

Zaiats, Liubomyr, Fedorchenko, Yuliya, Zukow, Walery. Dynamics of endogenous intoxication in experimental diabetes mellitus. *Journal of Education, Health and Sport*. 2022;12(10):52-59. eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2022.12.10.007>
<https://apcz.umk.pl/JEHS/article/view/40298>
<https://zenodo.org/record/7127730>

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 21, 2021. No. 32343. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences). Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 21 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przynależność do dyscypliny naukowej: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

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The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 01.09.2022. Revised: 02.09.2022. Accepted: 30.09.2022.

UDC:616.24+616.092.9+616.379-008.64

DYNAMICS OF ENDOGENOUS INTOXICATION IN EXPERIMENTAL DIABETES MELLITUS

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Abstract

Background. Nowadays, diabetes mellitus is defined as one of the global medical and social problems and shows a growing tendency. **The purpose** of the study was to analyze the dynamics of endogenous intoxication in experimental diabetes mellitus. **Materials and methods.** The experiments were performed on 88 white male Wistar rats weighing 170-210 g. Animals were divided into three groups: 1 - intact; 2 - control; 3 - experimental with a model of diabetes mellitus, which was reproduced by intraperitoneal injection of streptozotocin by "Sigma" company (USA), diluted in 0.1 M citrate buffer with a pH of 4.5, at a rate of 60 mg/kg body weight. The control group of animals received an intraperitoneal injection with an equivalent dose of 0.1 M citrate buffer solution with a pH of 4.5.

All studies were performed under thiopental-sodium anesthesia at a rate of 60 mg/kg body weight. To determine endogenous intoxication, blood sampling of middle mass molecules (MMM) was performed 14, 28, 42 and 70 days after the streptozotocin injection. Detection of MMM fractions was performed by spectrophotometer «SF46» at the wavelength of 254 nm (MMM₂₅₄) and 280 nm (MMM₂₈₀). The STATISTICA 10 program was used for statistical processing of the obtained results. **Results.** The conducted biochemical studies of blood serum showed that in animals with streptozotocin-induced diabetes mellitus there was an increase in the content of both MMM fractions compared to the similar indicators of the control group of animals at all stages of the experiment: in the 14 days increased by 32,9% - MMM₂₅₄, by 12,7%- MMM₂₈₀, in the 28 days increased by 44,3 % MMM₂₅₄, by 34,0 %

MMM₂₈₀, in the 42 days increased by 62,8 % MMM₂₅₄, by 64,8 % MMM₂₈₀, in 70 days increased by 76,1 % MMM₂₅₄, by 81,1% MMM₂₈₀. **Conclusions.** Streptozotocin-induced diabetes mellitus is accompanied by an increase in the level of endogenous intoxication which is manifested by an increase of both MMM fractions content in serum.

The severity of endogenous intoxication depends on the duration of the endogenous factor.

Keywords: Streptozotocin-induced diabetes, middle mass molecules.

INTRODUCTION

Nowadays, diabetes mellitus is defined as one of the global medical and social problems and shows a growing tendency [14, 16, 20, 22].

Numerous clinical and experimental studies have shown that the pathogenesis of many diseases is accompanied by the development of endogenous intoxication syndrome, the severity of which depends on the effectiveness of protective antitoxic mechanisms at the organ and tissue levels and the body as a whole [8, 11, 18]. Further depletion of protective antitoxic and regulatory systems leads to disruption of the structure and function of biological membranes, distortion of biosynthetic processes, accumulation of underoxidized metabolic products and is a reason of increased concentration of toxic metabolites [7, 19, 21].

According to the literature data, the universal biochemical marker that reflects the level of pathological protein metabolism in endogenous intoxication is middle mass molecules (MMM) level [2, 10, 11]. To date, it has been established that MMM are products of catabolism of endo- and exogenous proteins with a relative molecular weight of 500- 5000 daltons which sharply increases during pathological conditions. Convincing evidence of the important role of proteolysis in the accumulation of MMM is presented in a number of works [12, 15]. It is believed that the increase in the level of middle mass molecules in plasma or blood serum is an integral feature of many pathological processes [1, 2, 4, 9, 13]. Peculiarity of middle mass peptides is their high biological activity.

Depending on their composition and specific conditions, MMM are able to influence on different levels of regulation. As evidenced by numerous literature data [7, 15] middle mass peptides disrupt the physicochemical properties of cell membranes and membrane transport, inhibit the processes of protein biosynthesis, disconnect the processes of oxidation and phosphorylation, have neurotoxic activity. MMM also promote erythrocyte hemolysis, reduce hemoglobin synthesis and DNA synthesis in erythroblasts, suppress phagocytosis, cellular and humoral immunity.

The purpose of the study: analysis of the dynamics of endogenous intoxication in experimental diabetes mellitus.

MATERIALS AND METHODS

Experiments were conducted on 88 white male Wistar rats 170- 210 g weight, which were kept on a standard diet with free water access. Animals were divided into three groups: 1-intact (n=10); 2-control (n=40); 3-experimental (n=38) with the model of diabetes mellitus which was reproduced by intraperitoneal injection of streptozotocin "Sigma" (USA) at a dose of 60 mg/kg body weight diluted 0,1 M citrate buffer (pH 4,5). The control group of animals received intraperitoneal injection of the equivalent dose of 0,1 M citrate buffer solution (pH 4,5).

All procedures were performed in accordance with with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes (Strasbourg, 1986), the Law of Ukraine on the "Protection of Animals from Cruelty" (2006), "General Ethical Principles of animal experiments" approved by the Fifth National Congress on Bioethics (Kyiv, 2013).

All studies were performed under the thiopental sodium anesthesia (60 mg/ kg body weight). To determine endogenous intoxication, blood sampling of MMM was performed in 14, 28, 42 and 70 days after the streptozotocin injection. The content MMM was determined by a modified method of Gabrielian and co-authors. (1981) [6]. The method is based on the precipitation of serum proteins with 10% trichloroacetic acid and quantitative determination of middle mass peptides in the supernatant obtained by centrifugation. Detection of MMM fractions was performed by spectrophotometer «SF46» at the wavelength of 254 nm (to determine chain amino acids) and 280 nm (to determine aromatic amino acids). The results of middle mass molecules evaluation were expressed in conventional units of optical density.

During statistical processing of the obtained results, the STATISTICA 10.0 program was used. Using the possibilities of descriptive statistics, all the quantitative data obtained in the study were distributed adequately by Shapiro-Wilk test. Since the vast majority of these data corresponded to the normal Gaussian law to describe the central trend the arithmetic mean (M)+ standard errors (SE) (M±m) were chosen, to assess the difference reliability of obtained data in the comparison groups (experimental and control) and to test the null hypothesis -parametric t- test (Student t-criterion) was chosen. To assess the reliability of data changes in the dynamics (14, 28, 42, 70 days) within each of the comparison groups nonparametric method was implicated (for three or more groups of equations) - Friedman analysis of variance and Kendall's competitiveness coefficient (Friedman ANOVA and Kenall Coef. Of Concordance).

RESULTS AND DISCUSSION

The results of biochemical studies showed significant deviations in the concentration of MMM₂₅₄ fractions (Table 1, Figure 1) and MMM₂₈₀ fractions (Table 2, Figure 2) in the blood serum compared to the control group of animals at all stages of the experiment from 14 to 70 days.

Table 1. The content of MMM₂₅₄ fraction (c.u.) in the serum of rats in experimental diabetes mellitus

| Group | 14 days | | 28 days | | 42 days | | 70 days | | p ₂ |
|----------------|-------------|-------|---------|-------|---------|-------|---------|-------|----------------|
| | M | ±m | M | ±m | M | ±m | M | ±m | |
| experimental | 0,335* | 0,007 | 0,396* | 0,006 | 0,442* | 0,006 | 0,511* | 0,007 | <0,01 |
| control | 0,252 | 0,003 | 0,261 | 0,001 | 0,261 | 0,001 | 0,255 | 0,001 | >0,05 |
| p ₁ | <0,001 | | <0,001 | | <0,001 | | <0,001 | | x |
| Intact | 0,257±0,002 | | | | | | | | |

Notes: 1. p₁- the reliability of the data difference between experimental and control groups.

2. p₂-the reliability of data within the group in the dynamics

3. * the reliability of the data difference compared to the intact group

On the 14th day after diabetes mellitus modeling, the average content of middle mass peptides, which was determined in the serum at a wavelength of 254 nanometers, increased by 32,9% (p<0,001) compared with the control group of animals. At this time of the study there

also was an increase by 12.7% ($p < 0,001$) of MMM fraction, which was determined at a wavelength of 280 nanometers.

With the increase of the experiment duration (28 days), the content of MMM₂₅₄ fractions in the serum of experimental animals increased by 44,3 % ($p < 0,001$) compared with the control group of animals. Similar changes were observed during the study of another MMM fraction. So, the level of MMM₂₈₀ fractions increased by 34% ($p < 0,001$) compared to the control group of animals.

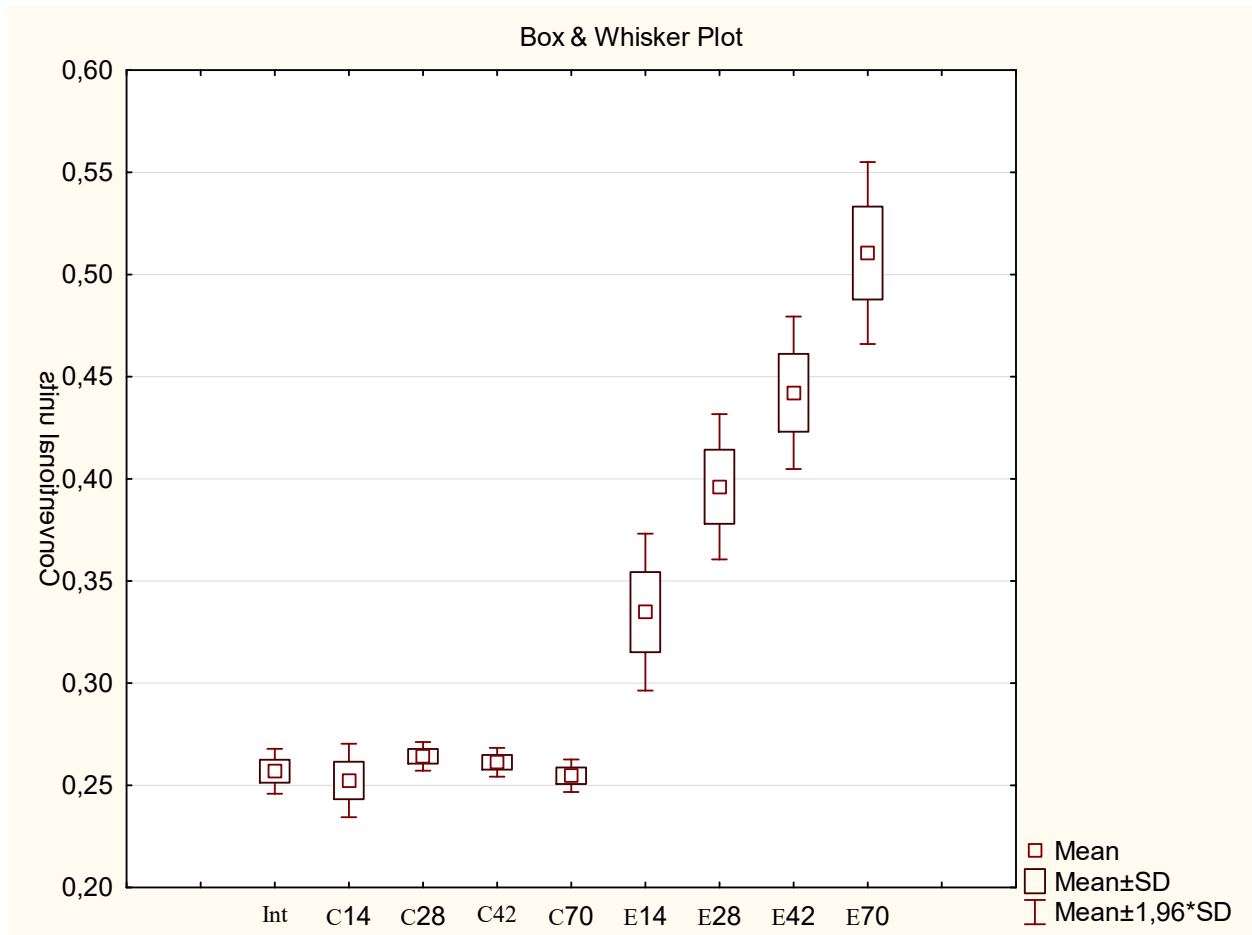


Fig.1. Dynamics of the content of MMM₂₅₄ fraction (c.u.) in the serum of rats in experimental diabetes mellitus

Notes: groups of animals: Int- intact; C-control; E-experimental.
14, 28, 42, 70- days of the experiment

In 42 days after the diabetes mellitus modelling, serum fraction of MMM₂₅₄ was increased by 62,8 % ($p < 0,001$) compared with the control group of animals.

We also noted an increase of concentration of MMM₂₈₀ in the serum by 64,8 % ($p < 0,001$) compared to control data. The maximum increase of both fractions of of MMM in

serum was observed in 70 days after the beginning of research in animals with experimental streptozotocin-induced diabetes mellitus. The content of the MMM₂₅₄ fraction in the serum was by 76% more than in the control group of animals.

Table 2. The content of MMM₂₈₀ fraction (c.u.) in the serum of rats in experimental diabetes mellitus

| Group | 14 days | | 28 days | | 42 days | | 70 days | | p ₂ |
|----------------|-------------|-------|---------|-------|---------|-------|---------|-------|----------------|
| | M | ±m | M | ±m | M | ±m | M | ±m | |
| experimental | 0,186* | 0,002 | 0,217* | 0,005 | 0,262* | 0,004 | 0,319* | 0,006 | <0,01 |
| Control | 0,165 | 0,001 | 0,162 | 0,001 | 0,159 | 0,001 | 0,164 | 0,001 | >0,05 |
| p ₁ | <0,001 | | <0,001 | | <0,001 | | <0,001 | | x |
| intact | 0,160±0,001 | | | | | | | | |

Notes: 1. p₁- the reliability of the data difference between experimental and control groups.

2. p₂-the reliability of data within the group in the dynamics

3. * the reliability of the data difference compared to the intact group

Changes of a similar nature were observed of the MMM₂₅₈₀ fraction which was increased by 81,1% (p<0,001) compared to the control group of animals.

Comparing the experimental groups with each other, we found a significant increase (p<0,001) of both fractions of MMM in the blood serum at all stages of the experiment.

Analysis of the experimental study results evidence unidirectional changes in both fractions of MMM and indicate a violation of metabolic processes that are manifested in varying degrees of endogenous intoxication, which is consistent with the data of other researchers [2, 8].

Under the conditions of modelled diabetes there is a significant increase in the level of endogenous intoxication, which is manifested by an increase of the content of MMM in the blood serum compared to the control group of animals. A similar increase in blood counts of MMM in various pathological conditions have been noted by several other scientists [3, 5, 17].

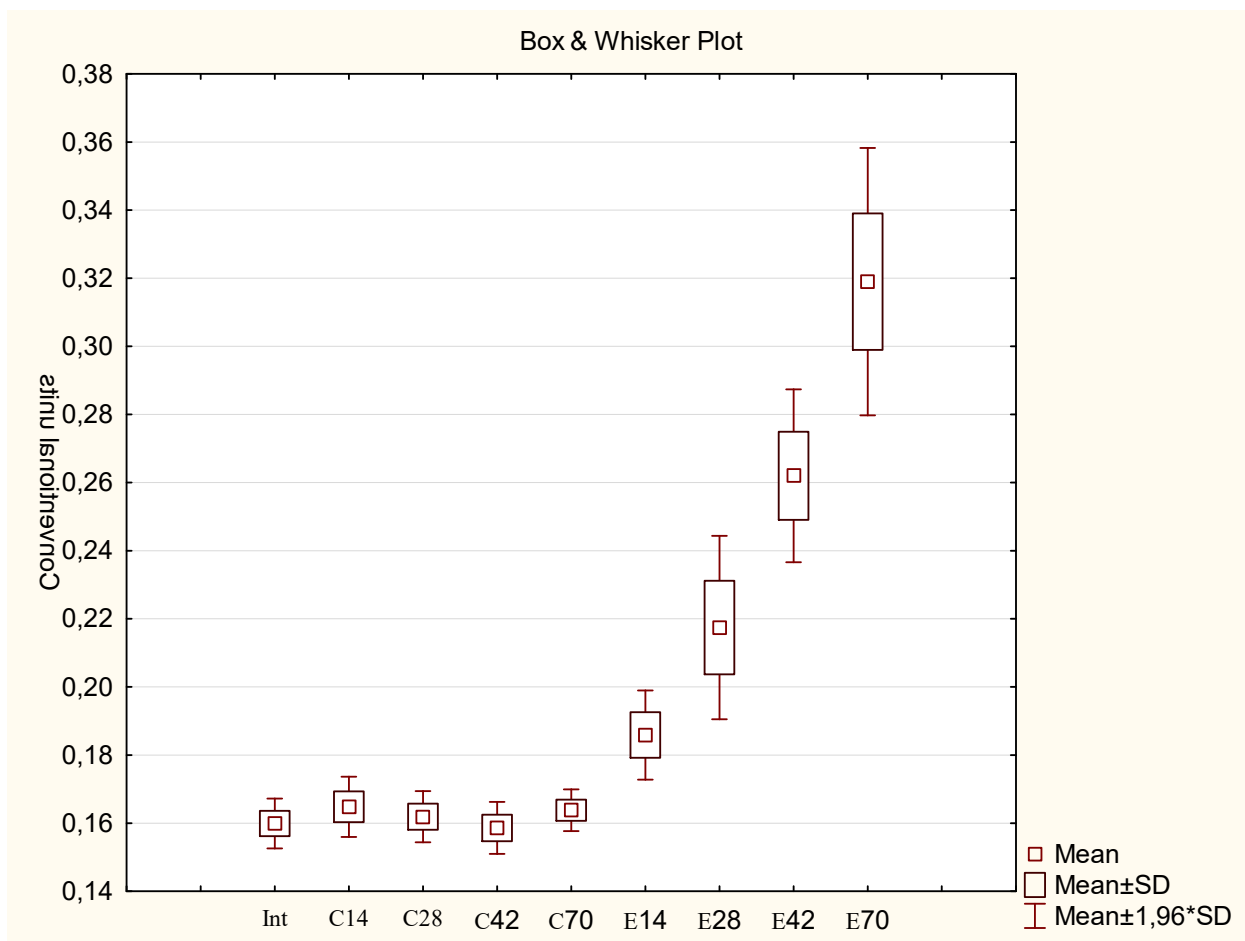


Fig.2. Dynamics of the content of MMM₂₈₀ fraction (c.u.) in the serum of rats in experimental diabetes mellitus

Notes: groups of animals: Int- intact; C-control; E-experimental.
14, 28, 42, 70- days of the experiment

CONCLUSIONS

1. Streptozotocin-induced diabetes mellitus is accompanied by an increase in the level of endogenous intoxication which is manifested by an increase of both MMM fractions content in serum.
2. The severity of endogenous intoxication depends on the duration of the endogenous factor.

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