UDC 616.379, 616.43:616-008:612.621.31+612.616.31 doi: https://doi.org/10.15407/ubj96.04.033

SEX DIFFERENCES IN RESPIRATION AND REDOX HOMEOSTASIS OF HEART MITOCHONDRIA IN RATS ON HIGH-FRUCTOSE DIET

O. IVANOVA[1], N. GORBENKO[1], O. BORIKOV[2], T. KIPRYCH[1], K. TARAN[1]

[1]SI "V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine", Kharkiv, Ukraine;
[2]V. N. Karazin Kharkiv National University, Kharkiv, Ukraine;
✉e-mail: ivolga3006@ukr.net

Received: 05 April 2024; **Revised:** 27 May 2024; **Accepted:** 25 July 2024

Sex hormones play a leading role in the sexual dimorphism of mitochondrial dysfunction and oxidative stress that are associated with Metabolic Syndrome (MetS) and considered as possible causes of cardiovascular disease. The aim of the work was to determine mitochondrial respiration and redox homeostasis in the heart mitochondria of high-fructose diet-fed (HFD) rats depending on sex. MetS was induced in Wistar rats by 8 weeks intake of fructose (200 g/l) with drinking water. The experiment was performed on 30 rats divided into five groups: control males, control females, HFD fed males, HFD fed females with intact ovaries, ovariectomized HFD fed females. Heart mitochondria were isolated and indicators of redox homeostasis as well as mitochondrial oxygen consumption rate were determined. Heart mitochondria of intact female rats were characterized by a lower intensity of lipid peroxidation, a higher activity of antioxidant defense system and state 3 respiration in comparison with control males. HFD was shown to induce more expressed oxidative stress due to significant inhibition of enzymatic and non-enzymatic components of antioxidant defense and more pronounced dysregulation of mitochondrial respiration in the heart mitochondria of ovariectomized females as compared to males. This data may partially explain the greater cardiovascular risk in women with low estrogen sufficiency and justify the necessity of new sex-specific prevention and treatment of cardiovascular risk approaches.

Keywords: heart mitochondria, mitochondrial respiration, oxidative stress, antioxidant defense system, sex differences, rats.

It is known that a complex cluster of cardio-metabolic risk factors, named Metabolic Syndrome (MetS), affects about one in four individuals worldwide [1]. MetS is greatly associated with an increased risk of cardiovascular disease (CVD), diabetes mellitus, and higher all-cause and cardiovascular mortality. Age and sex may differentially affect some components of MetS, which can result in remarkable variances in the risk of CVD and mortality in men and women [2]. Clinical studies have shown that MetS is more strongly connected with an increased risk of suffering from major cardiovascular events in women, especially in the menopause period, than in men [3-6]. However, data of experimental and clinical studies have not yet given substantial evidence regarding the positive or negative impact of sex hormones on the development of CVD.

It is increasingly acknowledged that oxidative stress and mitochondrial dysfunction are associated with many metabolic diseases and considered as possible causes of the CVD. It has been confirmed that mitochondrial dysfunction was verified in many different organs affected by MetS, such as adipose tissue, skeletal muscle, the liver, the heart, blood vessels, and pancreatic islet beta cells [7].

It is supposed that sex hormones, in particular estrogens, play leading role in the sexual dimorphism of mitochondrial function, modulating ATP production, reactive oxygen species (ROS) generation, antioxidant defenses, mitochondrial membrane potential, and calcium handling [8]. In addition, estrogens control mitochondrial biogenesis, mitochondrial quality control and mitophagy. In some pathological states, including estrogen deficiency and MetS, the regulatory effects of hormones may be declined, which leads to the restriction of estrogen functions in the heart [8].

Although sex differences in the prevalence and manifestation of CVD and mortality risk have been fully described, the sex-related reasons for the cardiac disparities in physiological and pathological states remain still unclear.

The aim of the work was to determine mitochondrial respiration and redox homeostasis in the cardiomyocites of high-fructose diet-fed rats, depending on sex.

Materials and Methods

Chemicals. All chemicals used were of analytical reagent grade quality and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Experimental design. The present study was carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and approved by the bioethics committee of the "V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine" (Kharkiv, Ukraine).

The experiment was performed on 12 male and 18 female Wistar rats (12 week-old, 160–190 g body weight), which were housed in plexiglas cages (3 animals per cage) at a temperature of (22 ± 1)°C in a constant 12-hour light/dark cycle.

Estrogens deficiency was reproduced by bilateral ovariectomy in female rats under light ether anaesthesia and was confirmed by the uterine weight. Females with intact ovaries were sham-operated.

MetS was induced in male and female rats with different levels of estrogen deficiency by chronic (for 8 weeks) intake of fructose with drinking water at a concentration of 200 g/l started on the 15^{th} day after the surgical procedure [9]. Control intact animals were fed a standard diet ad libitum during 8 weeks. The animals had free access to water.

Experimental rats were divided into five groups: control males (n = 6), control females (n = 6), males fed high-fructose diet (HFD) (n = 6), females with intact ovaries fed HFD (n = 6), ovariectomized (OVX) females fed HFD (n= 6).

After 8 weeks, all animals were sacrificed according to the protocol of the ethics committee.

Physiological and metabolic variables. Body weight gain and visceral fat, calculated as the sum of epigonadal, epinephric and mesenteric fat, were measured.

The intraperitoneal glucose tolerance test (IPGTT) was performed on overnight fasted rats. Blood glucose concentrations were initially measured at the basal condition (0 min), then the animals were administered an intraperitoneal injection of glucose (3 g/kg b.w.). Subsequently, tail blood glucose levels were measured at 15, 30, 60, 90 and 120 min after the glucose load.

Insulin sensitivity was determined using the intraperitoneal insulin tolerance test (IPITT) (insulin (Actrapid, Novo Nordisk, Agsvaerd, Denmark) 0.5 U/kg body weight, glucose 2 g/kg in 10 min after insulin administration) [10].

Tail blood glucose levels were measured using a glucose analyzer Eksan-G (Analita Firm JSC Ltd., Vilnius, Republic of Lithuania). Areas under glycemic curves (AUC) were calculated using the Mathlab computer program.

Mitochondria isolation. Mitochondria were isolated by conventional procedures of differential centrifugation. Freshly excised rat hearts were homogenized in a medium containing 250 mM sucrose, 10 mM EDTA, 0.5% BSA, 10 mM Tris-HCl, pH 7.4. To remove EDTA and albumin, mitochondrial pellets were washed twice with buffer containing 250 mM sucrose, 10 mM Tris-HCl (pH 7.4) and resuspended in wash buffer [11].

Spectrophotometry. All spectrophotometry was performed using the double-beam UV-VIS spectrophotometer Shimadzu UV-1800 (Shimadzu Corporation, Kyoto, Japan).

Mitochondrial respiration rate. Mitochondrial oxygen consumption rate was measured polarographically at 30°C using a Clark-type oxygen electrode in the incubation medium: 150 мM sucrose, 75 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$, 10 mM Tris-HCl (pH 7.4) using 5 mM glutamate+malate or 8 mM succinate as energy substrates of Complex I or II, respectively. ADP was added to the incubation medium at a concentration of 200 µM. The rate of mitochondrial respiration in metabolic states 3 and 4 according to Chance B. (V_4, V_3 in nanoatoms of oxygen (natoms O) per mg of protein per minute) and coefficient of respiratory control (V_3/V_4) were calculated from the oxygen absorption curves [11].

Glutathione peroxidase activity. The enzymatic reaction was used to measure glutathione peroxidase (GPX) activity. 3 ml of reaction mixture (pH 7.0) contained: 0.1 mg of mitochondrial protein, 0.56 mM NADPH, 1.0 U glutathione reductase, 7.5 mM sodium azide, 5 mM GSH, 5 mM EDTA and 0.05 M phosphate buffer. 23 mM tert-butyl hydroperoxide as a substrate was added to initiate the enzymatic reaction. The rate of oxidized glutathione (GSSG) formation was determined by decreasing of the reaction mixture absorbance at 340 nm [12].

Mn-superoxide dismutase activity. The Mn-superoxide dismutase (Mn-SOD) activity in mitochondrial suspension was detected by the inhibition of nitroblue tetrazolium (NBT) reduction caused by the xanthine–xanthine oxidase system as the superoxide generator and the absorbance was finally determined at 560 nm. The reaction mixture contained 0.02 mg of mitochondrial protein, 0.05 M carbonate buffer (pH 10.2), 0.1 mM EDTA, 0.1 mM xanthine, 25 μM NBT, 1 mU/ml xanthine oxidase. The results were expressed in arbitrary units (a.u.) of Mn-SOD activity per mg protein. One a.u. means the enzyme amount causing 50% inhibition of the NBT reduction rate [12].

Cytochrome c oxidase activity. Cytochrome *c* oxidase activity was measured in potassium phosphate buffer, pH 7.2, with the addition of 10 μM reduced cytochrome c. The oxidation of cytochrome *c* was measured as the decrease in absorbance at 550 nm and was used to calculate enzyme activity. Cytochrome c oxidase activity was expressed in micromoles of cytochrome c oxidized per minute per mg protein [13].

Reduced glutathione level. The spectrophotometric method for reduced glutathione (GSH) involves the oxidation of GSH by the sulfhydryl reagent 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm [14].

Malondialdehyde level. Malondialdehyde (MDA) can react with thiobarbituric acid to form 3,5,5-trimethyloxazol-2,4-dione at high temperature. According to the extinction value at 532 nm, the MDA content can be calculated (ε_{532} = 155 $l \cdot mol^{-1} \cdot cm^{-1}$). The values were expressed as nmol/mg protein [15].

Protein content measurement. For the measurements, 10 μl mitochondrial suspension and 5 ml Bradford solution were mixed and incubated for 5 min. A standard curve was made of BSA (0, 0.0625, 0.125, 0.25, 0.5 and 1 $g \cdot l^{-1}$) and absorbance was read at 595 nm [16].

Statistical analysis. Data are presented as mean ± standard error of mean (SEM). The Shapiro-Wilk test was used to check normality of data distribution. For multiple comparisons of data with a normal distribution, a parametric two-way analysis of variance (ANOVA) was performed and the Student-Newman-Keuls method was used to test differences in means. Values were considered statistically significant at $P < 0.05$.

Results

We previously showed that HFD did not change the fasting glycemia in experimental animals, but caused the development of glucose intolerance and insulin resistance in male and OVX female rats compared to control animals of the corresponding sex. At the same time, neither glucose homeostasis nor insulin sensitivity were affected by HFD in sham-operated female rats (Table 1) [17].

HFD led to greater body weight gain and significantly higher levels of relative mass of total visceral fat in both males and OVX females in comparison to control animals. Sham-operated HFD-fed females have not shown the sings of obesity and were excluded from experiment (Table 1).

Statistical analysis showed significant effect of sex, as well as HFD on cellular redox homeostasis in isolated heart mitochondria of experimental rats.

It was established that the intensity of lipid peroxidation, assessed by the level of MDA in cardiomyocytes mitochondria of control animals, was higher in male compared to female rats. HFD was accompanied by the development of the oxidative stress in cardiomyocytes of both sexes' animals, but OVX female rats exhibited significantly higher MDA level in the mitochondria compared with males (Table 2).

We observed the significant sex differences in the antioxidant system of rats' heart mitochondria. The mitochondria of intact female rats were characterized by a higher activity of complex enzymatic and non-enzymatic components of the defense pathways, which was confirmed by higher level of GSH and GPX activity compared to males of the control group (Table 2).

It was found that the HFD-induced metabolic disturbances led to the attenuation of the antioxidant defense in male rats, confirmed by a reduction of Mn-SOD activity by 20% and GSH level by 45% in isolated mitochondria of cardiomyocytes in comparison with control animals. However, the activity of GPX remained unchanged in the heart mitochondria of male rats fed HFD (Table 2).

Compared with HFD-fed males, OVX HFD-fed females showed more pronounced alterations of

Table 1. Indexes of glucose homeostasis and obesity in male and female rats, n = 6

Indexes	Control		HFD		
	males	females	males	females	
				sham-operated	OVX
Basal glucose level, mmol/l	4.69 ± 0.28	4.30 ± 0.12	4.62 ± 0.35	4.39 ± 0.34	4.33 ± 0.12
AUC during IPGTT, mmol/l·min	683.1 ± 27.4	560.5 ± 31.7	1043.7 ± 82.5^{ab}	592.6 ± 30.3^{b}	802.3 ± 20.1^{abc}
AUC during IPITT, mmol/l·min	379.9 ± 34.2	373.6 ± 25.4	547.1 ± 18.8^{ac}	427.5 ± 30.5^{b}	536.1 ± 31.3^{ac}
Body weight gain, %	12.24 ± 2.05	13.67 ± 1.25	21.19 ± 1.64^{ab}	14.79 ± 0.78^{b}	28.96 ± 2.00^{abc}
Relative mass of total visceral fat, %	1.68 ± 0.13^{b}	4.07 ± 0.29^{b}	3.95 ± 0.31^{ab}	4.65 ± 0.34	8.27 ± 0.46^{abc}

Note. Data are shown as mean ± SEM. $^{a}P < 0.05$ vs control rats of the corresponding sex; $^{b}P < 0.05$ vs HFD rats of the opposite sex; $^{c}P < 0.05$ vs HFD sham-operated female rats

Table 2. Indexes of cellular redox homeostasis in isolated heart mitochondria of male and female rats, n = 6

Indexes	Control		HFD	
	males	females	males	OVX females
MDA level, nmol/mg protein	0.82 ± 0.06^{b}	0.56 ± 0.02^{b}	1.18 ± 0.14^{ab}	1.51 ± 0.08^{ab}
Mn-SOD activity, a.u./mg protein	48.43 ± 2.06	44.91 ± 1.17	38.65 ± 1.31^{ab}	22.94 ± 2.10^{ab}
GPX activity, nmol/min/mg protein	24.31 ± 1.16^{b}	31.53 ± 1.50^{b}	26.67 ± 0.93^{b}	19.64 ± 1.55^{ab}
GSH level, nmol/mg protein	3.84 ± 0.28^{b}	5.01 ± 0.30^{b}	2.10 ± 0.27^{a}	2.49 ± 0.28^{a}

Note. Data are shown as mean ± SEM. $^{a}P < 0.05$ vs control rats of the corresponding sex; $^{b}P < 0.05$ vs HFD rats of the opposite sex

both enzymatic and non-enzymatic components of antioxidant protection. Statistical analysis revealed that Mn-SOD and GPX activities were significantly lower in the heart mitochondria of female rats compared with males ($P < 0.002$) (Table 2).

Mitochondrial respiration was determined in the heart tissue of experimental animals using succinate or glutamate/malate as substrates in both state 3 (stimulated by ADP) and state 4 (in the absence of ADP).

Our study has shown significant sex differences in mitochondrial function of control animals. Respiration rates and state 3 respiration that depend on the glutamate/malate oxidation pathway in isolated heart mitochondria were higher in control females in comparison with control males (Table 3).

Our results presented considerable impact of HFD as well as sex on the respiration indexes in the isolated heart mitochondria, induced by respiratory donors glutamate/malate. It was established that HFD suppressed stimulated respiration (state 3) and significantly decreased respiratory control in male heart mitochondria compared to control animals. At the same time, HFD-fed OVX females showed more substantial impairment of cardiomyocyte mitochondrial respiration, induced by glutamate/malate, that was confirmed by significant speed of unstimulated oxygen absorption up (state 4) and an almost twofold decrease in respiratory control index. Statistical analysis showed that V_4 was significantly higher in the isolated heart mitochondria of OVX female rats as compared with control females or HFD-fed males ($P < 0.002$) (Table 3).

It should be noted that neither respiratory control nor rate of oxygen absorption in states 3 and 4 with succinate as a donor in the heart mitochondria

T a b l e 3. Indexes of respiration, induced by malate/glutamate in isolated heart mitochondria of male and female rats, n = 6

Indexes	Control		HFD	
	males	females	males	OVX females
V_3, natoms O/min/mg protein	84.99 ± 4.61^b	108.67 ± 10.16^b	61.21 ± 3.12^{ab}	122.46 ± 9.87^b
V_4, natoms O/min/mg protein	14.11 ± 0.99	12.21 ± 0.88	14.44 ± 1.18^b	24.53 ± 1.43^{ab}
V_3/V_4	6.07 ± 0.20^b	8.90 ± 0.59^b	4.31 ± 0.21^a	4.99 ± 0.24^a

Note. Data are shown as mean ± SEM. $^aP < 0.05$ vs control rats of the corresponding sex; $^bP < 0.05$ vs HFD rats of the opposite sex

T a b l e 4. Indexes of respiration, induced by succinate in isolated heart mitochondria of male and female rats, n = 6

Indexes	Control		HFD	
	males	females	males	OVX females
V_3, natoms O/min/mg protein	177.23 ± 11.88	180.58 ± 19.18	178.34 ± 13.21	189.41 ± 11.71
V_4, natoms O/min/mg protein	76.85 ± 5.44	63.15 ± 7.70	86.05 ± 5.59	96.95 ± 7.01^a
V_3/V_4	2.35 ± 0.18	2.91 ± 0.13	2.07 ± 0.05	1.97 ± 0.03^a

Note. Data are shown as mean ± SEM. $^aP < 0.05$ vs control rats of the corresponding sex

T a b l e 5. Activity of cytochrome c-oxidase in isolated heart mitochondria of male and female rats, n = 6

Index	Control		HFD	
	males	females	males	OVX females
Cytochrome c-oxidase activity, μmol/min/mg protein	11.44 ± 0.24	11.01 ± 0.52	10.34 ± 0.59^b	7.44 ± 0.64^{ab}

Note. Data are shown as mean ± SEM. $^aP < 0.05$ vs control rats of the corresponding sex; $^bP < 0.05$ vs HFD rats of the opposite sex

of control animals were influenced by sex. However, metabolic disorders induced by HFD caused impairment of mitochondria respiration, depended on succinate, only in females with estrogen deficiency. HFD-fed OVX rats have shown an acceleration of state 4 respiration of cardiomyocyte mitochondria, which was accompanied by a decrease in respiration control (Table 4).

The activity of the component of electron transport chain (ETC) - cytochrome c-oxidase (complex IV) in the cardiomyocytes mitochondria did not differ between control male and female rats. HFD did not changed this index in male rats, however OVX females have exhibited significant decrease in activity of cytochrome c-oxidase in isolated heart mitochondria in comparison with control animals of the corresponding sex (Table 5).

Discussion

It is known that metabolic disorders induced by HFD may lead to oxidative stress in the mitochondria in different ways, in particular, due to the activation of NADPH oxidase, inhibition of aconitase and citrate overload [18].

In addition, *in vitro* studies have confirmed that high concentrations of fructose can cause mitochondrial dysfunction due to energy metabolism disturbance, suppression of mitochondrial enzymes, decline of mitochondrial membrane potential and disruption of ETC function. Consequently, oxidative stress and mitochondrial dysfunction may lead to an increase in reactive oxygen and nitrogen species and cause mitochondrial apoptosis [19, 20].

We revealed more pronounced lipid peroxidation in isolated heart mitochondria of control males in comparison with female rats. The causes of these variances could be explained by the regulatory role of sex hormones, particularly estrogens, in the processes of mitochondrial functioning. It is known that females heart mitochondria, compared to males, have a higher oxidative capacity and produce less ROS [21], due to estrogen-induced post-translational modifications of some mitochondrial proteins, which take part in maintaining the redox balance [8].

We established that HFD-induced metabolic abnormalities led to oxidative stress in the heart mitochondria in rats of both sexes, but OVX females showed more pronounced disturbances compared to males fed HFD, which could specify the key role of sex hormones in regulating the production of oxidant molecules.

It is well known that metabolic disorders, in particular insulin resistance, are associated with the development of oxidative stress in the mitochondria due to the increase in the generation of free radicals, as well as the activation of NADPH oxidase [22, 23]. The excess of ROS causes disruption of mitochondrial function, damage to the mitochondria and acceleration of their division, which leads to a decrease in oxidative phosphorylation and subsequent generation of ROS [24].

Estrogen deficiency is considered an additional factor that predisposes women to the development of oxidative stress. *In vivo* and *in vitro* experimental studies indicate that estrogens can deter the disturbance of redox homeostasis by preventing ROS hyperproduction and scavenging free radicals [25].

Our results confirmed the theory that the most remarkable difference between female and male mitochondria lies in antioxidant defense. Intact female rats' mitochondria were characterized by higher activity of antioxidant defense system in comparison with males. Estrogen receptors and their signaling cascades are implicated in the regulation of many antioxidant enzymes expression, including Mn-SOD, GPX, and glutathione reductase. As a result, estrogen deficiency makes females more vulnerable to disturbance of redox homeostasis due to more pronounced alterations of both the enzymatic and non-enzymatic components of the antioxidant system compared to males [26].

It is known that mitochondrial dysfunction in cardiomyocytes, which is associated with modifications in oxidative phosphorylation and respiration, is considered one of the leading mechanisms of CVD, such as cardiac hypertrophy and aging, heart failure, and ischemia [27, 28].

The present study reported marked sex differences among control animals regarding mitochondrial function in the heart. In fact, state 3 respiration was significantly higher in female cardiac tissue samples compared with that in male ones when the respiratory donor substrates glutamate and malate were used, whereas it was similar when succinate was used.

Our results correspond to the data of experimental studies, which showed no significant sex differences in the mitochondrial respiration in intact animals, with the exception in respiration stimulated by glutamate and malate [29, 30]. This can be connected with the impact of sex and sex hormones on cardiac mitochondrial functionality. It is known that cardiomyocytes of female rats contain fewer mitochondria but these mitochondria are more differentiated than male ones. Female mitochondria exhibit greater functional efficiency and more productive work of the respiratory chain, providing less electron accumulation by ETC complexes and consequent reduction in ROS production [31]. Despite a lower mitochondrial content in females compared to males, the mitochondrial oxidative capacity was shown to be similar in both sexes, probably due to higher phosphorylative efficiency in females, allowing them to retain the oxygen consumption rate at the same level as males [32].

Furthermore, estradiol regulates the transcription of genes of mitochondrial complexes I and IV, preventing the development of mitochondrial dysfunction in female rats, which may explain the more efficient cellular metabolism and mitochondrial respiration in cardiomyocytes of women compared to men [33].

In our study, metabolic abnormalities induced by HFD significantly decreased mitochondrial control of respiration, stimulated by glutamate/malate, in rats of both sexes. We found that HFD affected mitochondria respiration differently depending on sex: inhibited ADP-stimulated respiration (state 3) in males, while significantly speeded up the unstimulated oxygen absorption (state 4) in OVX females compared with control animals. In addition, acceleration of state 4 respiration, induced by succinate, and a decrease in respiration control were shown only in the heart mitochondria of OVX rats.

It is known that an increase in the rate of respiration can lead to the more intensive generation of ROS in the mitochondria, and our results are approved with more pronounced oxidative stress in HFD-fed OVX females compared to HFD-males (Table 2).

Data from clinical studies have shown a decrease in the mitochondria respiratory function, when using pyruvate/malate, in patients with dysglycemia and type 2 diabetes mellitus compared to ones without signs of these disturbances, which may indicate inhibition or damage of some mitochondrial pathways. Respiration in isolated mitochondria, induced by pyruvate/malate, can be caused by a complex interaction between the activity of the Krebs cycle, ETC, changes in the membrane potential, the functionality of ATP synthase, and the activity of adenine nucleotide translocase. It remains unclear which factor is responsible for the reduction of state 3 respiration in patients with type 2 diabetes mellitus compared with control subjects. Research data confirmed the association between long-term hyperglycemia and mitochondrial dysfunction induced by overproduction of ROS and attenuation of antioxidant protection that lead to oxidation of mitochondrial respiratory complexes proteins [34].

At the same time, it is known that insulin-resistant states are accompanied by a decrease in the number of mitochondria and the levels of mitochondrial enzymes, abnormal morphology of mitochondria and disruption of ATP synthesis both *in vivo* [35] and *ex vivo* in human muscle biopsies [36]. Several studies have shown a relationship between changes in mitochondrial morphology and mitochondrial dysfunction, in particular, a decrease in ATP production and impaired activation of mitochondrial ATP-dependent potassium channels in the heart [37, 38]. Thus, an increased number of morphologically abnormal mitochondria in myocardial tissue, observed in models of insulin resistance in rodents, was associated with the development of cardiac hypertrophy [39, 40].

It is known that estrogen deficiency can cause a disruption of the mitochondria structure and lead to the development of mitochondrial dysfunction due to the loss of the estradiol regulatory effect. The results of experimental studies indicate structural damage and an abnormal shape of mitochondria, a significant decrease in the content of mitochondrial ETC proteins, inhibition of oxidative phosphorylation, disruption of lipid and carbohydrate metabolism, as well as the development of oxidative stress and apoptosis of the mitochondria in OVX females' cardiomyocytes [33].

It can be supposed that specific sex-dependent changes in the respiration of isolated mitochondria when several energy substrates are used reflect differences in the expression of the complexes involved in the processes of respiration and oxidative phosphorylation in cardiomyocytes of HFD-fed experimental rats. It is known that the respiratory substrates malate and glutamate activate dehydrogenases with the following reduction of NADH, which donates electrons to complex I of ETC and through Q-cycle – to complex IV and final acceptor O2. Succinate is oxidized at the level of ETC complex II by succinate dehydrogenase, which is a FAD-dependent enzyme that directly transfers electrons to ubiquinone, bypassing the NADH-dependent complex I [41].

Thus, in HFD-fed male rats, respiration rates decreased when malate/glutamate as the sources were used and remained unchanged when succinate was added. We can suppose that the disruption of mitochondrial respiration occurs mainly at the level of ETC complex I.

However, the decline in respiratory rates in OVX females fed HFD, when using malate and glutamate, as well as succinate, indicates more significant disturbances of the respiratory function in cardiomyocyte mitochondria, in particular at the levels of ETC complex I and II that can be associated with metabolic disorders and estrogen deficiency.

This hypothesis is also supported by the unchanged activity of another component of ETL – cytochrome *c*-oxidase (complex IV) – in the heart mitochondria of HFD-fed male rats compared to control animals. In contrast to males, metabolic disorders induced by HFD and estrogen deficiency led to the inactivation of this enzyme in OVX females that point to the key role of sex hormones and their levels in the regulation of metabolism in the mitochondria.

The results of experimental studies indicate that mitochondrial dysfunction is accompanied by a decrease in the expression of mitochondrial proteins, which are encoded both by the nuclear (succinate dehydrogenase and pyruvate dehydrogenase) and mitochondrial genome, in particular cytochrome *c*-oxidase [40]. Addition of estradiol in vitro to the pituitary tumor cell line GH4C1 led to an increase in the expression of RNA for subunit II of cytochrome *c*-oxidase, demonstrating an important role of sex hormones in the regulation of mitochondrial function [33].

As a result of our study, it was established that metabolic disorders induced by HFD lead to inhibition of ATP-stimulated mitochondrial respiration in male rats when using glutamate and malate as a substrate, that is possibly due to dysregulation of ETC at complex I level. HFD did not change the activity of ETC complex IV – cytochrome c-oxidase in the mitochondria of cardiomyocytes of male animals compared to the control group.

At the same time, it was revealed that HFD causes more significant disturbances in the ETC of cardiomyocyte mitochondria of ovariectomized female rats, in particular at the complexes I, II and IV, that was evidenced by acceleration of unstimulated mitochondrial respiration, both when using malate, glutamate, and succinate, and also by a decrease in the activity of cytochrome *c*-oxidase.

Thus, HFD-induced MetS in our study has led to impairment of mitochondrial respiration in both rat sexes, but these disturbances were more pronounced in ovariectomized females, that may partially explain the greater cardiovascular risk in estrogen-deficient women.

Conclusions

1. We revealed that sex and estrogen sufficiency have a great impact on the development of oxidative stress and impairment of the mitochondrial respiration, induced by high-fructose diet in rats.

2. A high-fructose diet in females with estrogen deficiency caused a more intensive production of reactive oxygen species and a more significant inhibition of both enzymatic and non-enzymatic components of antioxidant defense compared to males.

3. It was established that metabolic disorders induced by high-fructose diet lead to dysregulation of mitochondrial respiration at the level of complex I electron transport chain in male rats.

4. High-fructose diet-fed females with estrogen deficiency have exhibited more pronounced impairment of electron transport chain, in particular at the levels of complex I, II and IV, compared to males that may partially explain the greater cardiovascular risk in women with low estrogen sufficiency.

5. The obtained data justify the need to develop new sex-specific prevention and treatment cardiovascular risk approaches aimed at maintaining the cardiomyocyte mitochondria functional state and pro-/antioxidative balance at the initial stages of dysglycemia.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. The study was conducted within the framework of the state budget theme "Determination of sex differences in cardiometabolic risk and justification of the efficiency of new preparations for its correction under the conditions of prediabetes" (state registration No. 0121U111554).

СТАТЕВІ ВІДМІННОСТІ В ДИХАННІ ТА ОКИСНО-ВІДНОВНОМУ ГОМЕОСТАЗІ МІТОХОНДРІЙ СЕРЦЯ У ЩУРІВ НА ДІЄТІ З ВИСОКИМ ВМІСТОМ ФРУКТОЗИ

О. Іванова[1], Н. Горбенко[1], О. Боріков[2], Т. Кіприч[1], К. Таран[1]

[1]ДУ «Інститут проблем ендокринної патології ім В. Я. Данилевського НАМН України», Харків, Україна;
[2]Харківський національний університет імені В. Н. Каразіна, Харків, Україна;
✉e-mail: ivolga3006@ukr.net

Статеві гормони відіграють провідну роль у статевому диморфізмі мітохондріальної дисфункції та окисного стресу, які асоціюються з метаболічним синдромом і розглядаються як можливі причини серцево-судинних захворювань. Метою роботи було визначення мітохондріального дихання та окисно-відновного гомеостазу у мітохондріях серця щурів, яких утримували на високофруктозній дієті (ВФД), в залежності від статі. Метаболічний синдром індукували у щурів Wistar надходженням фруктози (200 г/л) з питною водою протягом 8 тижнів. Експеримент проводили на 30 щурах, розділених на п'ять груп: контрольні самці, контрольні самиці, самці на ВФД, самиці на ВФД із інтактними яєчниками, оваріектомовані самиці на ВФД. В ізольованих мітохондріях серця визначали показники окисно-відновного гомеостазу, а також швидкість споживання кисню. Встановлено, що мітохондрії серця контрольних самиць мали нижчу інтенсивність перекисного окислення ліпідів, вищу активність системи антиоксидантного захисту та швидкість дихання у стані 3 порівняно з інтактними самцями. Показано, що ВФД індукує більш виразний окислювальний стрес за рахунок значного пригнічення ензимних та неензимних компонентів антиоксидантного захисту та більшу дисрегуляцію дихання в мітохондріях серця самиць із дефіцитом естрогенів порівняно з самцями. Отримані дані

можуть частково пояснити підвищений серцево-судинний ризик у жінок із дефіцитом естрогенів та обґрунтовують необхідність розробки нових напрямів профілактики та лікування серцево-судинних захворювань з урахуванням статі.

Ключові слова: мітохондрії серця, мітохондріальне дихання, оксидативний стрес, система антиоксидантного захисту, статеві відмінності, щури.

References

1. Alberti G, Zimmet P, Shaw J, Grundy SM. The IDF consensus worldwide definition of the metabolic syndrome. IDF. 2006; 24 p. Available at https://idf.org/media/uploads/2023/05/attachments-30.pdf.
2. Guembe MJ, Fernandez-Lazaro CI, Sayon-Orea C, Toledo E, Moreno-Iribas C, RIVANA Study Investigators. Risk for cardiovascular disease associated with metabolic syndrome and its components: a 13-year prospective study in the RIVANA cohort. *Cardiovasc Diabetol*. 2020; 19(1): 195.
3. Pucci G, Alcidi R, Tap L, Battista F, Mattace-Raso F, Schillaci G. Sex- and gender-related prevalence, cardiovascular risk and therapeutic approach in metabolic syndrome: A review of the literature. *Pharmacol Res*. 2017; 120: 34-42.
4. Barrett-Connor EL, Cohn BA, Wingard DL, Edelstein SL. Why is diabetes mellitus a stronger risk factor for fatal ischemic heart disease in women than in men? The Rancho Bernardo Study. *JAMA*. 1991; 265(5): 627-631.
5. Sergi G, Dianin M, Bertocco A, Zanforlini BM, Curreri C, Mazzochin M, Simons LA, Manzato E, Trevisan C. Gender differences in the impact of metabolic syndrome components on mortality in older people: A systematic review and meta-analysis. *Nutr Metab Cardiovasc Dis*. 2020; 30(9): 1452-1464.
6. Ramezankhani A, Azizi F, Hadaegh F. Gender differences in changes in metabolic syndrome status and its components and risk of cardiovascular disease: a longitudinal cohort study. *Cardiovasc Diabetol*. 2022; 21(1): 227.
7. Prasun P. Mitochondrial dysfunction in metabolic syndrome. *Biochim Biophys Acta Mol Basis Dis*. 2020; 1866(10): 165838.
8. Ventura-Clapier R, Piquereau J, Veksler V, Garnier A. Estrogens, Estrogen Receptors Effects on Cardiac and Skeletal Muscle Mitochondria. *Front Endocrinol (Lausanne)*. 2019; 10: 557.
9. Mamikutty N, Thent ZC, Sapri SR, Sahruddin NN, Mohd Yusof MR, Suhaimi FH. The establishment of metabolic syndrome model by induction of fructose drinking water in male Wistar rats. *Biomed Res Int*. 2014; 2014: 263897.
10. Lundholm L, Bryzgalova G, Gao H, Portwood N, Fält S, Berndt KD, Dicker A, Galuska D, Zierath JR, Gustafsson JA, Efendic S, Dahlman-Wright K, Khan A. The estrogen receptor α-selective agonist propyl pyrazole triol improves glucose tolerance in ob/ob mice: potential molecular mechanisms. *J Endocrinol*. 2019; 243(2): X1.
11. Chance B. Quantitative aspects of the control of oxygen utilization / transl. from engl. by Dorfman VA; ed. by Kaplansky SY. Regulation of cell metabolism. M.: Izd-vo inostr. lit-ry, 1962. P. 111-154. (In Russian).
12. Arutiunian AV, Dubinina EE, Zybina NN. Methods for assessing free radical oxidation and the body's antioxidant system. SPb.: IKF “Foliant”, 2000. 104 p. (In Russian).
13. Straus W. Colorimetric determination of cytochrome c oxidase by formation of a quinoedimonium pigment from dimethyl-p-phenylenediamine. *Biochim Biophys Acta*. 1956; 19(1): 58-65.
14. Putilina FE. Determination of reduced glutathione content in tissues / Prokhorova MI. Biochemical research methods (lipid and energy metabolism). L.: Izd-vo Leningr. un-ta, 1982. P. 183-187.
15. Ohkawa H, Ohishi N, Yagi K. Reaction of linoleic acid hydroperoxide with thiobarbituric acid. *J Lipid Res*. 1978; 19(8): 1053-1057.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72: 248-254.
17. Ivanova O, Gorbenko N, Borikov O, Kiprych T, Taran K, Plekhova E. The impact of sex on metabolic and functional abnormalities, induced by high-fructose-diet in rats. *Probl Endocr Pathol*. 2023; 80(3): 85-93.
18. Lanaspa MA, Sanchez-Lozada LG, Choi YJ, Cicerchi C, Kanbay M, Roncal-Jimenez CA, Ishimoto T, Li N, Marek G, Duranay M, Schreiner G, Rodriguez-Iturb B, Nakagawa T, Kang DH, Sautin YY, Johnson RJ. Uric acid induces hepatic steatosis by generation of

mitochondrial oxidative stress: potential role in fructose-dependent and -independent fatty liver. *J Biol Chem.* 2012; 287(48): 40732-40744.
19. Jaiswal N, Maurya CK, Arha D, Avisetti DR, Prathapan A, Raj PS, Raghu KG, Kalivendi SV, Tamrakar AK. Fructose induces mitochondrial dysfunction and triggers apoptosis in skeletal muscle cells by provoking oxidative stress. *Apoptosis.* 2015; 20(7): 930-947.
20. Lemos GO, Torrinhas RS, Waitzberg DL. Nutrients, Physical Activity, and Mitochondrial Dysfunction in the Setting of Metabolic Syndrome. *Nutrients.* 2023; 15(5): 1217.
21. De Oliveira MC, Campos-Shimada LB, Marçal-Natali MR, Ishii-Iwamoto EL, Salgueiro-Pagadigorria CL. A Long-term Estrogen Deficiency in Ovariectomized Mice is Associated with Disturbances in Fatty Acid Oxidation and Oxidative Stress. *Rev Bras Ginecol Obstet.* 2018; 40(5): 251-259.
22. Henriksen EJ, Diamond-Stanic MK, Marchionne EM. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free Radic Biol Med.* 2011; 51(5): 993-999.
23. Masenga SK, Kabwe LS, Chakulya M, Kirabo A. Mechanisms of Oxidative Stress in Metabolic Syndrome. Int J Mol Sci. 2023;24(9):7898.
24. Li A, Zheng N, Ding D. Mitochondrial abnormalities: a hub in metabolic syndrome-related cardiac dysfunction caused by oxidative stress. Heart Fail Rev. 2022;27(4):1387-1394.
25. Jia G, Aroor AR, Sowers JR. Estrogen and mitochondria function in cardiorenal metabolic syndrome. *Prog Mol Biol Transl Sci.* 2014; 127: 229-249.
26. Klinge MC. Estrogenic control of mitochondrial function and biogenesis. *J Cell Biochem.* 2008; 105(6): 1342-1351.
27. Liu M, Lv J, Pan Z, Wang D, Zhao L, Guo X. Mitochondrial dysfunction in heart failure and its therapeutic implications. *Front Cardiovasc Med.* 2022; 9: 945142.
28. Bisaccia G, Ricci F, Gallina S, Di Baldassarre A, Ghinassi B. Mitochondrial Dysfunction and Heart Disease: Critical Appraisal of an Overlooked Association. *Int J Mol Sci.* 2021; 22(2): 614.
29. Moulin M, Piquereau J, Mateo P, Fortin D, Rucker-Martin C, Gressette M, Lefebvre F, Gresikova M, Solgadi A, Veksler V, Garnier A, Ventura-Clapier R. Sexual dimorphism of doxorubicin-mediated cardiotoxicity: potential role of energy metabolism remodeling. *Circ Heart Fail.* 2015; 8(1): 98-108.
30. Ribeiro RF Jr, Ronconi KS, Morra EA, Do Val Lima PR, Porto ML, Vassallo DV, Figueiredo SG, Stefanon I. Sex differences in the regulation of spatially distinct cardiac mitochondrial subpopulations. *Mol Cell Biochem.* 2016; 419(1-2): 41-51.
31. Colom B, Oliver J, Roca P, Garcia-Palmer FJ. Caloric restriction and gender modulate cardiac muscle mitochondrial H_2O_2 production and oxidative damage. *Cardiovasc Res.* 2007; 74(3): 456-465.
32. Ventura-Clapier R, Moulin M, Piquereau J, Lemaire C, Mericskay M, Veksler V, Garnier A. Mitochondria: a central target for sex differences in pathologies. *Clin Sci (Lond).* 2017; 131(9): 803-822.
33. Mooga VP, White CR, Giordano-Mooga S. Estrogen and mitochondrial function in disease. Eds. Taskin E, Guven C, Sevgiler Y. Mitochondrial Diseases. IntechOpen, 2018. Ch. 18. Available at https://www.intechopen.com/chapters/58585.
34. Mogensen M, Sahlin K, Fernström M, Glintborg D, Vind BF, Beck-Nielsen H, Højlund K. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes.* 2007; 56(6): 1592-1599.
35. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med.* 2004; 350(7): 664-671.
36. Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol Endocrinol Metab.* 2000; 279(5): E1039-E1044.
37. Ozcan C, Bienengraeber M, Dzeja PP, Terzic A. Potassium channel openers protect cardiac mitochondria by attenuating oxidant stress at reoxygenation. *Am J Physiol Heart Circ Physiol.* 2002; 282(2): H531-H539.
38. Santos DL, Palmeira CM, Seiça R, Dias J, Mesquita J, Moreno AJ, Santos MS. Diabetes and mitochondrial oxidative stress: a study using heart mitochondria from the diabetic Goto-Kakizaki rat. *Mol Cell Biochem.* 2003; 246(1-2): 163-170.

39. Duncan JG, Fong JL, Medeiros DM, Finck BN, Kelly DP. Insulin-resistant heart exhibits a mitochondrial biogenic response driven by the peroxisome proliferator-activated receptor-alpha/PGC-1alpha gene regulatory pathway. *Circulation*. 2007; 115(7): 909-917.
40. Kim JA, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. *Circ Res.* 2008; 102(4): 401-414.
41. Chowdhury SR, Djordjevic J, Albensi BC, Fernyhough P. Simultaneous evaluation of substrate-dependent oxygen consumption rates and mitochondrial membrane potential by TMRM and safranin in cortical mitochondria. *Biosci Rep.* 2015; 36(1): e00286.