УДК 576.385.7:576.311.347:576.344:577.171.4
https://doi.org/10.20538/1682-0363-2024-2-123-132

The role of estrogens in mitochondrial metabolism

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ABSTRACT

Central organelles in cells are mitochondria, which are essential for many fundamental biological processes. In the course of evolution, mitochondria have been transformed into signaling centers in biological systems that can cause changes in the cell via secreted factors and affect physiology of humans and animals.

Along with performing many key functions for the cell, mitochondria have also evolved into active hubs that can both control cellular programs through interaction with other compartments, such as the endoplasmic reticulum, and affect tissues, determining the health of the body via mechanisms that we are only beginning to understand.

Keywords: estrogens, mitochondria, sex differences, malignant tumors

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

For citation: Kit O.I., Frantsiyants E.M., Shikhlyarova A.I., Neskubina I.V., Ilchenko S.A. The role of estrogens in mitochondrial metabolism. *Bulletin of Siberian Medicine.* 2024;23(2):123–132. https://doi.org/10.20538/1682-0363-2024-2-123-132.

Роль эстрогенов в метаболизме митохондрий

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РЕЗЮМЕ

В клетках центральными органеллами являются митохондрии, которые необходимы для многих фундаментальных биологических процессов. Митохондрии в процессе эволюции трансформировались в сигнальные узлы в биологических системах, способные вызывать изменения в клетке посредством секретируемых факторов и влияющие на физиологию человека и животных.

В дополнение к выполнению множества ключевых функций для клетки, митохондрии также превратились в активные центры, которые могут контролировать не только клеточные программы посредством взаимодействия с другими компартментами, например, с эндоплазматическим ретикулумом, но также воздействовать на ткани, определяя здоровье организма с помощью механизмов, которые мы только начинаем понимать.

Ключевые слова: эстрогены, митохондрии, половые различия, злокачественные новообразования

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Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Для цитирования: Кит О.И., Франциянц Е.М., Шихлярова А.И., Нескубина И.В., Ильченко С.А. Роль эстрогенов в метаболизме митохондрий. *Бюллетень сибирской медицины.* 2024;23(2):123–132. https://doi.org/10.20538/1682-0363-2024-2-123-132.

INTRODUCTION

Sex differences in human morbidity are partly due to the number of endogenous sex steroids that are involved in the regulation of mitochondrial metabolism. Although the mechanisms and targets by which estrogens directly or indirectly regulate mitochondrial function are not fully understood, it is clear that estradiol (E2) regulates the metabolism and morphology of mitochondria through nuclear and mitochondrial-mediated events, including stimulation of transcription factors that bind to genomic and mitochondrial DNA.

E2 and other estrogens, as well as synthetic GPER1 agonists, regulate mitochondrial bioenergetics, fusion, and division processes. Estrogens control the expression of genes, which, in turn, regulate mitochondrial functions, such as metabolism, OXPHOS, apoptosis, UPR^{mt}, division, and fusion. The mechanism of these events involves binding of E2 and other estrogens by receptors – the estrogen receptor α (ERα) and the estrogen receptor β (ERß) to regulate the transcription of nuclear genes and signaling cascades. In addition, estrogens activate protein G – a protein related to GTPases and functioning secondary intermediaries in intracellular signaling, coupled with the GPER1 receptor, which also regulates intracellular signaling events, including through cross–interaction with endothelial growth factor (EGFR) [1].

ESTROGENS AND THEIR FUNCTIONS IN THE BODY

Estrone (E1) and estriol (E3) were first isolated in 1930–1931 from the urine of pregnant women by Edward A. Doisy. E2 was later isolated by Dr. Doisy from the follicular fluid of pigs [2]. Subsequently, the metabolism of E2, its tissue-specific capture, cloning of ERα, as well as the discovery and cloning of ERß were described [1].

There are three primary estrogens – E1, E2, and E3. E2 is considered as the most active type because it has the highest affinity for ERα and is the dominant estrogen in women of reproductive age. Estrogens E1 and E3 have lower affinity for ERα. E2 is synthesized in the ovaries, whereas E1 is synthesized from androstenedione in the adrenal cortex, and E3 is mainly of placental origin, although each of them can be synthesized from androgenic precursors depending on tissue expression of the aromatase CYP19 [3].

Postmenopausal obese and overweight women have a higher level of circulating estrogens produced by adipose tissue compared to slim women [4]. Estrogens bind the ERα and ERß, which are conservative nuclear receptors (NR) with high identity in the DNA-binding and ligand-binding domains [5]. In addition to the full-sized ERα and ERß, each receptor subtype has a variety of splicing variants. ERα and ERß were identified in the mitochondria of various cell types, where they bind mtDNA. It was found that ERα also indirectly interacts with nuclear DNA through direct (protein : protein) coupling with other transcription factors associated with DNA [6].

Currently, there is a great interest in understanding sex differences in the disease in order to personalize treatment. The National Institutes of Health (NIH) mandates each grant application to consider “gender as a biological variable.” The list of diseases showing sex differences is too long and includes, for example, hypertension [7], ischemic stroke and myocardial infarction [8], as well as neurodegenerative and neuropsychiatric diseases [9].

Each of these diseases is associated with mitochondrial dysfunction. Differences in the prevalence of diseases that are associated with higher E2 levels in premenopausal women include type 1 and type 2 diabetes (T1DM1 and T2DM). Premenopausal women have lower incidence of metabolic disorders, while postmenopausal women are more likely to develop diabetes mellitus, cardiovascular diseases, and kidney diseases than men [10].

Mitochondrial dysfunction is involved in many diseases, while it is known that defects in hundreds

of genes involved in the mitochondrial biology cause pathologies [11]. These defects cover mutations in the mitochondrial genome itself, in nuclear genes encoding mitochondrial components, and in genes belonging to various functional classes affecting the mechanism of mtDNA replication, mitochondrial division and fusion, oxidative phosphorylation (OXPHOS) or biosynthesis of iron – sulfur clusters [12, 13]. Mitochondrial dysfunction is associated with insulin resistance [14] and multiple endocrine disorders [15]. The existence of sex differences depends not only on estrogens and androgens, but also on genes encoded by sex chromosomes [16].

Experimental and clinical studies found that E2 increases fat oxidation, inhibits lipogenesis, and regulates immune system cells, such as B cells, T cells, natural killer (NK) cells, neutrophils, and macrophages [17]. More than 75% of autoimmune diseases are more common in women. Recent studies have shown a direct relationship between the expression of ERα in T cells in the development of T-cell-dependent colitis in mice and a decrease in T cell proliferation [18]. The role of estrogens and ERα in systemic autoimmune diseases, where B cells and T cells are affected, has been described [19].

Hepatocellular carcinoma (HCC) is more common in men than in women, since estrogens have a protective effect against the emergence and progression of HCC [20]. Sex-dependent differences in liver metabolism cover the expression of cytochrome P450 liver enzymes, as well as transcription factors (TF), including ERα, the aryl hydrocarbon receptor (AHR), the peroxisome proliferator-activated receptor (PPAR) α, and the farnesoid X receptor (FXR), leading to differences in drug responses and metabolism in men and women [21].

Non-alcoholic fatty liver disease (NAFLD) is more common in men than in premenopausal women, but increases in postmenopausal women [22]. With drug-induced liver injury (DILI), sex-dependent differences also manifest themselves: 41 drugs affect the liver, and DILI is detected predominantly in women (only in premenopausal women). Interestingly, drugs for the treatment of DILI in women have a more pronounced effect on mitochondria, which is associated with the formation of reactive metabolites and a greater potential for inhibition of mitochondrial transporters [23]. The model of immune-mediated DILI in BALB/c mice showed that the production of proinflammatory hepatic cytokines (interleukin (IL)-6) in females was higher than in males, and hepatitis in male mice was more severe. This fact suggests that E2 and IL-6 may be responsible for a decrease in the protective and regulatory function of T cells [24].

There are three factors that emphasize the deep-rooted biological links between mitochondria and gender as a biological trait. Firstly, mammalian mitochondria are inherited through the maternal lineage, which means that they are transmitted exclusively through the egg. In the course of animal experiments, it was suggested that transfer of mitochondria to female and male organisms has different effects on metabolism and life expectancy [25].

Secondly, it is an underestimated fact that the stage limiting the rate of synthesis of all sex hormones, including estrogens, progestins, and testosterone, occurs in mitochondria, located mainly in the ovaries and testes [26]. The first enzymatic stages of the synthesis of all steroids, which also include glucocorticoids and mineralocorticoids, occur in the mitochondrial matrix [27].

Thirdly, mitochondria contain receptors for sex hormones. Both ERß receptors and androgen receptor (AR) move into the mitochondrial matrix, where they interact with mtDNA and affect many areas of the mitochondrial biology [28, 29]. Thus, the mitochondria of the genital organs have such a molecular mechanism that contributes to the development of and canonical mechanisms for hormone perception of sexual differentiation.

At the same time, A. Junker et al. (2022) [30] showed that stable binary sex-dependent differences were determined in greater mitochondrial content in female urine and isolated leukocyte subpopulations and higher ROS production in male skeletal muscles. Other measurements showed inconsistent sex-dependent differences with large discrepancies in the strength and direction of research, experimental conditions (for example, metabolic substrates), and evaluated tissues.

MITOCHONDRIAL HORMONE-REGULATING FUNCTIONS

Mitochondria are tightly packed dynamic organelles of bacterial and endosymbiotic origin [31]. Mitochondria support life by converting metabolites of food fuel into ATP, CO_2, and H_2O, while releasing heat and providing adaptation to stress for survival. The origin of mtDNA from oocytes leads to hereditary disorders that are transmitted through the maternal

lineage [31]. Paternal mitochondrial transmission is extremely rare [32].

Mitochondria contain their own DNA of the mitochondrial genome in the matrix. The mitochondrial genome is inherited through the maternal lineage and exists in the form of circular double-stranded DNA consisting of 16,569 base pairs in humans [33]. Since the mitochondrial genome encodes a small number of mitochondrial genes, including transport RNAs, mitochondrial ribosomal RNAs, and protein subunits of complexes with an electron transport chain, many mitochondrial genes are encoded in the nucleus. Thus, coordination of transcription events between mitochondria and the nucleus is necessary to maintain metabolic homeostasis [34].

During ATP production, electron transport also generates reactive oxygen species (ROS), which damage macromolecules, including mtDNA, proteins, and lipids. Estrogens and androgens protect mitochondria from the degenerative effects of aging in a tissue-specific way through activation of the corresponding receptors [35]. ROS contributes to mitochondrial stress and abnormal protein conformation. Misfolded proteins and aggregates accumulate in the inner membrane space (IMS) and the mitochondrial matrix, which leads to activation of the mitochondrial unfolded protein response (UPR ^{mt}).

Regulation of E2 – ERα by means of UPR ^{mt} is known. Recent studies show that breast cancer cells co-opt mitohormesis, the process of increasing basal UPR^{mt} and reducing oxidative stress, leading to increased invasion and metastasis, and, therefore, to worse survival of breast cancer patients with the UPR^{mt} gene signature [36]. E2 – ERα increases the transcription of sirtuin 3 (SIRT3) localized in mitochondria, where it weakens ROS by deacetylation of manganese superoxide dismutase (MnSOD, SOD2) and interacts with forkhead box protein O3 (FOXO3A) to activate its translocation into the nucleus. Next, the expression of genes encoding PGC-1α, a coregulator necessary for transcription, and MnSOD takes place [37].

REGULATION OF APOPTOSIS IN MITOCHONDRIA BY HORMONES

Mitochondria not only produce energy, but are also the site of synthesis of all steroid hormones, including E2 in granulosa cells of the ovaries [38, 39]. The participation of steroid hormones in the regulation of apoptosis has been established, so E2 is able to inhibit apoptosis via a variety of mechanisms. Under mitochondrial stress, mitochondria also produce and secrete mitokines, for example, humanin, a stress-sensitive peptide encoded by the *MT-RNR2* gene in mtDNA, and fibroblast growth factor 21 (FGF-21), which regulates energy metabolism [40]. Estrogen deficiency disrupts regulation of the L-type Ca2+ channel, the ryanodine receptor, the sarcoplasmic / endoplasmic reticulum Ca2+-ATPase (SERCA), and the Na+ – Ca2+ exchanger, causing impairment of Ca2+ homeostasis, which leads to cardiovascular diseases [41].

Another recent review summarized sex-dependent differences in human skeletal muscles [42]. It was found that many mitochondrial functional genes are expressed differently, and this correlates with known inter-sex differences in the composition of muscle fibers in women with a higher percentage of type I muscle fibers with a more oxidative phenotype [1]. Differences in gene expression in skeletal muscles of men and women are partly due to epigenetic changes, including differences in DNA methylation, histone modifications, and microRNA expression [42].

Activation of ER and G-protein-coupled estrogen receptor (GPER) preserves mitochondrial function and reduces mitophagy after injury (ischemia / reperfusion) by signaling dependent on mitochondrial permeability and activation of mitogen-activated protein kinase (MEK) regulated by extracellular signal-regulated kinase (ERK), thus reducing apoptosis by preservation of the mitochondrial integrity. In this regard, the administration of estrogen in *in vivo* models before ischemia / reperfusion reduces the infarct size and improves myocardial contractility [43].

Sex-dependent differences in mitochondria and mitochondrial function in various organs were considered, mainly in rodents [44-46]. A recent study reported that the effect of gender on gene expression and mitochondrial metabolism in adipose tissue depended on the mouse lineage when studying 100 inbred mouse lines [47].

The role of mitochondrial ERα and ERß in the transcription of mtDNA genes and the function of mitochondria depend on the type of cells, which is consistent with their specific localization. A group of researchers found that retrograde signaling via activation of the ROS-AKT pathway in response to UPR^{mt} activates ERα and increases nuclear respiratory factor-1 (NRF-1) signaling [48]. However, there are still many unresolved questions about the protective effects of estrogens in mitochondria that have yet to be fully elucidated.

THE ROLE OF ESTROGENS IN THE REGULATION OF LPO – ANTIOXIDANTS SYSTEM

Mitochondrial metabolism inevitably leads to the formation of ROS, which, in turn, cause mitochondrial dysfunction. E2 is known to cause a decrease in ROS levels and increase the amount of antioxidant proteins, including superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD2) and glutathione peroxidase (GPx) [49]. On the other hand, in the vascular network, GPER modulates ROS by reducing NADPH oxidase 4 (NOX4), prostaglandin – endoperoxide synthase 2 (PTGS2), and GPx1, as well as by increasing the amount of antioxidant proteins, such as SIRT3 and glutathione S-transferase Kappa 1 (GSTK1) [49]. Consequently, as described in several studies, women differ from men in the level of antioxidants localized in the mitochondria, thus producing fewer free radicals and, in turn, less oxidative damage to the heart [50].

In this regard, some studies reported that female mitochondria produce half as much hydrogen peroxide as male ones and have higher levels of mitochondrial reduced glutathione. However, the mechanism through which E2 exerts these effects, as well as the involvement of other cell organelles have not yet been fully elucidated [51].

Another interesting feature that may be related to the modulation of ROS is the participation of E2 in the regulation of Ca2+ levels. Two studies showed that in female OVX mice, mitochondria had a reduced ability to retain Ca2+, which was restored after the introduction of E2, thus improving normal contraction and relaxation of the heart [41]. Similarly, several studies found that the regulation of mitochondrial homeostasis was of key importance for attenuating the damaging effects of various pathological processes in cardiovascular diseases. Certain proteins, such as the peroxisome proliferator-activated receptor-γ coactivator 1 α (PGC-1α), AMP-activated protein kinase (AMPK), and several genes involved in the electron transport chain (ETC), are regulated by sex hormones and, more specifically, by estrogen signaling [49].

ERα and ERß have been identified in mitochondria and reported to regulate mtDNA transcription [6]. E2 increases the transmission of redox signals in MCF-7 breast cancer cells containing ERα. This process is considered as part of the oncogenic process in breast cancer and involves the activation of AKT signaling, which leads to the initiation of NRF-1 [52]. E2 rapidly increased the temporal localization of ERα in mitochondria in MCF-7 cells and stimulated the direct ERα – MnSOD interaction, which was found using confocal imaging and co-immunoprecipitation [53]. The mitochondrial localization of ERα and the ERα – MnSOD interaction were blocked by fulvestrant, which suggested the importance of ERα conformation for the described interactions. Induced migration of ERα into mitochondria in MCF-7 cells is considered as a non-genomic E2 response to increased MnSOD acetylation of K68, which leads to inhibition of MnSOD activity. It was reported that the E2 – ERα – MnSOD bond blocks the MnSOD – SIRT3 interaction, increasing the superoxide level and activating mTORC2 [53].

Mitochondria contain NR – thyroid hormone receptor (TR), androgen receptor (AR), retinoid X receptor (RXR), RAR, glucocorticoid receptor (GR), and gamma receptor activated by the peroxisome proliferator-activated receptor (PPARG, PPARy2) [54]. ERß was identified in the mitochondria of the human heart. A recent study reported that low levels of mitochondrial ERß (mitoERß) were associated with an increased risk of breast cancer recurrence [55]. Transfection of MCF-7 breast cancer cells using GST-ERß followed by GST pull-down identified HSPA9 (mitochondrial heat shock protein 70; also called GRP75) associated with ERß.

MALDI-TOF mass spectrometry identified ERß and HSPA9 in the purified complex, and knockdown and overexpression studies showed that HSPA9 moved ERß into the mitochondria of MCF-7. Transfection of triple-negative breast cancer (TNBC) cells MDA-MB-231 by mitochondria-directed ERß expression vector reduced cell proliferation, invasion, and migration *in vitro* and tumor formation *in vivo*. A higher level of ERß was determined in mitochondrial fractions from ectopic endometrial tissues compared to uterine fibroids or controls without lesions [56]. Given the uncertainty regarding the specificity of some antibodies to ERß [57], further studies on the localization and activity of ERß in mitochondria are required.

The naturally occurring variants of ERα splicing, ERα36 and ERα46, are the result of the use of a differential promoter and splicing, which leads to shortened forms of ERα devoid of N-terminal domains A and B, which make up AF-1. ERα36 also lacks the F-domain at the C-end of the full-size ERα66 and has a shortened LBD [58]. It was reported that ERα 36 is localized mainly in the mitochondria of the human

uterine leiomyoma (UtLM) and smooth muscle cell lines and interacts with inhibin (PHB) [59].

B.N. Radde et al. reported that E2 (10 nM) stimulated the baseline oxygen consumption rate (OCR) and baseline extracellular acidification rate (ECAR) in breast cancer cells MCF-7 and T47D lumen A (ERa+) and activated ATP-bound OCR, while not affecting the maximum mitochondrial reserve capacity. The authors suggested that E2 did not affect the tolerance to cellular stress in these cell lines [60]. Medroxyprogesterone acetate (MPA) inhibited the potentiation of E2 primary neurons of the rat hippocampus and mitochondrial reserve capacity of glial respiration *in vitro*, however, the mechanisms of this phenomenon have not been disclosed. In the meantime, a recent study showed that ERα knockout in CD4+ T cells reduced mitochondrial reserve capacity, and it was assumed that ERα regulated mitochondrial metabolism in T cells [18].

A search in PubMed for articles investigating the effect of estrogens on mitochondrial bioenergetics revealed relatively few results. Thus, one group of researchers found that overexpression of ERα in SK-N-BE(2) MYCN-amplified (MNA) neuroblastoma (NB) cells suppressed the growth of a tumor xenograft, blocking many processes associated with oncogenesis of NB [61].Glycolysis (measured as ECAR in the Seahorse bioanalyzer), maximum glycolytic capacity, and glycolytic reserve were significantly reduced in cells overexpressing ERα, and treatment with E2 and nerve growth factor had no additional effect on any of these parameters in NB cells [61].

Similarly, the baseline level of OCR, ATP-bound OCR, and mitochondrial bioenergetic reserve capacity were increased in ERα cells overexpressing SK-N-BE(2) MNA NB, which was partially mediated by suppression of fatty acid utilization. Overexpression of the ERß-labeled mitochondrial-targeting sequence in primary human endometrioid cells increased basal OCR and mitochondrial reserve capacity. ERß knockdown reduced the expression of NRF1, TFAM, MT-CO1, and MT-ATP6 transcripts in endometrioid cells and increased the anti-apoptotic protein BCL-2, thereby helping cells avoid mitochondrial apoptosis caused by oxidative stress [56].

Studies on female mice with muscle-specific Esr1 (ERα) ERα (MERKO) knockout showed that glucose homeostasis was impaired in mice of this line, and obesity was present in combination with aberrant mitochondrial morphology, increased ROS, impaired mitochondrial division, and an imbalance of calcium and ATP production. These data indicate a key role of the mitochondrial function of ERα in muscles. It was shown that the level of ERα was reduced in the muscles of women with metabolic syndrome. Transmission electron microscopy revealed elongated hyperfused mitochondria with an increased content of inactive diamine-related protein 1 (DRP1) phosphorylated by the inhibitory serine residue (SER 637).

The authors also observed an increase in regulator of calcineurin 1 (Rcan1) and calcineurin inhibitor leading to mitophagy disorders and increased ROS, which causes inflammation and insulin resistance [62]. The E2 – ERα bond was also necessary to maintain the number of satellite cells (muscle stem cells) in the muscles of female rodents and humans [63]. Indeed, in mice with ERα knockout (Esr1 -/-), a decrease in fatty acid oxidation in muscles, increased overall obesity, impaired mtDNA replication, mitophagy, and autophagy, failure of insulin signaling (including glucose utilization), high levels of H_2O_2 and superoxide, lipid accumulation, and inflammation were recorded. The authors noted the importance of identifying methods and selecting therapeutic agents for modulation of tissue-specific pathways regulated by ERα, which will adjust the energy balance and glucose homeostasis, especially in postmenopausal women [64].

Replacement therapy using E2 in female mice after ovariectomy improved the activity of mitochondrial complex I (CI) and decreed H_2O_2 in skeletal muscles, but increased CI-mediated H_2O_2 production and decreased the intensity of OXPHOS in the liver. The authors stated that "the mechanism(s) of tissue specificity of E2 effect on mitochondrial function remains unknown" [65]. At the same time, transcriptome profiling revealed microRNAs controlling glycolysis and oxidative metabolism in the muscle fibers of male mice [66]. The role of estrogens in the regulation of these microRNAs is still known.

Interestingly, studies examining miRNAs in skeletal muscles of homozygous twins with discordant use of hormone replacement therapy (HRT) revealed miR-182, miR-233, and miR-142-3p targeting IGF-R1, FOXO3A, and inflammatory signaling [67]. In another study, this group of scientists also identified E2 regulation of muscle energy pathways in women receiving HRT [68].

In mice with the ERα – MERKO knockout, a change in the morphology of mitochondria was demonstrated, and mitochondrial elongation occurred. In addition, impairment of mitochondrial division by pronounced

suppression of signal transmission took place. In fact, ERα deficiency leads to suppressed phosphorylation of DRP1, a key factor in mitochondrial division. That is, the mitochondrial dysfunction phenotype in muscles prevails in MERKO mice. Obviously, ERα is necessary to maintain mitochondrial function and protects against mitochondrial-related health disorders in women [69].

In vitro experiments using molecular methods determined that E2 increased the levels of mRNA transcripts MFN1, MFN2, OPA1, and DRP1, while reducing FIS1 during 4-hour treatment of MCF-7 cells, and these transcriptional responses were inhibited by antiestrogenic fulvestrant (ICI 182.780). The authors reported that E2 induced mitochondrial fusion in MCF-7 cells, reduced the expression of OXPHOS complex proteins, and increased ATP levels.

Similar results were recorded in T47D cells treated with E2. In addition, overexpression of ERß in T47D cells was found to increase the number of OXPHOS complex proteins and reduce division, while increasing fusion [70]. On the contrary, activation of the E2 – Erα pathway in MCF-7 cells increased phosphorylation of DRP1 at ser616 to induce DRP1 activity leading to mitochondrial division [71]. There was a need for ERα, since knockdown blocked E2 and induced phosphorylation of DRP1, but the authors did not evaluate whether this was mediated by genomic or non-genomic activation.

From the standpoint of aging, it was established that E2 protects against cellular aging and mitochondrial dysfunction. This fact was revealed in experiments using human umbilical vein cells and vascular smooth muscle cells in female C57BL/6 mice [72]. The ability of E2 to increase mitochondrial autophagy and maintain mitochondrial function, thereby slowing down aging is known. However, E2 does not modulate the microtubule-associated protein 1 light chain 3 (LC3), as well as the deficiency of the autophagy-related protein 7 (ATG7). Moreover, E2-mediated effects on mitochondrial autophagy were eliminated using either Unc-51-like kinase-1 (Ulk-1) or Ras-related protein Rab-9 (Rab9).

These results showed that E2-mediated mitochondrial autophagy is associated with Rab9-dependent alternative autophagy. In addition, E2 enhances the regulation of sirtuin 1 (SIRT1) and activates liver kinase B1 (LKB1), AMPK, and Ulk1, which indicates that the effect of E2 on the induction of Rab9-dependent alternative autophagy is mediated by the SIRT1/LKB1/AMPK/Ulk1 pathway. Compared with sham-operated mice, mice with ovariectomy (OVX) are characterized by reduced mitochondrial autophagy, increased mitochondrial dysfunction, and aging of the arteries, all of which was successfully blocked by E2 [72].

CONCLUSION

Mitochondria are organelles inherited through the maternal lineage, which have the most important tissue-specific functions, including hormone synthesis and energy production, affecting human development, health, and aging. However, it still remains unclear whether mitochondria of women and men are characterized by stable biological differences, which is a serious gap in knowledge. Solving this issue is of paramount importance for the development of clinically specific indicators of mitochondrial biology and the construction of comprehensive human health models that include mitochondrial bioenergetics.

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Received 17.08.2023;
approved after peer review 10.10.2023;
accepted 02.11.2023