Impact of Aging on Mitochondrial Respiration in Various Organs

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Summary

Mitochondria are considered central regulator of the aging process; however, majority of studies dealing with the impact of age on mitochondrial oxygen consumption focused on skeletal muscle concluding (although not uniformly) a general declining trend with advancing age. In addition, gender related differences in mitochondrial respiration have not been satisfactorily described yet. The aim of the present study was to evaluate mitochondrial oxygen consumption in various organs of aging male and female Fischer 344 rats at the ages of 6, 12 and 24 months. Mitochondrial respiration of homogenized (skeletal muscle, left and right heart ventricle, hippocampus, cerebellum, kidney cortex), gently mechanically permeabilized (liver) tissue or intact cells (platelets) was determined using high-resolution respirometry (oxygraphs O2k, Oroboros, Austria). The pattern of age-related changes differed in each tissue: in the skeletal muscle and kidney cortex of both sexes and in female heart, parameters of mitochondrial respiration significantly declined with age. Resting respiration of intact platelets displayed an increasing trend and it did not correlate with skeletal muscle respiratory states. In the heart of male rats and brain tissues of both sexes, respiratory states remained relatively stable over analyzed age categories with few exceptions of lower mitochondrial oxygen consumption at the age of 24 months. In the liver, OXPHOS capacity was higher in females than in males with either no difference between the ages of 6 and 24 months or even significant increase at the age of 24 months in the male rats. In conclusion, the results of our study indicate that the concept of general pattern of age-dependent decline in mitochondrial oxygen consumption across different organs and tissues could be misleading. Also, the statement of higher mitochondrial respiration in females seems to be conflicting, since the gender-related differences may vary with the tissue studied, combination of substrates used and might be better detectable at younger ages than in old animals.

Key words

Aging • Mitochondria • Gender differences • High-resolution respirometry

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Introduction

Aging is a complex process affected by both intrinsic and extrinsic factors, like genetic background, metabolic fitness, age-related diseases, nutrition, lifestyle or mobility [1]. There is ample evidence that mitochondria play an important role in the aging process [2-4]. The function of the electron transport system, located in the inner mitochondrial membrane is an important subject of the aging research not only as a source of high-energy substrates for the cellular metabolic processes but also as a major reactive oxygen species (ROS) producer. On the one hand, mitochondrial theory of aging suggests that the organelle is both the source and the target of harmful and aging promoting ROS [5]. On the other hand, ROS have an indispensable role in the cellular signalling and some studies even indicate a positive correlation between higher ROS and longevity [6].

Although most studies dealing with age-related mitochondrial oxygen consumption in rodents state that the efficiency of oxidative phosphorylation decreases with age [7,8], they are mainly based on analysis of the skeletal muscle mitochondria concluding that alterations in bioenergetics situation in different tissues follow a common general pattern. However, the results of research performed on other organs and tissues suggested that the dysfunction of mitochondrial electron transporting system might not be an automatic process associated with aging [9]. In addition, studies dealing with analysis of mitochondrial bioenergetics in more than two organs are extremely rare and they mostly focus on the male population only [10-12]. Tissue-specific morphology and function of mitochondria, method of sample preparation (isolated mitochondria, permeabilized or homogenized tissue), differences in methodology (spectrophotometry, oxygraphy, different media and substrates), species, gender and age of experimental animals used, represent the factors contributing to difficulties in comparison of results of different studies [13] and make the conclusion about the overall decline of mitochondrial respiration with age problematic.

Over the last decade, respirometric evaluation of platelets has become popular since they are easily available in humans and some studies suggested that their respiratory activity might reflect mitochondrial fitness of some metabolically active organs like heart, liver and brain [14]. In our recent paper, we described a decrease in routine and uncoupled respiration of aged human platelets [15]. Other studies reported significant defects in complex I [16]. The data on the impact of aging on platelet mitochondrial oxygen consumption in rodents are not available yet.

In the present study, we decided to perform a complex respirometric analysis of various organs and tissues (left and right heart ventricle, hippocampus, cerebellum, skeletal muscle, cortex of the kidney, liver, and platelets) in 6, 12 and 24 months old sedentary Fischer 344 male and female rats. The aims were not only to make the comparison of the impact of aging on mitochondrial activity across various organs possible, but also to reveal potential sex-related differences in more age categories.

Materials and Methods

Solutions and chemicals

The composition of solutions used in the experiments was: BIOPS preservation solution – CaK_2EGTA 2.77 mmol/l, K_2EGTA 7.23 mmol/l, imidazole 20 mmol/l, taurine 20 mmol/l, 50 mmol/l, MES hydrate, 50 mmol/l dithiothreitol, $MgCl_2 \cdot 6H_2O$ 6.56 mmol/l, Na_2ATP 5.77 mmol/l, Na_2phosphocreatine 15 mmol/l, pH 7.1; MIR05 respiration medium: EGTA 0.5 mmol/l, $MgCl_2 \cdot 6H_2O$ 3 mmol/l, potassium lactobionate 60 mmol/l, taurine 20 mmol/l, KH_2PO_4 10 mmol/l, HEPES 20 mmol/l, sucrose 110 mmol/l, fatty acid free bovine serum albumin 1 g/l, pH 7.0 [17]; PBSG medium: $Na_2HPO_4 \cdot 7H_20$ 7.5 mmol/l, $NaH_2PO_4 \cdot H_20$ 2.5 mmol/l, NaCl 137 mmol/l, KCl 2.7 mmol/l, glucose 10 mmol/l; procaine solution: procaine hydrochloride 86 mmol/l and NaCl 34 mmol/l. If not stated otherwise, chemicals were from Sigma-Aldrich (St. Louis, MO, United States). All chemicals were of analytical grade.

Animals

For experiments, we used Fischer 344 rats obtained from local breeding colony at the Faculty of Medicine in Pilsen, Charles University. Animals were divided into 6-, 12- and 24-month-old cohorts. The rats were housed in pairs on wood chip bedding at 23±2 °C under 12-hr light/dark cycles and provided with food and water ad libitum. All the experiments were performed in accordance with the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU) and were approved by the Animal Welfare Advisory Committee at the Faculty of Medicine in Pilsen and by the Ministry of Education, Youth and Sports of the Czech Republic (approval ID: MSMT 19751/2020-2).

Experimental protocols

At the day of experiment, the animals were weighed and anaesthetized with intraperitoneal urethane (1 g/kg body weight). Then, the rats were sacrificed by decapitation; organs of interest (hippocampus, cerebellum, heart, kidney, liver, skeletal muscle) were quickly excised and kept on ice in the preservation solution BIOPS. For subsequent respirometric analyses, the tissue samples (muscle, heart, kidney cortex, brain) were homogenized by PBI-Shredder O2k-Set (Oroboros instruments; Innsbruck Austria) or gently mechanically permeabilized with a pair of sharp forceps (liver). Samples were taken from the following locations: mixed red and white fibers from the soleus muscle, proximal anterior free wall of the right and left ventricles, lobus hepatis dexter medialis (liver), cortex of the kidney and

cerebellum and the whole hippocampus. Whole blood collected after cervical dislocation was mixed with 100 µl K_2EDTA (75 mmol/l) and prostaglandin E1 (62.5 ng/ml) to prevent blood clotting and activation of platelets during the procedure. Platelets were isolated from platelet-rich plasma (PRP) obtained by centrifugation at 300× g for 20 min at 25 °C with no brake. PRP was then centrifuged again at 1500× g for 10 min at 25 °C and the platelet pellet was resuspended in the final medium consisting of 50 % of PBSG and 50 % of rat's own plasma. Platelets were counted in Bürker's chamber after washing in the procaine solution for 10 min. Final platelet concentration was adjusted to 150-350×10^9/l.

Evaluation of mitochondrial respiration using high-resolution respirometry (HRR; O2k)

Mitochondrial respiration of the tissue samples (mass per chamber: heart ventricles 0.8 mg; hippocampus 3 mg; cerebellum 2.5 mg; skeletal muscle 3 mg; kidney cortex 1 mg; liver 1.5 mg; results were then normalized to tissue wet mass) was analyzed in 4 calibrated O2k oxygraphs (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria) in the MiR05 medium at 37 °C using the following substrate-uncoupler-inhibitor-titration protocols (SUIT) [17] for the heart, muscle, kidney, and liver samples – malate (M; 0.1 mmol/l) and palmitoyl-L-carnitine (Pcar; 0.04 mmol/l) – adenosine diphosphate (D; 5 mmol/l) – cytochrome c (c; 10 µmol/l) – glutamate (G; 10 mmol/l) – pyruvate (P; 5 mmol/l) – succinate (S; 50 mmol/l) – carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP; 0.5 µmol/l titrations) – rotenone (Rot; 0.5 µmol/l) – antimycin A (Ama; 2.5 µg/ml) – N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD; 0.5 mmol/l) in the presence of ascorbate (Asc; 2 mmol/l) – sodium azide (Azd; 100 mmol/l). The respiratory states induced by individual titrations started with non-phosphorylating leak state when metabolism of fatty acids was activated after addition of M and Pcar (L_{FAO}), continued with active phosphorylating respiration after D and c (OXPHOS-FAO; P_{FAO}), coupled phosphorylating respirations after injection of G and P providing electrons to Complex I (OXPHOS-FAO+I; P_{FAO+I}) and S as a substrate of Complex II (OXPHOS-FAO+I+II; $P_{FAO+I+II}$). FCCP titrations induced the uncoupled state providing the information about the capacity of the electron-transporting system (ETSC-FAO+I+II; $E_{FAO+I+II}$); after injection of the Complex I inhibitor Rot, oxygen consumption was reduced to the Complex II only (ETSC-II; E_{II}); Complex III inhibitor Ama allowed to measure residual oxygen consumption (ROX), and TMPD/Asc after subtraction of Azd oxygen consumption enabled the estimation of the capacity of Complex IV (C_{IV}). For the brain samples (hippocampus and cerebellum), the following SUIT protocol was used: M – G (L_I) – D – c – P (P_I) – S (P_{I+II}) – FCCP (E_{I+II}) – Rot (E_{II}) – Ama (ROX) – Asc/TMPD – Azd (C_{IV}). Oxygen consumption of the intact platelets was measured in 50 % of PBSG and 50 % of rat's own plasma without no addition (routine respiration; ROUT; R), after ATP-synthase inhibitor oligomycin (Omy; 2 µg/ml) injection (L), FCCP titrations (ETSC; E), and additions of Rot and Ama (ROX).

Data presentation and statistics

Data are presented as means ± SD with 10 to 90 percentile ranges. Normality of distribution was tested using Smirnov-Kolmogorov test and, if needed, the data were processed by logarithmic transformation followed by two-way ANOVA (factors age and gender). *Post hoc* comparisons were made with *post hoc* Tukey test. Spearman's correlation coefficients were calculated for skeletal muscle and platelet respirometric parameters. The analyses were performed using the software Origin 2017 (OriginLab, Corp., Northampton, MA, United States). Values of $p<0.05$ were considered significant.

Results

Skeletal muscle and platelets

As shown in Figure 1, parameters of mitochondrial respiration in the skeletal muscle significantly declined with age in both sexes (P_{FAO}, P_{FAO+I}, $P_{FAO+I+II}$, C_{IV}). Samples isolated from younger animals (6M and 12M) displayed also significant sex-related differences with higher oxygen consumption rates in males. This difference disappeared at the age of 24 months (Fig. 1A-D). The capacity of the electron transporting system ($E_{FAO+I+II}$) was by 5-15 pmol/s/mg higher than the respective $P_{FAO+I+II}$ and displayed the same trends, i.e. decline with age occurring earlier in females than in males with no difference at the age of 24 months. The age-related pattern of the intact platelets respiration was different: ROUT respiration increased between the ages of 6 and 12 months in both sexes and it remained higher in 24-month-old female rats. No gender differences were detected at any age category. Capacity of the electron transporting system did not show any differences between individual age categories, but it was

significantly higher in males than females at the age of 12 months (Fig. 1E, F). No significant correlation was revealed between the skeletal muscle and platelet respirometric parameters.

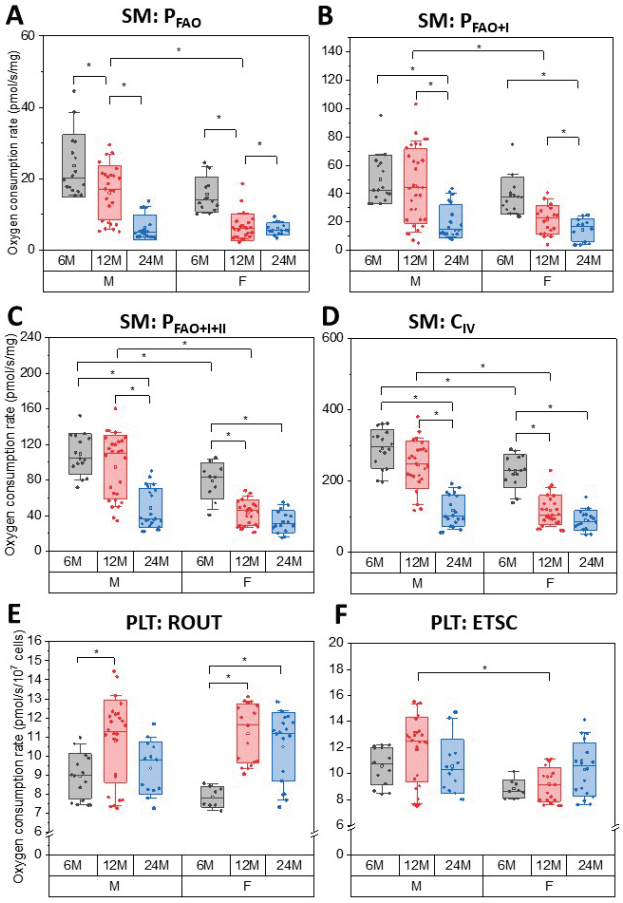

Fig. 1. Oxygen consumption rate of skeletal muscle (SM; **A-D**) normalized to mg of tissue wet weight and platelets (PLT; **E, F**) normalized to 10^7 cells in 6-, 12- and 24-month-old male (M) and female (F) Fischer 344 rats. (**A**) P_{FAO}=OXPHOS state after injection of malate (M), palmitoyl-L-carnitine (Pcar), ADP (D) and cytochrome c (c). (**B**) P_{FAO+I}=OXPHOS after M, Pcar, D, c + glutamate (G) and pyruvate (P). (**C**) $P_{FAO+I+II}$=OXPHOS after injection of M, Pcar, D, c, G, P and succinate (S). (**D**) Complex IV (C IV) capacity after Complex III inhibition and addition of TMPD with ascorbate (sodium azide-sensitive portion) and sodium azide. (**E**) ROUT=Routine respiration of intact platelets in mixed PBS+glucose and plasma (1:1) solution. (**F**) ETSC=maximum capacity of uncoupled respiration chain of platelets (after titration of uncoupler FCCP). The boxes are mean ± SD with horizontal line denoting median and whiskers showing 10 to 90 percentile range, * p<0.05.

Heart

In general, oxygen consumption rates were slightly lower in the right compared to the left ventricles of both sexes and significant gender-related differences were observed in P_{FAO} and C_{IV} in both ventricles of 12-month-old rats with slightly lower oxygen consumption rates in female rats. The impact of age was substantially different in males than in females. In male rats, respiratory states remained relatively stable over analyzed age categories in both ventricles with the exception of C_{IV} that was significantly lower at the age of 24 months compared to 12-month-old rats. In female rats, the declining trends in P_{FAO} and C_{IV} respiratory states were observed (Fig. 2A-F). Respiratory rates at the state $E_{FAO+I+II}$ were slightly higher than at $P_{FAO+I+II}$ and they did not display any significant differences related to age.

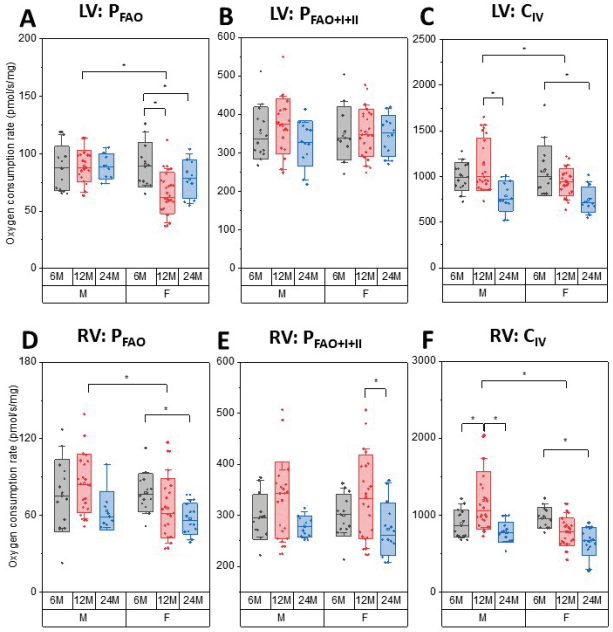

Fig. 2. Oxygen consumption rate of the left (LV; **A-C**) and right ventricle (RV; **D-F**) normalized to mg of tissue wet weight in 6-, 12- and 24-month-old male (M) and female (F) Fischer 344 rats. (**A, D**) P_{FAO}=OXPHOS state after injection of malate (M), palmitoyl-L-carnitine (Pcar), ADP (D), and cytochrome c (c). (**B, E**) $P_{FAO+I+II}$=OXPHOS after injection of M, Pcar, D, c, glutamate (G), pyruvate (P) and succinate (S). (**C, F**) Complex IV (C IV) capacity after complex III inhibition and addition of TMPD with ascorbate (sodium azide-sensitive portion) and sodium azide. The boxes are mean ± SD with horizontal line denoting median and whiskers showing 10 to 90 percentile range, * p<0.05.

Brain

The values of mitochondrial respiration were higher in the cerebellum than hippocampus in all analyzed respiratory states and they displayed minimum changes over age in the hippocampus. In the cerebellum, OXPHOS capacity related to Complex I was significantly lower at the age of 24 months compared to both 6-month and 12-month age categories in both sexes, whereas OXPHOS capacity related to Complex I+II was lower at

the age of 24 months compared to 12 months. The only gender-related difference was revealed in CIV activity in 12-month-old animals (Fig. 3A-F). Oxygen consumption rates at the state E_{I+II} were by ~30 pmol/s/mg higher than at P_{I+II} and they did not display any significant differences related to age in both hippocampus and cerebellum.

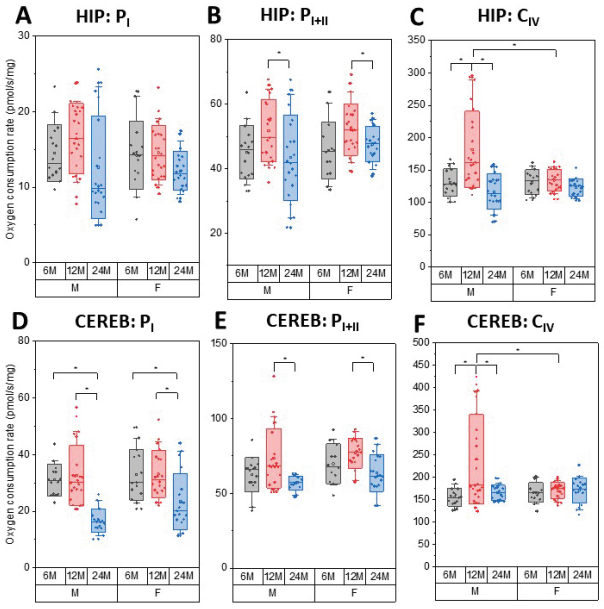

Fig. 3. Oxygen consumption rate of hippocampus (HIP; **A-C**) and cerebellum (CEREB; **D-F**) normalized to mg of tissue wet weight in 6-, 12- and 24-month-old male (M) and female (F) Fischer 344 rats. (**A, D**) P_I=OXPHOS state after injection of malate (M), glutamate (G), ADP (D) and cytochrome c (c). (**B, E**) P_{I+II}=OXPHOS after injection of M, G, D, c, pyruvate (P) and succinate (S). (**C, F**) Complex IV (C IV) capacity after complex III inhibition and addition of TMPD with ascorbate (sodium azide-sensitive portion) and sodium azide. The boxes are mean ± SD with horizontal line denoting median and whiskers showing 10 to 90 percentile range, * $p<0.05$.

Kidney and liver

In the kidney cortex, there was a declining trend in mitochondrial respiration linked to FAO and Complex I in both sexes (Fig. 4A-C). This difference disappeared if succinate was added to fully stimulate the Complex II-linked respiration that was even significantly higher in 12M compared to 6M males. Accordingly, $E_{FAO+I+II}$ was higher in males at the age of 12 months compared to 6M and 24M age categories. In the liver, P_{FAO} and P_{FAO+I} were lower at the age of 12 months compared to 6 months in animals of both sexes, but the values returned back or at least closer to 6-month-old values at the age of 24 months. In addition, $P_{FAO+I+II}$ was the highest at the age of 24 months. Exactly the same pattern was observed for the state $E_{FAO+I+II}$. At the ages of 6 and 12 months, there were significant differences between males and females in the respiratory states P_{FAO} and $P_{FAO+I+II}$ (Figure 4D-F).

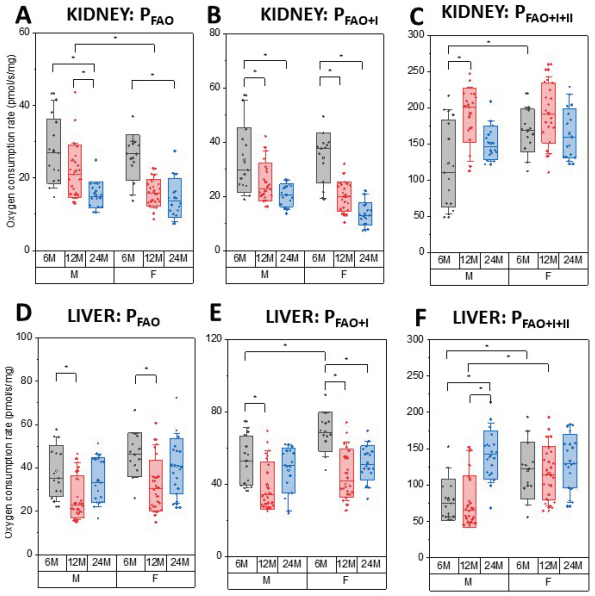

Fig. 4. Oxygen consumption rate of the kidney cortex (KIDNEY; **A-C**) and liver (**D-F**) normalized to mg of tissue wet weight in 6-, 12- and 24-month-old male (M) and female (F) Fischer 344 rats. (**A, D**) P_{FAO}=OXPHOS state after injection of malate (M), palmitoyl-L-carnitine (Pcar), ADP (D) and cytochrome c (c). (**B, E**) P_{FAO+I}=OXPHOS after injection of M, Pcar, D, c, glutamate (G), and pyruvate (P). (**C, F**) $P_{FAO+I+II}$=OXPHOS after injection of M, Pcar, D, c, G, P, and succinate. The boxes are mean ± SD with horizontal line denoting median and whiskers showing 10 to 90 percentile range, * $p<0.05$.

Discussion

In the present study, we assessed mitochondrial respiration in eight different tissues; heart, brain, liver and kidney belong to organs with the highest oxygen demands, skeletal muscle represents the most widely studied tissue in the aging research and platelets seem to emerge as a tool allowing insights into the bioenergetic situation of energy demanding organs [18,19]. In addition, heart, brain, and skeletal muscle are composed of post-mitotic cells; kidney cortex and liver cells can divide if they are challenged by toxins, hypoxia and other injuries [20]. We analyzed mitochondrial respiration in three age categories (6, 12, and 24 months) and separately in males and females to reveal potential sex-related differences. The maximum age of 24 months was selected as a median life span of Fischer 344 rats. Older ages are associated with dramatically increasing mortality [21].

Our study verified the age-related decline in

phosphorylating mitochondrial respiration in the skeletal muscle [7,8] and unlike other studies, it revealed significant gender-related differences in mitochondrial respiration: in the male rats, $P_{FAO+I+II}$ and C_{IV} were higher than in females at the ages of 6 and 12 months. In addition, respiratory fluxes were relatively stable in males at younger ages (6 and 12 month), whereas in females, the most prominent decline was noticed between the ages of 6 and 12 months. Some studies dealing with gender-related differences in mitochondrial functions of the skeletal muscle indicated higher oxygen consumption rates, higher mitochondrial content and higher activities of mitochondrial enzymes in female skeletal muscle, especially if the organism was challenged by caloric restriction or endurance training [22,23]. However, these studies were mostly performed on rats younger than 6 months. Another research declaring higher oxidative capacity of female skeletal muscle was performed on 15-month-old rats that, if subjected to high fat diet, displayed higher increase in mitochondrial oxygen consumption in females, but did not show any significant differences between the control male and female rats [24]. The aging studies on rat skeletal muscle are further complicated by the experimental approach (isolated mitochondria vs. permeabilized muscle fibres) and the type of muscle used. Analyses of isolated mitochondria seem to exaggerate the impact of age on mitochondrial oxygen consumption [25]. Mitochondrial dysfunction could be not only fiber type-specific [26], but also dependent on localization of isolated mitochondria since an increase in mitochondrial proton leak, a decrease in mitochondrial coupling and a decrease in mitochondrial membrane potential in subsarcolemmal but not interfibrillar mitochondria of senescent animals was reported [27,28].

In humans, skeletal muscle biopsies are relatively easily available and remain one of the rare sources of the fresh tissue samples in aging research. Both age-related decrease and no change in skeletal muscle mitochondrial function have been reported in humans [9,13,29]. In addition, some studies concluded that age-dependent decline in mitochondrial function may depend on the muscle type analyzed and result rather from low physical activity [28,30] or compromised cardiorespiratory fitness than chronological age per se [9]. An excellent meta-analysis focused on the putative gender-related differences in mitochondrial morphology and functions in humans concluded that respiration normalized to mass in human skeletal muscle does not differ between men and women [31], although some data indicate the trend towards higher values in younger women and older men [32].

Research in the field of mitochondrial respiration of platelets has indicated that platelets might serve an easily accessible pool of mitochondria that mirrors bioenergetics fitness of various organs and emerges as a promising tool in early diagnosis of some neurodegenerative diseases [18,19,33]. However, the results of other studies are completely opposite indicating no significant correlation between mitochondrial functional parameters in platelets and skeletal muscle in humans [34,35]. Our present study documents not only the age-related changes in platelet mitochondrial respiration in rats, but also very poor correlation between platelet and skeletal muscle mitochondrial respiratory parameters. Routine respiration of rat platelets increased between 6 and 12 months of age and then remained stable. Capacity of the electron transporting system did not change over all ages analyzed, but at the age of 12 months, it reached significantly higher values in males than in females, which is in line with observations on adult humans [36,37].

Relatively widely analyzed organ in association with mitochondrial aging research is the heart; studies mostly report a decrease in the electron-transporting capacity of the mitochondrial respiratory system in the male rats [38]. However, spectrophotometric analysis indicated no changes in activities of respiratory Complexes I and III, increase in Complex II and a decrease in complex IV [13]. Gender differences were only rarely studied, preferentially in younger animals [39,40]. In addition, data on the impact of aging on the intact female heart are mostly missing and little is known about putative interventricular differences in mitochondrial respiratory activity, although functional studies indicate substantial differences in the right vs. left ventricular contraction force or expression of cardiac markers of stress and metabolism [41,42]. Our study documents that in the female rat heart, mitochondrial respiration linked to fatty acid oxidation decreases with age in both ventricles as does the capacity of the Complex IV. In the male heart, P_{FAO} and $P_{FAO+I+II}$ remained stable over all ages studied and Complex IV capacity decreased at the age of 24 months, which is in line with spectrophotometric analyses [13]. Interestingly, individual respirometric parameters did not significantly differ between the right and left ventricle, although a general trend towards the lower values in the right

ventricle could be observed.

Mitochondrial dysfunction is frequently taken as a culprit in many neurodegenerative diseases that are associated with advanced age [43]. Thus, relatively many studies on age-dependent changes in mitochondrial respiration and/or activities of mitochondrial enzymes in rodents [44,45] are available. Nevertheless, they are in majority focused only on male population and deal with various parts of the brain, which makes them difficult to compare. In the study performed on male rats aged 4, 12 and 20 months, a continuous declining trend of oxygen consumption was observed in mitochondria isolated from hippocampus in the respiratory states linked to Complex I, where malate and glutamate were used as substrates and Complex II was stimulated with succinate [44]. Our respirometric protocol was slightly different: we used homogenized tissue and combination of malate, pyruvate and glutamate as substrates carrying electrons to the Complex I that was followed by addition of succinate to reach the OXPHOS capacity when both complexes are saturated. We have not revealed really substantially declining trend in mitochondrial respiration, although significant differences were noted between the ages 12 and 24 months in P_{I+II} in both sexes and C_{IV} in the male rats. However, values obtained from 24-month-old rats were not different from those measured in 6-month-old animals.

A slightly different pattern was observed in the cerebellum, where mitochondrial respiration linked to Complex I was significantly lower at the age of 24 months when compared to age categories of 6 and 12 months in both sexes. The same trend was revealed in $OXPHOS_{I+II}$ capacities, although the significance was reached only between 12- and 24-month-old animals of both sexes. The mitochondrial dysfunction of hippocampus is widely reported to be linked to various cognitive and mood disorders, whereas that of cerebellum to motor learning, regulation of balance and muscle coordination [46]. The age of 24 months in rats is usually compared to the human age around 60 years, when cognitive deficits cannot be taken as a general feature across the whole population, but muscle coordination could be already impaired, in particular in sedentary individuals [47].

The pattern of age-related changes in mitochondrial respiration in parenchymatous organs, i.e. liver and kidney, differed substantially in our study. Declining trend could be observed in kidneys, in particular in parameters P_{FAO} and P_{FAO+I}. The decrease was more pronounced in female rats resulting in significant difference between males and females at the age of 12 months. Our results are in good agreement with previously published research on rat isolated mitochondria or homogenized tissue [12,48]. In the liver, mitochondrial respiration in states P_{FAO+I} and $P_{FAO+I+II}$ was significantly higher in females than in males and P_{FAO} reached the highest values in males aged 24 months. The reports on impact of aging on the liver mitochondrial oxygen consumption are relatively rare showing either decrease of mitochondrial respiration in senescent animals [10,11,49] or no age-related changes [13]. Pandya *et al.* reported even an increase in mitochondrial oxygen consumption in the aged rat liver [50]. Interestingly, Justo *et al.* [51] documented significant gender-dependent differences in the morphology and function of the rat liver mitochondria.

In conclusion, the results of our study indicate that the concept of a general pattern of age-dependent decline in mitochondrial functions across different organs and tissues could be misleading. Also the statement of a higher mitochondrial respiration in females [52] seems to be conflicting, since the gender-related differences may vary with the tissue studied, combination of substrates used and might be better detectable at younger ages than in old animals.

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Conflict of Interest

There is no conflict of interest.

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