islets in the pancreas increased by a factor of 2.5 in diabetic Wistar rats and by 3.8 in hypertensive SHRs.

Morphometric analysis revealed distinct structural differences in pancreatic islets induced by the modulatory factors, as clearly illustrated by the distribution graphs of islet areas based on insulin- and glucagon-immunopositive material *(Fig. 5)*. Consistently across all experimental groups, the maximum islet area did not exceed ≈15,000 μm^2. Evaluation of the linear regression coefficients demonstrated that beta-cells predominated in control normotensive Wistar rats (72 %), hypertensive SHRs (80 %), and Wistar rats subjected to hypoxic training (up to 95 %). The observed higher proportion of beta-cells in SHR islets may contribute to the maintenance of normoglycemia despite a documented significant reduction in the total number of pancreatic islets. Importantly, hypoxic training in Wistar rats promoted substantial beta-cell dominance, increasing their proportion by 25 % of the endocrine population relative to control animals, although the total islet count was not markedly increased. In stark contrast, streptozotocin-induced diabetes in Wistar rats resulted in a complete reversal of the β/α endocrinocyte ratio, leading to a marked predominance of alpha-cells, which constituted over 60 % of the endocrine population.

Discussion

The findings of this study delineate several key features of pancreatic islet architecture. First, islet size exhibits heterogeneity, dominated by a significant proportion of small islets (measuring up to 1500 μm^2) throughout the pancreas. Second, most islets possess a bimodal cellular composition, consisting of both beta- and alpha-cells. Third, specialized monotypic cell clusters were identified within these structures, represented exclusively by beta-endocrinocytes or alpha-endocrinocytes, occurring either as solitary cells or as small, isolated islets with β^+/α^- or β^-/α^+ phenotypes. A central characteristic of pancreatic islets is their intrinsic plasticity, which allows for morphological and functional adaptation under various modulatory influences. As anticipated, the action of streptozotocin, a pharmacological agent exerting direct beta-cytotoxic effects, results in the ablation of the beta-endocrinocyte population, a decline in the overall islet count, and the consequent development of experimental diabetes in rats. This effect is a highly reproducible phenomenon and remains the standard method for using rodent models of diabetes [9].

A general consensus holds that the intrinsic architecture of pancreatic islets is fundamental to their optimal function. This functional necessity is achieved through the synchronization of individual beta-cells via intercellular oscillations of $Ca2^+$ currents and membrane electrical potential, which ensures the appropriate regulation of glucose-stimulated insulin secretion [1]. Crucially, this synchronization extends systemically across the entire pancreatic islet population, thereby maintaining the characteristic pulsatile rhythm of circulating insulin concentration observed following glucose challenge [1]. A resulting diminution in the total number of pancreatic islets, therefore, not only reduces the overall mass of insulin-producing beta-endocrinocytes but also critically compromises the physiological mechanisms responsible for the rhythmic control of insulin secretion.

Our study demonstrates that streptozotocin-induced diabetes in rats triggers a restructuring of the endocrine pancreas, extending beyond mere islet loss to include marked changes in islet morphology and cellular composition. These changes involve a 50 % shift toward smaller islets, the elimination of the β^+/α^- small islet population, and a severe reduction in solitary beta-endocrinocytes. This level of beta-cell diminution (50 % loss) is highly consequential, as documented literature confirms it results in irreversible desynchronization of beta-cell secretory function, which is a mechanism independent of the resulting hyperglycemia [10].

Streptozotocin-induced diabetes mellitus results in significant remodeling of pancreatic islet architecture, where the alpha-cell population becomes the predominant endocrinocyte type, often constituting ≥60 % of the total islet cell mass. This profound architectural alteration is considered a hallmark of the diabetic state [11]. The observed increase in the alpha-cell proportion is generally interpreted as being relative to the pathological decline in beta-cell mass characteristic of diabetes [6]. Furthermore, this proliferative phenomenon may be associated with enhanced expression of the c-Kit protein in alpha-cells, a mechanism known to stimulate cellular proliferation [12].

Consistent with these established findings, the present study observed a threefold increase in the number of β^-/α^+ small islets within the pancreas. This expansion of the alpha-endocrinocyte population in the diabetic state suggests a degree of functional plasticity within the islet system [13].

The comorbidity of arterial hypertension in diabetic patients represents a significant clinical challenge, often contributing to disease progression and exacerbated patient outcomes. Our study has revealed that hereditary hypertension in SHRs resulted in a diminished pancreatic islet count. Notably, this reduction was less severe than the islet loss observed in streptozotocin-induced diabetic Wistar rats. These quantitative alterations in SHR islet architecture are consistent with prior reports [14]. Intriguingly, while SHRs exhibit impaired glucose homeostasis and reduced glucose tolerance [15], the decrease in islet number does not result in a hyperglycemic state. We hypothesize that the maintenance of normoglycemia in SHRs is potentially

linked to a substantial preservation of beta-endocrinocytes within the remaining islets (up to 80 %) compared to the severely depleted beta-cell mass in the diabetic Wistar rats (less than 40 %).

The deterioration of beta-cell mass and function, coupled with systemic insulin resistance observed in the context of diabetes and arterial hypertension, constitutes a critical biomedical challenge. Consequently, research efforts are intensely focused on identifying innovative strategies to stimulate pancreatic insulin production.

In this context, hypoxic exposure has emerged as a significant etiological factor contributing to the development of these metabolic disorders, particularly insulin resistance and beta-cell dysfunction. While hypoxia has traditionally been considered detrimental to pancreatic islet function, its mechanistic role is complex.

The hypoxia-inducible factor (HIF-1β) exhibits a dual regulatory role. It activates essential genes that facilitate the adaptive cellular response to low oxygen conditions [16]. Conversely, HIF-1β suppresses the expression of the peroxisome proliferator-activated receptor-γ (PPARG) gene and its coactivator (PPARGCA). This antagonistic regulation is hypothesized to drive the pathology, promoting insulin resistance, attenuating oxidative phosphorylation, and inducing a metabolic shift toward glycolysis [17].

Epidemiological evidence consistently demonstrates a reduced prevalence of diabetes mellitus in populations residing at high altitudes compared to those living in lowland areas [18]. Furthermore, exposure to moderate altitude has been shown to induce several beneficial metabolic adaptations, including the reduction of glycemia toward the lower normal limit, enhanced tissue insulin sensitivity, and improved adipocyte function [19]. Concurrently, controlled hypoxic exposures of varying duration and intensity elicit a broad spectrum of metabolic effects in both healthy subjects and individuals with diabetes mellitus [20,21,22].

The collective evidence suggests that controlled exposure to exogenous hypoxia elicits a measurable impact on cellular glucose metabolism and positively modulates insulin resistance, thereby highlighting the potential therapeutic benefits of intermittent hypoxia as a sanogenic intervention. Specifically, our current study demonstrates that hypoxic training significantly enhanced the proportion of β^+/α^- islet phenotypes while concomitantly reducing the number of β^-/α^+ islets in the pancreas. Furthermore, the fractional mass of beta-endocrinocytes within the islets increased substantially, reaching approximately 90 %.

In conclusion, it must be emphasized that the architecture of pancreatic islets exhibits significant morphological plasticity in response to various metabolic stressors. While quantitative and qualitative structural changes in the pancreatic endocrine apparatus are established findings in diabetes, the observed alterations in islet morphology associated with hypertension may suggest a mechanism for the observed clinical comorbidity with diabetes and necessitate further research into the underlying cellular mechanisms. We strongly contend that the search for novel pharmacological and biomedical strategies for the treatment of both type 1 and type 2 diabetes should extend beyond achieving target glycemic control and improving insulin sensitivity to encompass the restoration of pancreatic islet architecture and beta-endocrinocyte mass in patients.

Conclusions

1. The endocrine pancreas primarily contains classical islets composed of both beta- and alpha-cells. Notably, small islets (defined as having an area ≤1500 μm^2) constitute approximately one-third of the total islet population.

2. The total specific density of pancreatic islets in both control and hypoxia-trained Wistar rats was approximately twofold higher compared to that observed in diabetic or spontaneously hypertensive rats.

3. Solitary alpha-endocrinocytes were identified within the pancreatic tissue, with their population density being approximately four times greater than that of solitary beta-endocrinocytes.

4. In the pancreas of normal Wistar rats, small islets (≤1500 μm^2 in area) exhibited an equal numerical distribution between the β^+/α^- and β^-/α^+ phenotypes. In contrast, pancreatic islets displaying the β^-/α^+ phenotype were found to be predominant in diabetic rat models.

5. In the hypoxia-trained Wistar rats, the density of islets presenting the β^+/α^- phenotype was sixfold greater than the density of β^-/α^+ islets. Conversely, the β^+/α^- islet population was completely absent in the diabetic rat group.

Ethical approval
The research programme was reviewed and approved by the Bioethics Commission of Zaporizhzhia State Medical and Pharmaceutical University (Protocol No. 10 dated 18 September 2025). The document was prepared on the basis of the "General Principles of Animal Experiments" (III National Congress on Bioethics, Kyiv, 2007) and brought into line with the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986), Directive 86/609/EEC and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" No. 3447-IV of 21 February 2006.

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Information about the authors:
Ivanenko T. V., MD, PhD, Associate Professor of the Department of Pathological Physiology with Course of Normal Physiology, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0001-6617-5178
Kolesnyk Yu. M., MD, PhD, DSc, Professor of the Department of Pathological Physiology with the Course of Normal Physiology, Rector of Zaporizhzhia State Medical and Pharmaceutical University, Honorary Scientist and Engineering Figure of Ukraine.
ORCID ID: 0000-0002-1556-5085
Abramov A. V., MD, PhD, DSc, Professor of the Department of Pathological Physiology with the Course of Normal Physiology, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0001-8520-2258

Відомості про авторів:
Іваненко Т. В., канд. мед. наук, доцент каф. патологічної фізіології з курсом нормальної фізіології, Запорізький державний медико-фармацевтичний університет, Україна.

Колесник Ю. М., д-р мед. наук, професор каф. патологічної фізіології з курсом нормальної фізіології, ректор Запорізького державного медико-фармацевтичного університету, заслужений діяч науки і техніки України.
Абрамов А. В., д-р мед. наук, професор каф. патологічної фізіології з курсом нормальної фізіології, Запорізький державний медико-фармацевтичний університет, Україна.

Taras Ivanenko (Тарас Іваненко)
ivanenko.tv@zsmu.zp.ua

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