Sarakhan L. V., Savytskyi I. V., Znamerovskyi S. H., Pandey S. A. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus against the background of obesity and determination of the efficacy of complex pathogenetic correction. Journal of Education, Health and Sport. 2025;85:72606. eISSN 2391-8306. https://dx.doi.org/10.12775/JEHS.2025.85.72606
https://apcz.umk.pl/JEHS/article/view/72606
https://zenodo.org/records/20384170

The journal has had 40 points in Minister of Science and Higher Education of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 05.01.2024 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical culture sciences (Field of medical and health sciences);
Health Sciences (Field of medical and health sciences). Punkty Ministerialne 40 punktów. Załącznik do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 05.01.2024 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).© The Authors 2025;

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 22.08.2025. Revised: 28.08.2025. Accepted: 19.09.2025. Published: 25.09.2025.

UDC. 616.379-008.64:617.735

THE ROLE OF OXIDATIVE STRESS IN THE PATHOGENESIS OF TYPE 2 DIABETES MELLITUS AGAINST THE BACKGROUND OF OBESITY AND DETERMINATION OF THE EFFICACY OF COMPLEX PATHOGENETIC CORRECTION

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Abstract

Introduction. Diabetes mellitus is currently a worldwide problem, one of the key pathogenetic links of which is the development of oxidative stress

Aim: to study the markers of oxidative stress in the pathogenesis of type 2 diabetes mellitus against the background of obesity and to determine the efficacy of complex pathogenetic correction

Materials and methods of the study: The study was carried out on white nonlinear rats of both sexes weighing 180 - 200 g. All experimental animals were divided into 5 groups. Group 1 - intact, animals kept on a standard diet and water regimen; Group 2 - animals in which type 2 diabetes mellitus was modelled. The streptozotocin model was used to reproduce type 2 DM.

Group 3 - animals with modelled diabetes mellitus and obesity. Group 4 - rats that, against the background of modelled diabetes mellitus and obesity, received hypoglycaemic therapy - metformin

Group 5 - rats that, against the background of modelled diabetes mellitus and obesity, received metformin together with daily intragastric administration of L-arginine, citicoline and vitamin E for 30 days; Group 6 - rats that, against the background of modelled diabetes mellitus and obesity, received metformin together with daily intragastric administration of L-arginine, citicoline and vitamin E for 60 days

Results: The results we obtained indicate the development of oxidative stress against the background of modelling streptozotocin-induced diabetes mellitus under the conditions of our experiment, as evidenced by an increase in both primary and secondary LPO products. An aggravating effect of obesity on the pathogenesis of experimental diabetes mellitus was established, in particular on the development of oxidative stress in this pathology, which is confirmed by an increase in the level of both diene conjugates and malondialdehyde and all TBA-active products in general in Group 3. The analysis of the data of Group 4 (in which the pathological conditions were corrected with metformin) established that correction of the pathological condition with hypoglycaemic agents has a positive result, but does not allow a pronounced correction of the disturbances of the prooxidant system, which once again confirms the need to involve additional means of correction. The results of Group 5 indicate that the inclusion of citicoline, a nitric oxide donor and vitamin E for 30 days in the correction of modelled diabetes mellitus combined with obesity markedly reduces the activity of oxidative stress, but the results do not reach normative values. The obtained data indicate the efficacy of long-term use of combined correction in DM, especially if it develops against the background of comorbid pathology. It was found that changes in primary LPO products are more pronouncedly amenable to correction in the modelled pathology than the level of the representative of the secondary ones – MDA.

Keywords: diabetes mellitus; obesity; experiment; diene conjugates; malondialdehyde; TBA-active products; correction; metformin; L-arginine; citicoline; vitamin E

Introduction. Diabetes mellitus is currently a worldwide problem, since according to the IDF in 2024 589 million adults suffered from this pathology. If we add to this another 1.1 billion people in the world with impaired glucose tolerance or increased fasting glycaemia, which is a potential risk of developing type 2 diabetes mellitus, then it becomes clear how

global this problem is for all of humanity. According to experts' forecasts, this trend will only increase and the number of patients will rise substantially over the next two decades [1]. It is well known that type 2 diabetes mellitus is the most common, accounting for more than 90 % of the total number of diabetes mellitus cases. It is directly associated with increased mortality from micro- and macrovascular complications. There are many studies that have proven that type 2 diabetes mellitus can be brought into remission and the disease prognosis improved by controlling hyperglycaemia and its associated risk factors. Early diagnosis and prevention can prevent the progression of this pathology in individuals at high risk. The danger of developing diabetes mellitus, despite modern methods of prevention and treatment, unfortunately continues to grow, and therefore the further search for highly effective, pathogenetically substantiated treatment is an urgent need of today. [2] One of the dangerous features of this pathology is that for a long time it is characterised by an asymptomatic course and may be diagnosed already at the late stages of the disease. This pathology is characterised by micro- and macrovascular complications, which lead to disability and lethality [3, 4]. Chronic stress, the current socio-economic situation and changes in the way of life of the population associated with martial law lead to the progression of metabolic disorders [5, 6, 7]. In 2025, according to the National Health Service of Ukraine, the electronic healthcare system (eHealth) contained 1.32 million patients with DM, among whom 1.26 million (95.0%) suffered from type 2 diabetes mellitus [8, 9].

At the same time, oxidative stress is a key link of pathogenesis that is able to activate destructive and inflammatory mechanisms in diabetes mellitus and trigger a vicious circle of multiple organ complications in this pathology [10, 11].

Taking into account the above, it is informative to study oxidative stress in type 2 diabetes mellitus and obesity with the aim of further analysing the efficacy of the methods of its correction under experimental conditions.

Aim: to study the markers of oxidative stress in the pathogenesis of type 2 diabetes mellitus against the background of obesity and to determine the efficacy of complex pathogenetic correction

Materials and methods of the study: The study was carried out on white nonlinear rats of both sexes weighing 180 - 200 g, which were kept on a standard diet and water regimen in accordance with sanitary and hygienic norms: at a temperature of 20-22 ºC, humidity of no more than 60-70 %, an air exchange volume (exhaust-inflow) of 8/10, a day/night light regimen in standard aluminium cages of no more than 5 animals each

(Directive 2010/63/EU of European Parlament and Council on the protection of animals used for scientific purposes).

All experimental animals were divided into 5 groups (10 animals in each group):

Group 1 - intact, animals kept on a standard diet and water regimen;

Group 2 - animals in which type 2 diabetes mellitus was modelled. The streptozotocin model was used to reproduce type 2 DM. For this purpose, the rats were given a single intravenous injection of streptozotocin («SigmaAldrich Chemie GmbH», Germany) at a dose of 65 mg/kg. The streptozotocin solution was prepared in 0.1 M citrate buffer pH 4.5. In order to reduce the diabetogenic effect of streptozotocin, 15 minutes before its administration, nicotinamide («Afton Pharma», India) was injected intraperitoneally at a dose of 230 mg/kg, which allows up to 40 % of the pancreatic insulin reserves to be preserved in the experimental rats, owing to which the animals develop moderate and stable basal hyperglycaemia [12,13]

Group 3 - animals with reproduced DM and obesity: modelling of type 2 DM in sexually mature six-month-old rats was carried out by administering a low dose of streptozotocin (30 mg/kg intraperitoneally, in citrate buffer pH=4.5) after 90 days of keeping the animals on a combined diet, which is a combination of a high-fat diet (a diet with an excessive content of saturated fats: proteins - 20.0 %, fats - 60.0 %, carbohydrates - 20.0 % of the total caloric value) and excessive carbohydrate consumption (free access to a fructose solution at a concentration of 200 g/l) [14, 15, 16, 17], with a natural change in the regimen of lighting, temperature and air humidity - according to vivarium standards

Group 4 - rats that, against the background of modelled diabetes mellitus and obesity, received hypoglycaemic therapy - metformin was administered intragastrically daily at a dose of 300 mg/kg of the animal's body weight throughout the experiment

Group 5 - rats that, against the background of modelled diabetes mellitus and obesity, received metformin together with daily intragastric administration of L-arginine, citicoline and vitamin E for 30 days;

Group 6 - rats that, against the background of modelled diabetes mellitus and obesity, received metformin together with daily intragastric administration of L-arginine, citicoline and vitamin E for 60 days;

The study of indicators that characterise the LPO system included the study of TBA-AP products, the level of diene conjugates, and malondialdehyde.

The following indicators were used to assess LPO: the level of diene conjugates (DC), intermediate products of LPO that are indicators of the intensity of the free-radical process, and TBA-reactants, which are the final product of degradation of unsaturated fatty acids of

membrane phospholipids. The concentration of malondialdehyde (MDA) was also determined [18] Determination of diene conjugates (DC). It was performed by measuring optical density, which is measured at a wavelength of 230 nm and is the sum of the optical densities of conjugated double bonds (the actual LPO products). The result was expressed in units of optical density per 1 mg of lipids or 1 ml of serum. The intensification of free-radical processes in the liver was assessed by the quantitative content in the organ homogenate of the final LPO product - TBA-reactants, the principle of which method consists in the formation of coloured complexes that are extracted with butanol upon the interaction of LPO products with thiobarbituric acid. The concentration of MDA was determined by the TBA method. The principle of the method consists in the formation of a coloured complex upon the interaction of MDA with thiobarbituric acid.

The study was carried out in accordance with the «Rules for performing work using experimental animals», approved by Order of the Ministry of Health of Ukraine No. 249 of 01.03.2012 and the Law of Ukraine No. 3447-IV «On the protection of animals from cruel treatment» (as amended on 15.12.2009 and on 16.10.2012).

Results of the study and their discussion

Results of the study of diene conjugates in the modelled pathological conditions and against the background of the methods of correction (Table 1)

In the study of diene conjugates, which are primary LPO products, it was established that in the group in which diabetes mellitus was modelled without correction, the level of the studied indicator statistically significantly exceeded the results of the intact group ($p<0.05$) - by 67 %. The comorbid pathology has a more aggravating effect on the development of oxidative stress - the DC level was higher in Group 3 by 85 % ($p<0.05$) and by 11 % compared with the group in which diabetes mellitus was modelled without obesity. In the study of the DC level in Group 4, in which modelled diabetes mellitus against the background of obesity was corrected by the administration of metformin, a decrease of this indicator was established by 27 % relative to the group with comorbid pathology without correction, by 19 % compared with modelled diabetes mellitus without obesity, but the indicator remains elevated by 35 % compared with the intact group, which indicates the insufficiency of monotherapy for normalising oxidative stress in the pathogenesis of diabetes mellitus.

In the group in which combined correction with metformin and complex correction with L-arginine, citicoline and vitamin E was applied, the results were more pronounced already on day 30 - the level of primary LPO products was statistically significantly ($p<0.05$) lower compared with Group 3 (in which diabetes mellitus and obesity were modelled without

correction) - by 32%. Compared with the data of the group in which diabetes mellitus was modelled without concomitant pathology, the level of the OS marker was lower by 25%; we can also state better results compared with the group in which monocorrection with metformin was applied - by 7%. However, the level of the indicator is 25 % higher compared with the normative values (Group 1).

The data obtained on day 60 indicate the expediency of a more prolonged use of complex therapy for the correction of diabetes mellitus: the DC level is lower by 41 % compared with the group of comorbid pathology without correction, by 35% lower compared with the DM group, and by 20% lower compared with the use of metformin alone. It should also be noted that the values of the studied marker on day 60 indicate a more pronounced efficacy compared with day 30 of the experiment - by 13 %. Compared with the intact animals, the level of the oxidative stress marker is higher by only 8 %.

Results of the study of TBA-active products in the modelled pathological conditions and against the background of the methods of correction

As a result of the study, a statistically significant increase of TBA-active products was established in Group 2, in which diabetes mellitus was modelled without further correction ($p<0.05$); in Group 3, in which diabetes mellitus was modelled concomitantly with obesity, a statistically significant increase of TBA-AP was also detected compared with the intact group, and this indicator was also 8 % higher compared with Group 2. In Group 4, which received metformin in modelled diabetes mellitus against the background of obesity, it was found that this therapy is insufficient for normalising the studied marker of oxidative stress: although its level decreased by 15% and 21 % (no statistical significance) compared with the groups without correction No. 2 and No. 3 respectively, it remained statistically significantly ($p<0.05$) elevated compared with the intact group (the level was higher by 46 %). In the analysis of the results of Group 5, which received correction with metformin, L-arginine, citicoline and vitamin E for 30 days against the background of the modelled pathology, the following results were obtained: the level of the prooxidant was statistically significantly lower compared with Group 3, in which the modelled pathology was not corrected; compared with Group 1 it is elevated by 24 %, compared with Group 2 the rise is less pronounced by 27.8%, compared with Group 4 (in which monocorrection with metformin was used) its rise was less pronounced by 15 %. The above allows us to state the effectiveness of the complex method of correction we chose already on day 30 of the experiment. On day 60, the application of complex correction with L-arginine, citicoline and vitamin E in combination

with metformin gave better results compared with the groups without correction - by 32% and 40 % compared with Group 2 and 3 respectively ($p<0.05$). The obtained data also indicate more pronounced results in the normalisation of oxidative stress in Group 6 compared with the application of complex correction over a less prolonged period (Group 5) - by 10 %. Compared with the intact animals, the level of TBA-AP was elevated by 12%, that is, we can state that the results of this group were closer to the norm indicators compared with all the previous groups, where the difference was more pronounced

Table 1. - Results of the study of oxidative stress markers in the pathogenesis of diabetes mellitus, obesity and against the background of the methods of correction

Animal group (n = 10)	Stat. indicator	TBA-AP, nmol/g	Diene conjugates, nmol/g	Malondialdehyde, μmol/l
I group	$\bar{x} \pm S_{\bar{x}}$	2.90 ± 0.05	21.80 ± 1.40	4.21 ± 0.27
II group	$\bar{x} \pm S_{\bar{x}}$ P_{I-II}	5.00 ± 0.16 < 0.05	36.41 ± 2.00 < 0.05	13.14 ± 1.20 < 0.05
III group	$\bar{x} \pm S_{\bar{x}}$ P_{I-III} P_{II-III}	5.40 ± 0.19 < 0.05 –	40.40 ± 2.19 < 0.05 –	15.60 ± 1.28 < 0.05 –
IV group	$\bar{x} \pm S_{\bar{x}}$ P_{I-IV} P_{II-IV} P_{III-IV}	4.25 ± 0.23 < 0.05 – –	29.50 ± 1.59 – – –	11.39 ± 0.83 < 0.05 – –
V group day 30	$\bar{x} \pm S_{\bar{x}}$ P_{I-V} P_{II-V} P_{III-V} P_{IV-V}	3.61 ± 0.19 – – < 0.05 –	27.30 ± 1.20 – – < 0.05 –	8.40 ± 0.39 – – – –
VI group day 60	$\bar{x} \pm S_{\bar{x}}$ P_{I-VI} P_{II-VI} P_{III-VI} P_{IV-VI} P_{V-VI}	3.24 ± 0.15 – < 0.05 < 0.05 – –	23.70 ± 1.00 – < 0.05 < 0.05 – –	6.51 ± 0.26 – < 0.05 < 0.05 – –

Results of the study of malondialdehyde in the modelled pathological conditions and against the background of the methods of correction

The results of the determination of secondary LPO products indicate the progression of the pathological effect of oxidative stress in experimental diabetes mellitus - the MDA level is higher by 212 % compared with the intact animals ($p<0.05$). The mutually aggravating effect of DM and obesity was also confirmed - the MDA level is higher in Group

3 by 270 % ($p<0.05$) compared with the intact group and by 19 % higher compared with the group in which diabetes mellitus was modelled without concomitant pathology. In the group that received correction with metformin, a decrease of the MDA level by 27% was detected compared with the group of comorbid pathology without correction and by 13 % compared with modelled DM, but the level of the marker exceeds the value of the intact animals by 170 % ($p<0.05$). In the group in which L-arginine, citicoline and vitamin E were added to metformin therapy, better results in the context of reducing the development of oxidative stress were detected already on day 30: by 46 % compared with the group of the same modelled comorbid pathology without correction and by 36 % compared with modelled DM without aggravation. Compared with the monocorrection group, the result is better by 26%. However, compared with the intact animals, the MDA level is elevated by 99%, which indicates the need for a more prolonged use of complex correction for the normalisation of secondary products of lipid peroxidation.

On day 60, the results are closer to the normative values: thus, compared with the group with the modelled combined pathology without correction, the MDA level is lower by 58 %, and 50 % lower compared with the group in which only DM was modelled. Compared with metformin monotherapy, the involvement of complex multicomponent correction demonstrated its efficacy by 43 %. A more pronounced effectiveness is also observed with the more prolonged use of the proposed method of correction compared with day 30 of the experiment - by 22 %. The difference compared with the intact group is less pronounced compared with the previous groups and amounts to 54 %.

The above data confirm the efficacy of long-term use of combined correction in DM, especially if it develops against the background of comorbid pathology. In turn, it should be noted that changes in primary LPO products are more pronouncedly amenable to correction in the modelled pathology than the level of the representative of the secondary ones - MDA. In the course of the study, the efficacy of the complex effect of the combination of L-arginine, citicoline and vitamin E and a drug for normalising blood glucose was confirmed, as was the aggravating effect of obesity on the course of oxidative stress in DM.

Conclusions:

1. The results we obtained indicate the development of oxidative stress against the background of modelling streptozotocin-induced diabetes mellitus under the conditions of our experiment, as evidenced by an increase in both primary and secondary LPO products under the conditions of our experiment

2. An aggravating effect of obesity on the pathogenesis of experimental diabetes mellitus was established, in particular on the development of oxidative stress in this pathology, which is confirmed by an increase in the level of both diene conjugates and malondialdehyde and all TBA-active products in general under the conditions of our study aggravating effect of obesity

3. The analysis of the data of Group 4 (in which the pathological conditions were corrected with metformin) established that correction of the pathological condition with hypoglycaemic agents has a positive result, but does not allow a pronounced correction of the disturbances of the prooxidant system, which once again confirms the need to involve additional means of correction

4. The results of Group 5 indicate that the inclusion of citicoline, a nitric oxide donor and vitamin E for 30 days in the correction of modelled diabetes mellitus combined with obesity markedly reduces the activity of oxidative stress, but the results do not reach normative values

5. The obtained data indicate the efficacy of long-term use of combined correction in DM, especially if it develops against the background of comorbid pathology. It was found that changes in primary LPO products are more pronouncedly amenable to correction in the modelled pathology than the level of the representative of the secondary ones - MDA.

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