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Ecdysterone treatment restores constitutive NO synthesis and alleviates oxidative damage in heart tissue and mitochondria of streptozotocin-induced diabetic rats

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Constitutive NO synthases (cNOS) are the primary targets of diabetes mellitus and the impairment of cNOS functioning in cardiovascular system is one of the hallmarks of this disease. The aim of this work was to study the effect of a plant sterol ecdysterone (20-β-hydroxyecdysterone) on the NO synthases functioning and RNS metabolism in heart mitochondria and the heart tissue in the rat model of streptozotocin-induced type I diabetes. Diabetes development resulted in cNOS dysfunction both in heart mitochondria and heart tissue. cNOS activity was dramatically suppressed, but 3-fold and 6-fold rise of iNOS activity was observed in mitochondria and heart tissue respectively. Also, in mitochondria there was ~2.5 time's increase in urea content and the activity of arginase 2 (ARG2), which could compete with NOS for the common substrate L-arginine. Total RNS production was dramatically elevated in mitochondria of diabetic animals, which well agreed with iNOS activation. Unlike this, in heart tissue dramatic increase of iNOS activity increased the content of nitrosothiols (RSNO), while total RNS production remained close to control. Both in the heart tissue and mitochondria, there was dramatic augmentation of superoxide production that correlated with sharp elevation of iNOS activity and steep rise of diene conjugates (DC) content, which indicated strong lipid oxidation. Ecdysterone treatment resulted in the reduction of iNOS activity and twofold elevation of mtNOS activity as compared to control. However, in the whole heart tissue eNOS was restored only by half of control level, which indicated specific action of ecdysterone on mtNOS isoform. RNS production returned to control in mitochondria, and was by half reduced in the heart tissue, which indicated the abolition of nitrosative stress. Correlation dependence between iNOS activity and superoxide production was found in mitochondria, which could indicate iNOS uncoupling. The restoration of cNOS activity and the reduction of iNOS activity to control level after ecdysterone treatment well correlated with the reduction of superoxide production and indicated possible 'iNOS re-coupling', which resulted in the reduction of DC formation to control level. So, STZ-induced type I diabetes dramatically up-regulated iNOS activity and suppressed cNOS activity. Ecdysterone treatment reduced iNOS activity and restored constitutive NO biosynthesis to control level, which abolished oxidative and nitrosative stress in cardiac mitochondria and heart tissue of STZ-induced diabetic animals. Possible pathways involved in ecdysterone action on constitutive NO biosynthesis were discussed.

Key words: diabetes mellitus; heart; mitochondria; cNOS; iNOS; ARG2; ROS; RNS; ecdysterone.

INTRODUCTION

Type I diabetes (diabetes mellitus) is in the first line of the causes of mortality worldwide. It is well known that diabetes mellitus causes deep impairments at systemic and functional level and deregulation of metabolic processes in the living organism. Insulin deficiency in type I diabetes, with following hyperglycemia, endothelial dysfunction and cardiomyopathy are the most common clinical markers of this disease [1]. The progress of diabetes results in oxidative and nitrosative stress [2, 3], impairment of fatty acid metabolism, activation of phospholipases and

arachidonic acid cascade [4-6], development of inflammation [7, 8], and triggering of multiple apoptosis-related pathways.

Endothelial NO synthase (eNOS) and mitochondrial NOS (mtNOS) are the key factors in the maintenance of vessel tone and heart functions. NO radical generated by NO synthases triggers a complex network of signaling pathways, primarily cGMP/PKG signaling, resulting in cardioprotection [9]. Both NO deficiency and NO overproduction under pathophysiological conditions result in dramatic deregulation of RNS and ROS metabolism [2, 3, 9-11]. NOS dysfunction (primarily, the so-called NOS uncoupling resulting from the shortage of NOS substrate and co-factors, such as L-arginine and tetrahydrobiopterine [2, 11-13]) leads to severe impairments of RNS and ROS metabolism. This condition could be aggravated by the activation of inducible Ca^{2+}-independent NOS isoform iNOS (NOS2), which functioning can result in RNS and ROS overproduction, and the activity of arginase, the enzyme which competes with NO synthases for the common substrate L-arginine. The impairment of eNOS functioning, disturbance of RNS and ROS metabolism results in the development of several pathophysiological states and conditions in cardiovascular system. Constitutive NO synthases (cNOS) are the primary targets of diabetes and the deregulation of constitutive NO biosynthesis is one of the key features of this disease [13]. An urgent task for clinical studies is a search for the effective therapeutic strategies aimed at the abolition of oxidative and nitrosative stress, which is common consequence of diabetes.

In this regard, a plant sterol ecdysterone (20-β-hydroxyecdysterone) could be a promising medicine to treat cardiovascular disorders. According to published data, ecdysterone could be involved in the regulation of several biological processes including the promotion of protein synthesis, cell proliferation and growth, improvement of liver secretory function, maintenance of anabolic state, and enhancement of lean muscle mass [6, 14, 15]. Also, a structural role of ecdysterone in the mitochondrial membranes was reported [6]. However, positive therapeutic effects of ecdysterone were doubted in the literature [16].

Previous data have shown that ecdysterone could be involved in the regulation of NO biosynthesis. Ecdysterone treatment effectively restored eNOS activity and abolished oxidative stress in heart, vessels, and cardiac mitochondria in rat model of type I diabetes [17, 18], but the mechanisms of ecdysterone actions on RNS and ROS production remained unexplored. So the aim of this work was to study the effects of ecdysterone on the NOS functioning, RNS metabolism and ROS production in heart mitochondria and the whole heart tissue in the rat model of streptozotocin-induced type I diabetes and to discuss possible mechanisms of ecdysterone treatment on NO biosynthesis.

METHODS

All procedures performed in the studies were in accordance with EU directive 86/609/EEC and the ethical standards approved by the Ethics Committee at A.A. Bogomoletz Institute of Physiology, NAS of Ukraine. Adult Wistar rats with 180-200 g body weight were used. The animals were separated in three groups: control (I), streptozotocin (STZ)-administered group (II), and STZ-adminitered animals treated with ecdysterone (III). STZ was applied at 5 mg/100 g weight; ecdysterone was supplied with drinking water (100 ng/100 g weight) for two months after STZ administration. Animals were kept on standard chow with free water access. Blood glucose content was monitored with glucometer 'Medisense' ("Abbott", USA).

Mitochondria were isolated by the standard procedure. Hearts were thoroughly washed with 0,9% KCl (2°C) minced and homogenized in standard isolation medium: 250 mM sucrose, 20 mM Tris-HCl buffer, 1 mM EDTA, pH 7.4. Homogenate was centrifuged 7 min at 700 g and, after removal of the pellet, 15 min at 11000

g. The sediment was resuspended in a small volume of EDTA-free medium and stored on ice. The protein content was determined by the Lowry method.

Enzymes activity was determined in tissue homogenates and mitochondrial fraction after the interruption of the reactions with $HClO_4$ and the removal of protein. Metabolite content too was determined in protein-free extracts of tissues and mitochondria. For this purpose, aliquots of mitochondrial suspensions were sampled and protein was removed by the addition of 0.5 M $HClO_4$ with consequent precipitation 10 min at 10000 g. After the pellet was discarded, supernatant was neutralized by the additions of 5 M KOH and centrifuged repeatedly for 5 min at 10000 g. Protein-free extracts were stored at -70°C and further used for the metabolite analysis.

Total NOS activity (cNOS+iNOS) was determined as described earlier [17] in the medium: 0.5 mM KH_2PO_4, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM NADPH, 2 mM L-arginine, 5 mM Tris-HCl buffer for 60 min at 37°C. Protein was added at 0.5-1.0 mg. The reaction was stopped by 0.3 ml 2N $HClO_4$. NOS activity was assessed based on nitrite concentrations found after the reaction with Griess reagent [19]. iNOS activity was determined in the same medium as total NOS activity without Ca^{2+} and with the addition of 0.5 mM EGTA. To find constitutive NOS activity iNOS activity was subtracted from total NOS activity.

Arginase activity was assessed based on the determination of urea, which is the end product of arginase reaction, with diacetyl monoxime using the urea kit ("Felicit diagnostic", Ukraine). Activity was expressed as the amount of urea, $nmol \cdot min^{-1} \cdot mg^{-1}$ protein.

NADH-dependent nitrate reductase activity was found based on the determination of nitrate with brucine method. Activity was expressed as the amount of reduced nitrate, $nmol \cdot min^{-1} \cdot mg^{-1}$ protein [20].

Nitrite concentrations were determined in protein-free extracts with Griess reagent [19]. Griess reagent was prepared by mixing equal volumes of 0.1% water solution of *N*-(1-naphthyl) ethylenediamine dihydrochloride with 1% sulfanilamide in 5% H_3PO_4 just before use. 0.5 ml aliquots of mitochondrial protein-free extractions were sampled and mixed with Griess reagent in a proportion 1:1 by volume. 5 min after absorbance at 546 nm was measured; nitrite concentration was determined from calibration curves.

Total *S*-nitrosothiol content was found based on *S*-nitrosothiol decomposition with $HgCl_2$ by the method of Saville [21]. Nitrite concentrations after *S*-nitrosothiols decompositionwas found with Griess reagent [19].

The determination of nitrate was conducted with brucine method based on the reaction of the nitrate with brucine sulfate in a 13 N H_2SO_4 solution at 100ºC. The color complex was measured spectrophotometrically at 410 nm with a nitrate kit ("Felicit diagnostic", Ukraine) [17].

Superoxide generation was monitored spectrophotometrically in homogenates by cytochrome *c* reduction at 550 nm in 5 mM Tris-HCl buffer (pH 7.4) at 37°C. Superoxide formation was quantified using molar extinction coefficient 21000 $mol^{-1} \cdot cm^{-1}$ [22]. Diene conjugates were determined spectrophotometrically in heptane extracts of tissues and mitochondrial preparations [18].

All reagents were from "Merck" (USA). Kits for determination of nitrate, Fe^{2+}, urea, and uric acid were supplied by "Felicit diagnostic" (Ukraine). Radiolabeled probes for the determination of eicosanoids were of "Amersham" (UK). Deionized water was used for solutions preparation.

The data were expressed as mean ± S.E. of 4-6 independent experiments. Correlation dependences and Pearson correlation coefficients R^2 and the reliability of correlations were found using Excel 7.0. Statistical analysis was performed using paired Student's t test; $P < 0.05$ was taken as the level of significance.

RESULTS

As it was shown in earlier research [17], ecdysterone treatment improved blood glucose

level in STZ-treated diabetic animals. After the experiment blood glucose level remained at 6.4 ± 0.6 mmol/l in control group and reached 21.1 ± 6.7 mmol/l in STZ-treated group. Ecdysterone treatment reduced it to 9.1 ± 0.7 mmol/l, which was near the control level.

As we observed, STZ-induced type I diabetes had a dramatic impact on the constitutive NO biosynthesis. Dramatic suppression of cNOS activity, but ~3- and ~6-fold increase in the iNOS activity was observed respectively (Fig. 1) showing a reciprocal regulation of cNOS and iNOS activities both in heart mitochondria and heart tissue of STZ-treated diabetic animals. Observed ratio of cNOS/iNOS activity was dramatically reduced in mitochondria and the whole tissue. iNOS activation in mitochondria was accompanied by strong activation of mitochondrial arginase (ARG2) (Fig. 1), which indicated speeding up of L-arginine metabolism.

The analysis of stable metabolites of ROS and RNS showed dramatic reduction of NO_2^- formation both in mitochondria and the whole heart tissue (Fig. 2). Nitrosothiols (RSNO) formation too was reduced by half in heart mitochondria of diabetic animals (Fig. 1). However, total RNS production in mitochondria, based on most abundant metabolite nitrate, was two-fold elevated as compared to control (Fig. 2). In mitochondria, there was a ~3-fold increase in superoxide production; this was in line with steep rise of diene conjugates' content, which indicated strong lipid oxidation (Fig. 3). Two-fold elevation of urea content in mitochondria well agreed with 2.5 time's increase of ARG2 activity (Figs. 1; 3).

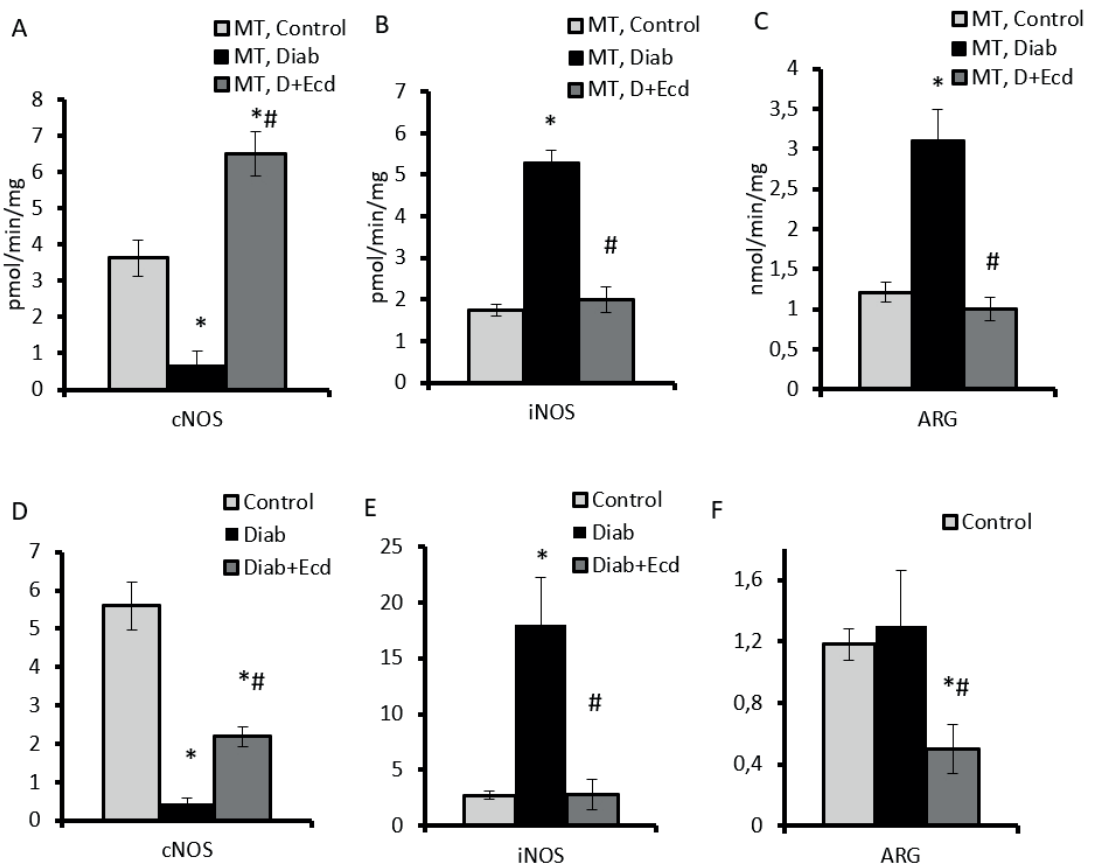

Fig. 1. The effect of ecdysterone treatment on cNOS, iNOS and ARG activity in mitochondria (A-C) and heart tissue (D-F) of STZ-induced diabetic animals. C – control, D – diabetes, D+E – ecdysterone treatment. M ± m, n = 10. *P < 0.05 as compared to control; #P < 0.05 as compared to diabetes

In the whole heart tissue reciprocal regulation of cNOS and iNOS activities was observed as well, however total arginase activity was practically unchanged (Fig. 1). Similarly to mitochondria, NO_2^- formation was dramatically reduced (Fig. 1D). Instead ~4.5-fold rise in RSNO formation was observed, which well agreed with extreme elevation of iNOS activity (Fig. 1B, D). However, unlike mitochondria, despite high level of RSNO, NO_3^- production in the whole heart tissue remained close to control level. Similarly to mitochondria, 2.5-fold elevation of superoxide production and similar increase in DC content in heart tissue was observed.

In mitochondria, a reliable correlation between iNOS activity and superoxide formation was found (Fig. 4A). Also, the increase of iNOS activity correlated with the elevation of NADPH-dependent nitrate reductase activity (Fig. 4B), which possibly could prevent an excess nitrate formation in the heart tissue (Fig. 2E).

Ecdysterone treatment resulted in the reduction of iNOS activity and the restoration of constitutive NO biosynthesis in mitochondria and the heart tissue. Twofold elevation of mitochondrial NOS activity was found, while in the whole heart tissue it was restored only by half of control level, which indicated possible specific action of ecdysterone on mitochondrial cNOS isoform (Fig. 1). NO_3^- production returned to control in mitochondria and was by half reduced in the heart tissue (Fig. 2). Reduction of iNOS activity to control level and the restoration of cNOS activity after ecdysterone treatment well correlated with the reduction of superoxide

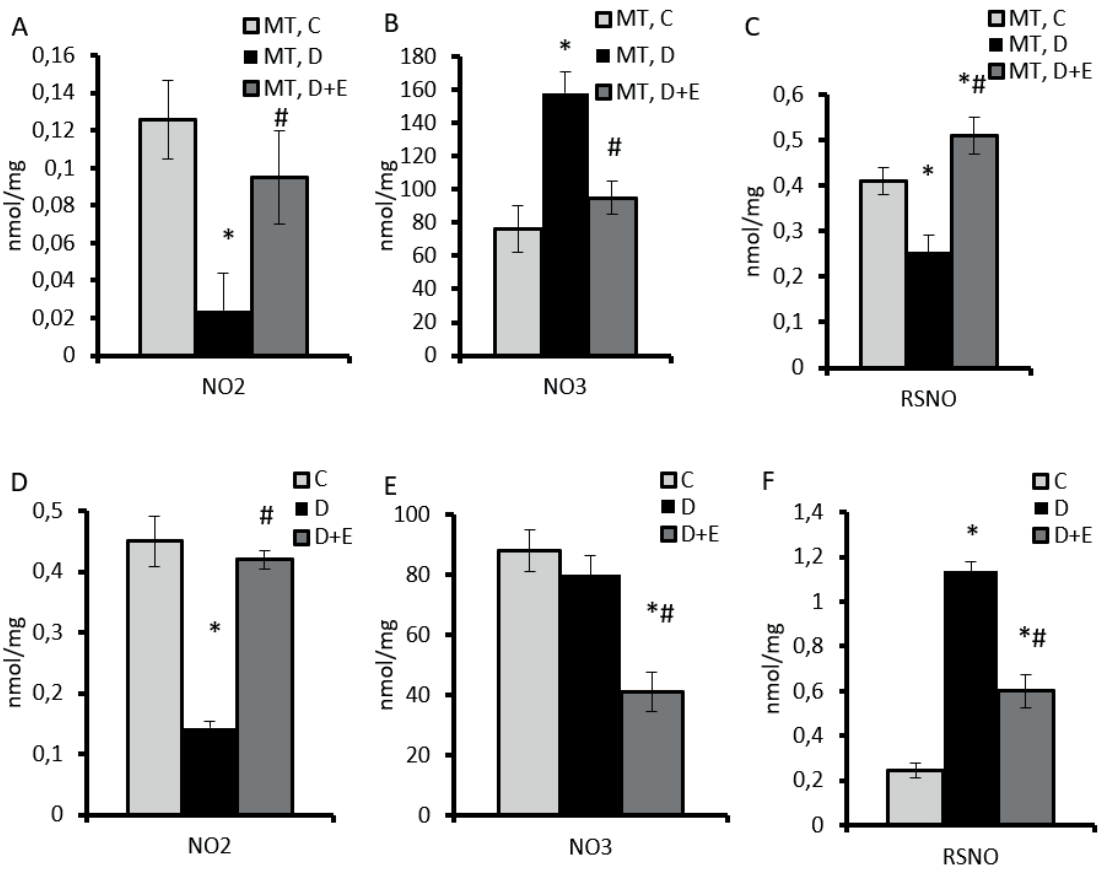

Fig. 2. The effect of ecdysterone treatment on stable RNS in mitochondria (A-C) and heart tissue (D-F) of STZ-induced diabetic animals. C – control, D – diabetes, D+E – ecdysterone treatment. M ± m, n = 10. *P < 0.05 as compared to control; #P < 0.05 as compared to diabetes

generation, which resulted in the reduction of DC formation to control level both in the whole heart and mitochondria (Fig. 3). Arginase activity was reduced to control in mitochondria, but suppressed in the whole heart tissue, which agreed with the reduction of urea content in heart tissue below control level (Fig. 3).

To find possible targets and pathways involved in the regulation of NOS functioning, RNS and ROS metabolism by ecdysterone, we constructed a protein-protein interactions (PPI) network using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://STRING-db.org) [23]. A PPI network of 20 interacting proteins of 1st shell of interactors containing 29 nodes and 37 edges was obtained (Fig. 5). To improve data reliability we used minimum interaction score ≥0.70. According to predicted pathways of ecdysterone action (Fig. 5), ecdysterone could bind to estrogen receptors ESR1 and ESR2, trigger activation of Akt1 kinase, positively regulate NOS3 activity, and regulate NOS2, ARG2 and RNS metabolism via several pathways and transcription factors, such as EGFR, PPARα, MTOR, STAT3, HIF-1α, and other pathways. Ecdysterone could regulate ROS metabolism by the binding to ESR1 receptor, targeting Akt1, EGFR, STAT3, Fox01, HIF-1α, and regulating SOD1 and NOX4 activity (Fig. 5). Several ecdysterone targets (NOS2, Fox01, Cav1, MTOR and others) are related to the development of diabetes mellitus (Human Gene Database (GeneCards; http://www.Genecards.org) [24], which could explain positive therapeutic effects of ECD treatment.

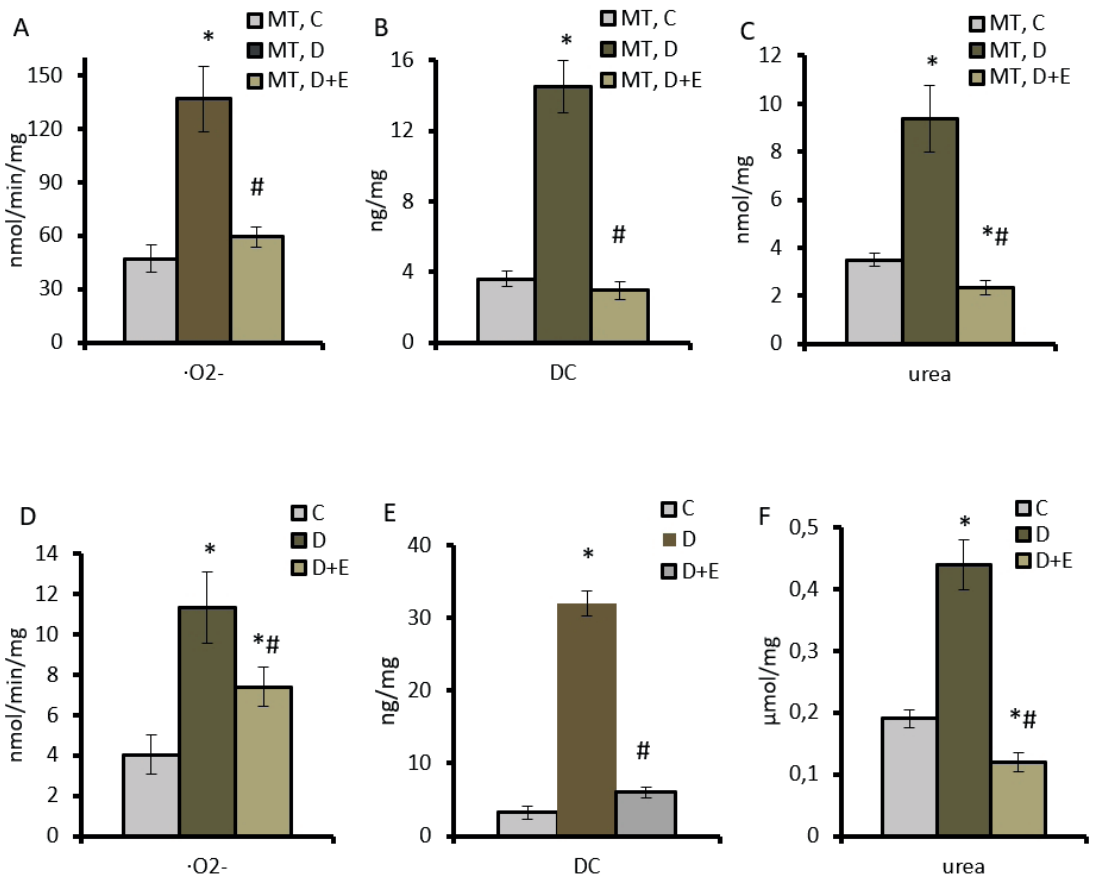

Fig. 3. The effect of ecdysterone treatment on the rate of superoxide formation and the level of diene conjugates and urea in rat heart mitochondria (A-C) and tissue (D-F) in STZ-induced diabetes. C – control, D – diabetes, D+E – ecdysterone treatment. M ± m, n = 10. *P < 0.05 as compared to control; #P < 0.05 as compared to diabetes

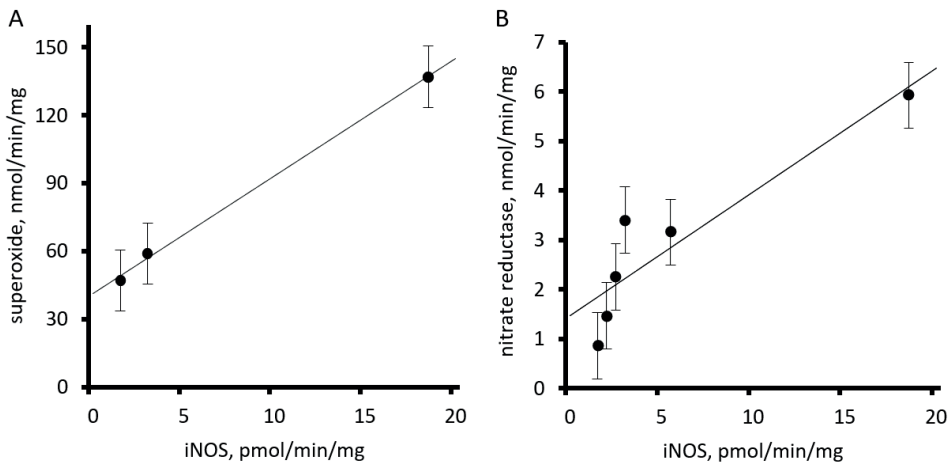

Fig. 4. Correlation dependences between iNOS activity, the rate of superoxide formation (A), and NADPH-dependent nitrate reductase activity (B) in heart mitochondria of STZ-induced diabetes animals. M ± m, n = 10. P < 0.05

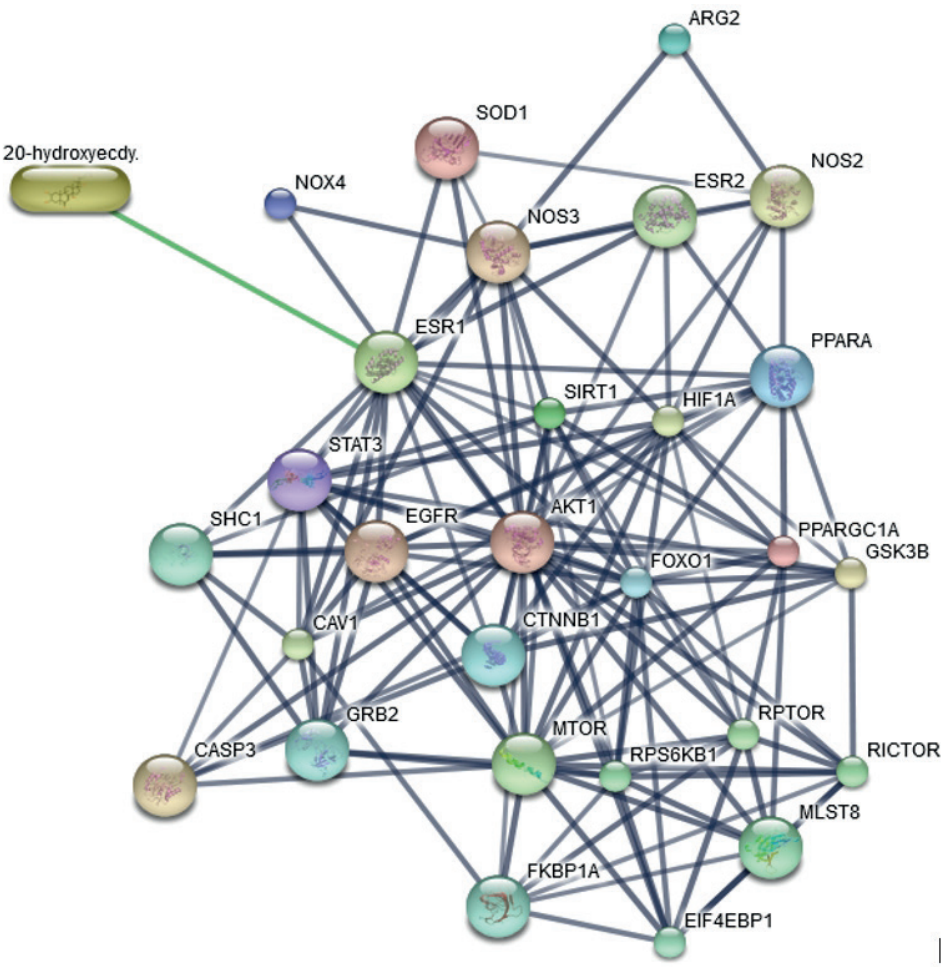

Fig. 5. Protein-protein interactions network related to ecdysterone actions. 20 interacting proteins of 1st shell of interactors are shown. Minimum interaction score ≥0.70

DISCUSSION

As showed the experiments, the development of STZ-induced type I diabetes resulted in severe impairments of cNOS functioning in heart tissue and mitochondria and RNS redistribution towards the formation of most oxidized product NO_3^-, which content in mitochondria was twofold higher as compared to control (Fig. 1B). Instead, steep rise of iNOS activity was observed in heart tissue and mitochondria (Fig. 1). Considering dramatic suppression of cNOS, it is reasonable to assume that most part of the observed RNS production both in mitochondria and the whole heart tissue of diabetic animals resulted from iNOS activity.

It was remarkable that in mitochondria 3-fold iNOS activation coincided with 2-fold rise of NO_3^- formation, instead RSNO and especially NO_2^- production was dramatically reduced (Fig. 2). Meanwhile, ~6-fold iNOS activation and high output of NO formation in the heart tissue preferably resulted in RSNO formation, without elevation of NO_3^- content (Fig. 2). Such redistribution towards the formation of most oxidized product (nitrate) in mitochondria could be explained by the high output of superoxide, which formation in mitochondria was by the order higher than in the whole heart tissue (Fig. 3).

It is well known that the primary sources of cellular ROS are xanthine oxidase, NADPH oxidase (NOX) and mitochondrial respiratory chain [25]. Worth mention, that neither NOX, nor xanthine oxidase are mitochondrial enzymes [26]. So, high output of superoxide production observed in mitochondria under diabetes primarily could result from mitochondrial dysfunction. However, one needs to consider that simultaneous 3-fold activation of iNOS and ~2.5 times' activation of ARG2 in mitochondria prepared ground for possible competition between NOS and ARG2 for the common substrate L-arginine [2, 12]. This state could be favorable for the so-called phenomenon of 'NOS uncoupling', i.e. production of superoxide by iNOS. Similarly, in the whole heart tissue 6-fold iNOS activation possibly could result in the fast depletion of L-arginine, too favorable for iNOS uncoupling.

As it is known, one of the most damaging consequences of NOS uncoupling is the formation of superoxide and still worse, peroxynitrite [27].·NO, which is primary product of NOS activity, can readily react with superoxide with following formation of peroxynitrite, which decomposition results in the formation of highly reactive ·OH radical [27]. So, steep rise in DC formation (Fig. 3), which indicated strong lipid oxidation could result not merely from superoxide production but from possible production of peroxynitrite and ·OH radical caused by 3- and 6-fold activation of iNOS and overproduction of nitric oxide radical.

Both in mitochondria and the whole tissue there was ~3 times' increase in superoxide production, which coincided with ~4-fold elevation of DC content (Fig. 3). Ecdysterone treatment returned iNOS activity, superoxide production, and lipid peroxidation to control level. Based on these observations, and a reliable positive correlation between iNOS activity and superoxide production found in mitochondria (Fig. 4A), it is tempting to hypothesize that ~3-6 times' elevation of iNOS activity, together with ~3-fold elevation of ARG2 activity in mitochondria prepared favorable conditions for iNOS uncoupling, which could largely contribute to superoxide production and peroxynitrite formation, which in turn could explain strong lipid oxidation observed in heart tissue and mitochondria of diabetic animals.

High iNOS activity in diabetes was reported in our previous research and the literature as well [13, 17, 18]. Worth notion, that both iNOS (NOS2) and endothelial NOS (NOS3) were found as diabetes-related targets [24]. However, in the literature there was little data about the impact of type I diabetes on mitochondrial NOS, which is not identical to other constitutive NO synthases (NOS1 and NOS3) [28]. As showed our experiments, both eNOS and mtNOS were

dramatically suppressed in experimental model of type I diabetes.

Ecdysterone application reversed disproportion between cNOS and iNOS activities: both iNOS and ARG2 returned to control level, whereas cNOS activity in mitochondria was even 1.5-2-fold elevated as compared to control (Fig. 1A). Accordingly, urea, superoxide and DC formation too returned to control level (Fig. 3). RSNO and NO_2^- formation was restored close to control; on the contrary, NO_3^- production was reduced to control level, which indicated alleviation of oxidative and nitrosative damage by ecdysterone (Fig. 2; 3). So, reduction of iNOS activity to control level and the restoration of cNOS activity after ecdysterone treatment well correlated with the reduction of superoxide production and indicated possible 'NOS re-coupling', which resulted in the reduction of DC formation to control level both in the whole heart and mitochondria. The reduction of iNOS and nitrate reductase activities by ecdysterone treatment, and the consequent reduction of superoxide production were in line with established correlation dependences (Fig. 4).

NOS, NO and RNS metabolism in diabetes could be regulated by multiple pathways, transcription factors, and bioactive mediators. As it was shown, in diabetes eNOS was negatively regulated by PKCα, p38MAPK, mTOR signaling [8, 29], NF-κB/NOX2 [30], glucose/Pin1/eNOS-NO [31] pathways, which resulted in pathophysiological conditions and promoted cellular apoptosis in cardiovascular system. Increased iNOS, Cav1, SOD1, SOD2, and down-regulated PGC-1α, PPARα and eNOS were found in diabetic mice model, which was accompanied by overproduction of NO and H_2O_2 [10]. Worth notion that high output of mitochondrial ROS could contribute to mtNOS dysfunction promoting NOS monomerization and uncoupling [29]. NOX2 activation too can largely contribute to ROS overproduction, which too may cause NOS uncoupling and endothelial dysfunction [3, 11]. Of pathways involved in positive regulation of eNOS functioning PI3K/Akt/eNOS signaling [32], AMPK/eNOS/NO signaling [33], Nrf2/NOX4 [30], PGC-1α, PPARα, PPARβ [10, 34], and other pathways were shown.

Many of the above pathways related to diabetes mellitus and eNOS dysfunction could be targeted by ecdysterone, which exhibits wide spectrum of biological activity. According to predicted pathways (Fig. 5), ecdysterone can positively regulate eNOS activity, and regulate iNOS, ARG2, RNS and ROS metabolism *via* ESR2, Akt1, PI3K signaling, insulin receptor signaling, mTOR, EGFR, PPARα, MAPK, STAT3, HIF-1α signaling, and the regulation of SOD1 and NOX4 activity [23].

Also, ecdysterone could suppress apoptosis by targeting ESR1, Akt1, NOS3, SOD1, EGFR, STAT3, caspase 3, Fox01 and GSK-3β (Fig. 5). Worth notion, that transcription factor Fox01 is one of the main targets of insulin signaling, which suppresses Fox01 activity [35]. Elevated expression of Fox01 was found in diabetes mellitus, which in turn correlated with the development of cardiomyopathy [35]. One more ecdysterone target related to apoptosis is glycogen synthase kinase 3β (GSK-3β), a protein related to the functioning of permeability transition pore (mPTP), a megachannel involved in triggering of apoptosis and cell death [36].

As showed earlier research conducted on ageing heart mitochondria [18], ecdysterone treatment inhibited mPTP opening. Abolition of oxidative and nitrosative stress by ecdysterone shown in this work, especially in mitochondria, too could suppress mPTP activity and contribute to cardioprotective effects of ecdysterone in experimental diabetes mellitus. Meanwhile, the effects of ecdysterone treatment on mPTP activity and mitochondrial functions in diabetes were not studied yet and remain a task for future research. Considering wide spectrum of ecdysterone targets (Fig. 5), there is a need for extensive further studies to find pathways involved in the regulation of NOS system by this bioactive compound in type I diabetes.

CONCLUSION

Constitutive NO synthases of cardiovascular system are the primary targets of diabetes mellitus. Dramatic reduction of cNOS and 6-fold elevation of iNOS activity was observed in rat model of STZ-induced diabetes. Ecdysterone treatment effectively abolished oxidative and nitrosative stress by down-regulation of iNOS and up-regulation of cNOS activity in heart mitochondria and the whole heart tissue. Reduction of superoxide production by ecdysterone and abolition of oxidative damage after the reduction of iNOS activity to control level indicated possible iNOS uncoupling and the contribution of iNOS to superoxide production in diabetes. So, ecdysterone treatment restored constitutive NO biosynthesis and abolished oxidative and nitrosative stress by suppression of iNOS activity in cardiac mitochondria and heart tissue of STZ-induced diabetic animals. Further research is required to study molecular basis underlying protective effects of ecdysterone on cardiac mitochondria and heart functions.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ЕКДИСТЕРОН ВІДНОВЛЮЄ КОНСТИТУТИВНИЙ СИНТЕЗ NO І ЗМЕНШУЄ ОКСИДАТИВНИЙ СТРЕС У ТКАНИНІ ТА МІТОХОНДРІЯХ СЕРЦЯ ЩУРІВ ЗА УМОВ СТРЕПТОЗОТОЦИНІНДУКОВАНОГО ДІАБЕТУ

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Порушення функціонування конститутивні NO синтази (cNOS) є одними з основних наслідків діабету. Метою роботи було вивчити дію рослинного стероїду екдистерону (20-β-гідроксиекдистерону) на активність NO-синтаз та метаболізм активних форм азоту (АФА) в мітохондріях і тканинах серця щурів за умов стрептозотоциніндукованого діабету 1-го типу. Розвиток діабету призводив до порушень функціонування конститутивної NO-синтази (cNOS) у мітохондріях і тканині серця. Активність cNOS пригнічувалась, тоді як 3- та 6-разова активація iNOS виявлялася в мітохондріях і тканині серця. Також виявлено 2,5-разове збільшення вмісту сечовини і активності аргінази 2, здатної конкурувати з NOS за спільний субстрат L-аргінін. У мітохондріях загальний вміст АФА за умов діабету стрімко зростав, що узгоджувалось з активацією iNOS. На відміну від цього, в тканині серця активація iNOS призводила до збільшення вмісту нітрозотіолів (RSNO), тоді як загальний вміст АФА залишався близьким до контролю. Як в тканині, так і в мітохондріях серця відмічався різкий приріст генерації супероксиду, що корелював зі зростанням активності iNOS та різким підвищенням вмісту дієнових кон'югатів (ДК), ознакою сильної пероксидації ліпідів. Дія екдистерону знижувала активність iNOS і натомість дворазово підвищував активність мітохондріальної NOS (mtNOS) порівняно х контролем. Проте в тканині серця відновлення активності cNOS становило тільки половину від контролю, вказуючи на специфічність дії екдистерону щодо mtNOS. Продукція АФА знижувалась до контрольних значень в мітохондріях та наполовину менш від контролю в тканині серця, що вказувало на усунення нітрозативного стресу. Встановлено кореляцію між активністю iNOS та утворенням супероксиду в мітохондріях, що є можливою ознакою роз'єднання iNOS. Відновлення активності cNOS та пригнічення активності iNOS за дії екдистерону добре корелювало із зменшенням продукції супероксиду, що може вказувати на відновлення функціонування NO-синтаз, внаслідок чого вміст ДК знижувався до контролю. Отже, стрептозотоциніндукований діабет 1-го типу різко підвищував активність iNOS та пригнічував cNOS. Застосування екдистерону пригнічувало iNOS та відновлювало конститутивний біосинтез NO до контрольного рівня, що усувало оксидативний та нітрозативний стрес у мітохондріях і тканині серця щурів за стрептозотоциніндукованого діабету. Обговорюються можливі шляхи дії екдистерону на конститутивний біосинтез NO.

Ключові слова: діабет 1-го типу; серце; мітохондрії; конститутивна NO-синтаза; індуцибельна NO-синтаза; активні форми азоту; аргіназа 2.

REFERENCES

1. Diagnosis and classification of diabetes mellitus. American Diabetes Association. Diabet Care. 2011;34 Suppl. 1:S62-69.
2. Rochette L, Lorin J, Zeller M, Guilland J-C, Lorgis L, Cottin Y, Vergely C. Nitric oxide synthase inhibition

and oxidative stress in cardiovascular diseases: possible therapeutic targets? Pharmacol Ther. 2013;140(3):239-57.
3. Meza CA, La Favor JD, Kim D-H, Hickner RC. Endothelial dysfunction: is there a hyperglycemia-induced imbalance of NOX and NOS? Int J Mol Sci. 2019;20(15):3775.
4. Wang B, Wu L, Chen J, Dong L, Chen Ch, Wen Zh, Hu J, Fleming I, Wang DW. Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets. Sig Transduct Target Ther. 2021;6:94.
5. Leslie ChC. Cytosolic phospholipase A_2: physiological function and role in disease. J Lipid Res. 2015;56:1386-402.
6. Syrov VN. Effect of phytoecdysteroids and nerobol on parameters of carbohydrate and lipid metabolism and phospholipid spectrum of liver mitochondrial membrane in experimental diabetes mellitus of rats. Ukr Biokhim Zh. 1992;64(4):6167. [Ukrainian].
7. Rom S, Zuluaga-Ramirez V, Gajghate S, Seliga A, Winfield M, Heldt NA, Kolpakov MA, Bashkirova YV, Sabri AK, Persidsky Y. Hyperglycemia-driven neuroinflammation compromises BBB leading to memory loss in both diabetes mellitus (DM) type 1 and type 2 mouse models. Mol Neurobiol. 2019 Mar; 56(3):1883-96.
8. Adoga JO, Channa ML, Nadar A. Type-2 diabetic rat heart: The effect of kolaviron on mTOR-1, P70S60K, PKC-α, NF-kB, SOD-2, NRF-2, eNOS, AKT-1, ACE, and P38 MAPK gene expression profile. Biomed Pharmacother. 2022;148:112736.
9. Andreadou I, Schulz R, Papapetropoulos R, Turan B, Ytrehus K, Ferdinandy P, Daiber A, Di Lisa F. The role of mitochondrial reactive oxygen species, NO and H2S in ischemia/reperfusion injury and cardioprotection. J Cell Mol Med. 2020;24:6510-22.
10. Juguilon C, Wang Zh, Wang Y, Enrick M, Jamaiyar A, Xu Y, Gadd J, Ch-L. W, Pu A, Kolz Ch, Ohanyan V, Chen Y-R, Hardwick J, Zhang Y, Chilian WM, Yin L. Mechanism of the switch from NO to H_2O_2 in endothelium-dependent vasodilation in diabetes. Basic Res Cardiol. 2022;117(1):2.
11. Youn JY, Gao L, Cai H. The p47phox- and NADPH oxidase organiser 1 (NOXO1)-dependent activation of NADPH oxidase 1 (NOX1) mediates endothelial nitric oxide synthase (eNOS) uncoupling and endothelial dysfunction in a streptozotocin-induced murine model of diabetes. Diabetologia. 2012;55(7):2069-79.
12. Gambardella J, Khondkar W, Morelli MB, Wang X, Santulli G, Trimarco V. Arginine and endothelial function. Biomedicines. 2020;8(8):277.
13. Van Dyke K, Jabbour N, Hoeldtke R, Van Dyke C, Van Dyke M. Oxidative/nitrosative stresses trigger type I diabetes: preventable in streptozotocin rats and detectable in human disease. Ann NY Acad Sci. 2010;1203:138-45.
14. Sláma KLR. Insect hormones – ecdysteroids: their presence and actions in vertebrates. Eur J Entoniol. 1995; 92:355-37.
15. Chermnykh N, Shimanovskii NL, Shutko GV, Syrov VN: The action of mehandrostenolone and ecysterone on the physical endurance of animals and on protein metabolism in the skeletal muscles. Farmakol Toksikol. 1988;51(6):57-60.
16. Wilborn, CD, Taylor LW, Campbell BI, Kerksick Ch, Rasmussen ChJ, Greenwood M, Kreider RB. Effects of methoxyisoflavone, ecdysterone, and sulfo-polysaccharide supplementation on training adaptations in resistance-trained males. J Int Soc Sports Nutr. 2006;3:19.
17. Korkach YuP, Kotsiuruba AV, Prysiazhna OD, Mohyl'nyts'ka LD, Sahach VF. NO-dependent mechanisms of ecdysterone protective action on the heart and vessels in streptozotocin-induced diabetes mellitus in rats. Fiziol Zh. 2007;53(3):3-8. [Ukrainian].
18. Korkach IuP, Rudyk OV, Kotsiuruba AV, Prysiazhna OD, Sahach VF. The role of nitric oxide and superoxide synthesis in protective mechanism of ecdysterone in the heart mitochondria of rats with streptozotocin-induced diabetes. Fiziol Zh. 2007;53(5):22-8. [Ukrainian].
19. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR: Analysis of nitrite, nitrate and [15N]nitrate in biological fluids. Anal Biochem. 1982;126:131-8.
20. Akopova OV, Korkach YuP, Sagach VF. The regulation of mitochondrial NO synthase activity under nitroglycerine application *in vivo*. Fiziol Zh. 2022;68(1):3-12
21. Percival MD, Ouellet M, Campagnolo Ch, Claveau D, Li Ch: Inhibition of cathepsin K by nitric oxide donors: evidence for the formation of mixed disulfides and a sulfenic acid. Biochemistry. 1999;38:13574-83.
22. Kuthan H, Ullrich U, Estabrook RW. A quantitative test for superoxide radicals produced in biological systems. Biochem J. 1982; 203(3):551-8.
23. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (https://STRING-db.org)
24. Human Gene Database. http:// www.Genecards.org
25. Hernansanz-Agustin P, Enriquez JA. Generation of reactive oxygen species by mitochondria. Antioxidants. 2021;10: 415.
26. Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol. 2004;555: 589-606.
27. Radi R, Beckman JC, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys. 1991;288:481-7.
28. Lacza Z, Pankotai E, Busija DW. Mitochondrial nitric oxide synthase: current concepts and controversies. Front Biosci. 2009;14(12):4436-43.
29. Zhao L, Zhang Ch-L, He L, Chen Q, Liu L, Kang L, Liu J, Luo J-Y, Gou L, Qu D, Song W, Lau ChW, Ko H, Mok VCT, Tian XY, Wang L, Huang Y. Restoration of autophagic flux improves endothelial function in diabetes through lowering mitochondrial ROS-mediated eNOS monomerization. Diabetes. 2022;71(5):1099-114.
30. Gajos-Draus A, Duda M, Beresewicz A. Exercise and

nitrite prevent and Nω-nitrol-L-arginine methyl ester reproduces imbalance in the nuclear factor-κB/NADPH oxidase 2 and nuclear factor erythroid 2-related factor 2/NADPH oxidase 4/endothelial nitric oxide synthase systems in diabetes. J Physiol Pharmacol. 2021;72(5).
31. Wang J-Zh. A novel glucose-Pin1-eNOS-NO signaling axis links diabetes mellitus with cardiovascular diseases. Int J Cardiol. 2018;271:262.
32. Wu Q, Dong J, Bai X, Jiang Y, Li J, Fan Sh, Cheng Y, Jiang G. Propionate ameliorates diabetes-induced neurological dysfunction through regulating the PI3K/Akt/eNOS signaling pathway. Eur J Pharmacol. 2022;925:174974.
33. Yan Zh, Cao X, Wang Ch, Liu Sh, Li Y, Lu G, Yan W, Guo R, Zhao D, Cao J, Xu Y. C1q/tumor necrosis factor-related protein-3 improves microvascular endothelial function in diabetes through the AMPK/eNOS/NO· signaling pathway. Biochem Pharmacol. 2022;195:114745.
34. Chuang Yang, Lai Xue, Yang Wu, Siman Li, Shangjun Zhou, Junxia Yang, Chengyan Jiang, Jianhua Ran, Qingsong Jiang. PPARβ down-regulation is involved in high glucose-induced endothelial injury via acceleration of nitrative stress. Microvascul Res. 2022;139:104272.
35. Wang J, Bai J, Duan P, Wang H, Li Y, Zhu Q. Kir6.1 improves cardiac dysfunction in diabetic cardiomyopathy via the AKT-FoxO1 signalling pathway. J Cell Mol Med. 2021;25(8):3935-49.
36. Zorov DB, Juhaszova M, Yaniv Y, Nuss HB, Wang S, Sollott SJ. Regulation and pharmacology of the mitochondrial permeability transition pore. Cardiovascul Res. 2009;83(2):213-25.

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