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Author manuscript

*Biochim Biophys Acta Bioenerg.* Author manuscript; available in PMC 2024 November 15.

Published in final edited form as:

*Biochim Biophys Acta Bioenerg.* 2024 November 01; 1865(4): 149494. doi:10.1016/j.bbabo.2024.149494.

## Sex-dependent differences in macaque brain mitochondria

Ivan Guerrero<sup>a</sup>, Belem Yoval-Sánchez<sup>a</sup>, Csaba Konrad<sup>a</sup>, Giovanni Manfredi<sup>a</sup>, Ilka Wittig<sup>b,c</sup>, Alexander Galkin<sup>a,\*</sup>

<sup>a</sup>Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, 407 East 61st Street, New York, NY 10065, USA

<sup>b</sup>Functional Proteomics, Institute for Cardiovascular Physiology, Goethe University, Frankfurt, Germany

<sup>c</sup>German Center for Cardiovascular Research (DZHK), Partner site Rhein Main, Frankfurt, Germany

### Abstract

Mitochondrial bioenergetics in females and males is different. However, whether mitochondria from male and female brains display differences in enzymes of oxidative phosphorylation remains unknown. Therefore, we characterized mitochondrial complexes from the brains of male and female macaques (*Macaca mulatta*).

Cerebral tissue from male macaques exhibits elevated content and activity of mitochondrial complex I (NADH: ubiquinone oxidoreductase) and higher activity of complex II (succinate dehydrogenase) compared to females. No significant differences between sexes were found in the content of  $\alpha$ -ketoglutarate dehydrogenase or in the activities of cytochrome *c* oxidase and F<sub>1</sub>F<sub>0</sub> ATPase. Our results underscore the need for further investigations to elucidate sex-related mitochondrial differences in humans.

### Keywords

Sex; Primates; Mitochondria; Mitochondrial complex I;  $\alpha$ -Ketoglutarate dehydrogenase; Succinate dehydrogenase; TCA cycle; ATPase; Cytochrome *c* oxidase; NADH:ubiquinone oxidoreductase

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Due to different hormonal statuses, adult healthy mammals exhibit sex differences in brain metabolism. Female hormone estradiol has a significant neuroprotective effect, increasing

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\*Corresponding author at: Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, 407 East 61st Street, New York, NY 10065, USA. [alg2057@med.cornell.edu](mailto:alg2057@med.cornell.edu) (A. Galkin).

CRedit authorship contribution statement

**Ivan Guerrero:** Writing – review & editing, Investigation, Data curation. **Belem Yoval-Sánchez:** Supervision, Methodology, Data curation. **Csaba Konrad:** Writing – review & editing, Methodology. **Giovanni Manfredi:** Writing – review & editing, Investigation. **Ilka Wittig:** Writing – review & editing, Methodology. **Alexander Galkin:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbabo.2024.149494>.

mitochondrial membrane fluidity and respiration [1]. In addition, estrogen receptors were found to be associated with brain mitochondria [2]. There are several, albeit controversial, reports on sex-dependence of the content and activity of brain mitochondrial enzymes in rodents [3–6] but very few reports on primates [7–9]. A recent meta-analysis [10] did not reveal any significant differences between sexes in humans, but tissues other than the brain were mostly considered. It is essential to perform an accurate evaluation of brain samples from non-human primates including both the content and function of the proteins involved in energy production. To address potential sex-related differences in the mitochondrial oxidative phosphorylation system, we analyzed the content and activity of respiratory chain enzymes in brain mitochondria from adult non-human primate *Macaca mulatta* using a novel approach recently developed in our laboratory [11,12].

The grey matter from snap-frozen prefrontal cortex was homogenized and processed for high-resolution Clear Native (hrCN) electrophoresis or activity measurements, as previously described [12,13] (see Supplementary data for details). Values of content and activity of several mitochondrial complexes for both sexes are shown in Fig. 1. After separation of the large membrane complexes by hrCN and fluorescent scanning of the gel, three main bands were determined: pyruvate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase and complex I (Fig. 1A). After calibration of the fluorescent signal [11],  $\alpha$ -ketoglutarate dehydrogenase content was not significantly different between male ( $44.3 \pm 12.0$  pmol/mg of protein) and female ( $44.4 \pm 5.8$  pmol/mg of protein) brains (Fig. 1B). A key control point for oxidative phosphorylation is mitochondrial complex I or NADH:ubiquinone oxidoreductase [14,15], which governs the electron entry flux to the respiratory chain and also determines the availability of  $\text{NAD}^+$  for TCA cycle dehydrogenases, such as pyruvate and  $\alpha$ -ketoglutarate dehydrogenase. In many pathologies, alterations of mitochondrial complex I function are a hallmark of central nervous system injury or degeneration. Macaque brains mitochondrial complex I content was found to be higher in males ( $6.9 \pm 1.0$  pmol/mg of protein) than in females ( $5.9 \pm 0.4$  pmol/mg of protein) (Fig. 1C). These values correspond to  $81 \pm 10$  and  $72 \pm 11$  pmol of complex I FMN per g of wet brain tissue in males and females, respectively (Supplementary data, Table 2S). Complex I content showed a trend towards increased levels in males and decreased levels in females with age (Fig. 1S). Specific complex I NADH oxidation activity with artificial acceptor hexaammineruthenium (HAR) was also significantly higher in male brain mitochondria (Fig. 1D), confirming the FMN content measurements. Next, we measured the activity of complex II (succinate dehydrogenase) and ATP hydrolysis by  $\text{F}_1\text{F}_0$  ATP-ase. In male brains, the activity of succinate dehydrogenase was higher than in female brains, contrary to the results reported in humans [8]. No statistically significant differences were found for complex IV cytochrome *c* oxidase or ATP hydrolytic activity of complex V.

Most of the mitochondrial studies in mammals regarding sex differences have been performed in rodents, whereas data on sex-related mitochondrial differences in primates or humans are limited. In this study, we analyzed binary sex differences in specific components of the mitochondrial oxidative phosphorylation machinery in brains of adult non-human primates. Unlike previous reports in rodents [5,6] and humans [8], we did not observe the elevated function of mitochondria enzymes in females. On the contrary, we found that the content of mitochondrial complex I was higher in males. Complex I provides the

electron entry to the respiratory chain and its activity defines overall flux through complexes I-IV as well as the rate of the TCA cycle via NAD<sup>+</sup> availability. Since mitochondria complex I is one of the major contributors to the production of reactive oxygen species in mitochondria [16], higher content of this enzyme could underlie elevated levels of oxidative stress in males as proposed for the rat brain [17]. Previously, higher content of other brain mitochondria components, such as uncoupling protein 1 was also reported in male brains [7]. It is worth noting that studies like the present one have limitations due to the difference in postmortem period, initial sample processing, storage time, and possible tissue heterogeneity. For example, due to the high variability between samples, it was not possible to estimate the content of pyruvate dehydrogenase (Fig. 1A, band 3).

Undoubtedly, the evidence of sex-dependent differences in mitochondria enzymatic activities in primates including humans is growing. With an increasing number of new mitochondria-targeted medicines, the physiologic and mechanistic implications of these differences remain to be appreciated. Recognizing the unique sex-based dissimilarities in mitochondrial function would have a big impact on the design of personalized drugs or therapeutic protocols and ensure more targeted and effective treatments for both men and women.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This work was supported by grants from the National Institutes of Health R01NS112381 and R21NS125466 (A. G.). This research was made possible in part using biomaterials from the NIA Nonhuman Primate Tissue Bank (<https://www.nia.nih.gov/research/dab/nonhuman-primate-tissue-bank>) at the Wisconsin National Primate Research Center, University of Wisconsin-Madison under contractual agreement with the National Institute on Aging (NIA). Work on the Typhoon scanner was partially supported by NIH grant S10OD030335.

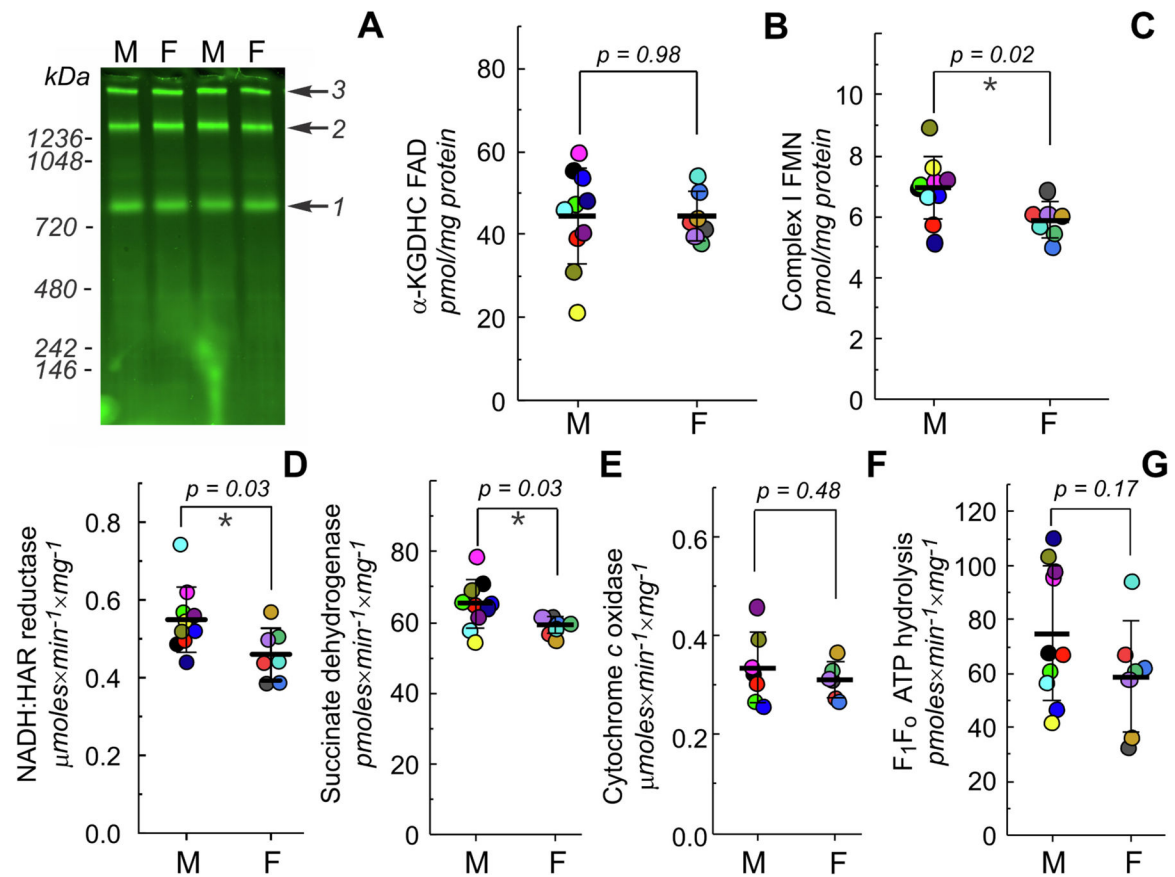
## Data availability

Data will be made available on request.

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**Fig. 1.**

Functional measurements of mitochondrial oxidative phosphorylation in males (M) and females (F) monkey's brain. A, representative image of an SDS-treated hrCN gel showing flavin fluorescence originated from complex I (1),  $\alpha$ -ketoglutarate dehydrogenase (2) and pyruvate dehydrogenase (3) complexes. B, C, quantitative measurement of  $\alpha$ -ketoglutarate dehydrogenase FAD (B) and complex I FMN (C) content. D–F, complex I NADH: hexaammineruthenium (HAR) reductase (D), complex II succinate dehydrogenase (E), complex IV ferrocyanide oxidase (F) activities and ATP hydrolysis by complex V (G) of brain mitochondria homogenates from males and females. Values of content and activity were normalized per tissue homogenate protein, mean  $\pm$  SD,  $n = 7$ –10 for each group, Student's *t*-test.