Mitochondrial Transfusion Improves Mitochondrial Function Through Up-regulation of Mitochondrial Complex II Protein Subunit SDHB in the Hippocampus of Aged Mice

A. Adlimoghaddam1 · T. Benson2 · B. C. Albensi1,3,4

Received: 29 March 2022 / Accepted: 23 June 2022 / Published online: 14 July 2022 © The Author(s) 2024, corrected publication 2024

Abstract
The mitochondrial theory of aging is characterized by mitochondrial electron transport chain dysfunction. As a hallmark of aging, an increasing number of investigations have attempted to improve mitochondrial function in both aging and age-related disease. Emerging from these attempts, methods involving mitochondrial isolation, transfusion, and transplantation have taken center stage. In particular, mitochondrial transfusion refers to the administration of mitochondria from healthy tissue into the bloodstream or into tissues affected by injury, disease, or aging. In this study, methods of mitochondrial isolation and transfusion were developed and utilized. First, we found a significant decrease ($p < 0.05$) in the expression of mitochondrial complex proteins (I-V) in aged (12 months old) mouse brain tissue (C57BL/6 mice) in comparison to healthy young brain tissue (1 month old). To investigate whether healthy young mitochondria taken from the liver could improve mitochondrial function in older animals, we intravenously injected mitochondria isolated from young C57BL/6 mice into aged mice from the same strain. This study, for the first time, demonstrates that mitochondrial transfusion significantly ($p < 0.05$) improves mitochondrial function via the up-regulation of the mitochondrial complex II protein subunit SDHB in the hippocampus of aged mice. This result has identified a role for mitochondrial complex II in the aging process. Therefore, mitochondrial complex II could serve as a putative target for therapeutic interventions against aging. However, more importantly, methods of mitochondrial transfusion should be further tested to treat a variety of human diseases or disorders and to slow down or reverse processes of aging.

Keywords Mitochondrial dysfunction · Bioenergetics · Complex II · Aging · Age-related disease · Mitochondrial transfusion · Brain · Neuroscience

Introduction
Many years ago, it was proposed that mitochondria play a crucial role in aging and age-related diseases. To this end, the targeting of mitochondria has become a critical strategy in several biological processes implicated in aging, such as oxidative stress via reactive oxygen species (ROS) generation, amino acid and lipid metabolism, regulation of apoptosis, and energy metabolism [1–3]. Mitochondria play a key role in energy-demanding brain functions [4]. Oxidative phosphorylation plays an essential role for mitochondria to produce adenosine triphosphate (ATP). A decline in mitochondrial function is associated with a decrease in oxidative phosphorylation capability and an increase in oxidative damage, which leads to aging and age-related diseases [3, 5]. Therefore,
there is a strong relationship between energy metabolism and aging and also between energy metabolism and brain disorders.

Several in vitro experiments show that incubation of cells with isolated mitochondria improved total ATP production, respiratory function, and cell proliferation indicating that mitochondrial transfer is beneficial in mitochondria-deficient cells [6–10]. Additionally, several in vivo experiments show that mitochondrial injections protect the heart, liver, and brain tissues from ischemic injury, and also rescue motor dysfunction in Parkinson’s disease and schizophrenia animal models [11–16]. These studies reveal that mitochondrial transplantation can be beneficial in different cell types and disorders [17].

Both the hippocampal and cortical regions of the brain, which are associated with key cognitive functions, are susceptible to aging and thus are susceptible to mitochondrial impairments [18]. Understanding oxidative phosphorylation changes is critical for the designing of novel strategies for therapeutic interventions for aging and even age-related disease. However, our knowledge of how specific mitochondrial mechanisms are affected in aging is currently poorly understood. Moreover, mitochondrial complexes (i.e., I–V) that make up the electron transport chain have been underexplored in a context of aging. Mitochondrial transfection therapy is a novel strategy that involves the transfer of functional mitochondria into defective brain cells for preventing age-related decline [19, 20]. In this study, we intravenously administered healthy young mitochondria obtained from the liver of young mice into aged mice to evaluate whether mitochondrial function improves in key aged brain tissues following mitochondrial transfection. This study provides novel insights for mitochondrial transfusion therapy on aged animals and yields new data for improving hippocampal function via the enhancement of mitochondrial bioenergetics during processes of aging.

Material and Methods

Animals

All animal procedures followed guidelines of the University of Manitoba Animal Care Committee, the Canadian Council of Animal Care rules, and the Institutional Animal Care and Use Committee (IACUC) standards. Mice were given ad libitum access to food and water and housed under the standard light/dark cycle (12 h light, 12 h dark at room temperature (22 °C)). In this study, we used 1-month-old and 12-month-old male C57BL/6 mice. All C57BL/6 mice were housed and aged in the pathogen-free animal facility at St. Boniface Hospital Research Centre.

Surgical Procedures

Each mouse was anesthetized by isoflurane inhalation (5% induction, 1.5–5% maintenance). Hair at incision sites was shaved and disinfected with 70% ethanol; eyes were also lubricated. Sterile technique was performed throughout the duration of all procedures according to IACUC requirements. The mouse was placed in dorsal recumbency on a heated table. A small bolster was placed beneath the animal’s head exposing the ventral aspect of the neck. An incision was made from the ramus of one side of the jaw to the tip of the sternum just lateral to the trachea/midline. The mouse was then gently dissected down through the salivary and lymphoid glands, adipose tissue, and fascia to the external jugular vein, which was superficial to most of the neck musculature; also elevated and cleaned the external jugular vein for a distance of 0.5 mm. Then, there was tie off of the distal (cephalic) end of the vein leaving tails 4–5 inches long. Two ligatures were then placed around the proximal (cardiac) end of the vein. Hemostats were placed on the cephalic suture and one cardiac suture was used to provide gentle counter traction to the vessel. A very small hole was made using vannas scissors. Hemorrhage was controlled with gentle traction on the cephalic ligature ends. The free end of the catheter was inserted into a hole in the vein wall, and was advanced gently to the level of the heart. The cephalic ligature was then tied around the catheter. Furthermore, a small access hole was created on the back of the animal in the mid-scapular region using a hemostat. The vascular access tubing was then led into this pocket and through the hole, allowing the catheter to reach over the neck to the external jugular vein with significant slack to permit free head and neck movement. Next, we attached the button to the tubular end, ensuring a snug fit. The catheter was secured to surrounding tissues using 5–0 absorbable sutures (Oasis Medical, IL), one for the underlying fascia and one to secure the vascular access button to the skin.

Group Design

The aged mice (12 months old) were randomly divided into three groups. The mice in the first and second groups were injected with the isolated mitochondria (10 mg/kg or 20 mg/kg body weight) via a catheter once every 2 days over the period of 2 weeks (seven total injections). The mice in the third group were injected with the same volume of phosphate-buffered saline-PBS (vehicle). All mice were sacrificed within 24 h after the last
day of mitochondrial or PBS transfusion. The mice were assigned (as \( n = 3 \)) in each group for immunoblotting analysis.

**Preparation of Isolated Mitochondria from Mouse Liver**

Two C57BL/6 mice of 30 days of age were euthanized by decapitation while under isoflurane anesthetic. Briefly, liver tissues were dissected and washed twice with ice-cold PBS (pH 7.4), and then transferred to a 50-ml falcon tube containing 5 ml ice-cold homogenizing buffer (300 mM sucrose, 10 mM K-HEPES, and 1 mM K-EGTA (pH 7.2)). Then, 250 µl of Subtilisin A (96.61 µM) was added to the homogenate, mixed by inversion, and incubated on ice for 20 min. Then, 5 ml of homogenizing buffer was added to the 40 µm filter and the filter was gently washed by pipetting up and down. Next, a pre-wet 40 µm filter was placed onto a 50-ml falcon tube and the homogenate was filtered into a new 50-ml falcon tube on ice. This step was repeated again with 40 µm and 10 µm filters. The filtrate was then transferred to 1.5-ml Eppendorf centrifuge tubes and centrifuged for 10 min at 10,000 × g for 4 °C. The supernatant was then discarded; the pellet was resuspended in 1 ml of ice-cold PBS. A final purification step consisted of the sequential passage of the resuspended mitochondria through 0.8-µm pore filters using a 3-ml syringe and transferred to a new 1.5-ml tube.

**Preparation of Isolated Mitochondria from Brain Tissue**

Mice were euthanized by decapitation while under isoflurane anesthetic. Brain (hippocampal and cortical) tissues were then removed. Mitochondria derived from transfused and control mice were isolated using the commercial isolation kits (110,170, Abcam, Cambridge, MA, USA) as reported previously [21]. Briefly, hippocampal and cortical tissues were washed twice with 1 × PBS and then homogenized in isolation buffer. Homogenates were centrifuged at 1000 × g for 5 min at 4 °C. Supernatants were centrifuged at 10,000 × g for 20 min at 4 °C. Pellets were resuspended with PBS. Furthermore, the protein concentrations of samples were measured utilizing a colorometric DC protein assay kit (Bio-Rad, Hercules, CA, USA) as described previously [22].

**Western Blotting**

Samples were added to 4X Laemmli buffer (40% glycerol, 16% SDS, 0.01% bromophenol blue, 20% β-mercaptoethanol, and 0.25 M Tris, pH 6.8) and heated for 8 min at 40 °C. Furthermore, 15 µg of protein from each sample was loaded in each well of a 10% sodium dodecyl sulfate–polyacrylamide (SDS-PAGE) gels (Bio-Rad, Hercules, CA, USA) and at 170 V for 60 min. Following electrophoresis, each was activated using a ChemiDoc imager (Bio-Rad, Hercules, CA, USA). Afterwards, proteins were transferred from gels into the nitrocellulose membranes (Bio-Rad, Hercules, CA, USA) using the Trans-Blot Turbo Transfer System (Bio-Rad, Hercules, CA, USA). Following the transfer, total proteins on the membranes were detected using the ChemiDoc imager. After imaging, the membranes were incubated in TBS-T buffer with 5% bovine serum albumin (BSA) for 1 h at room temperature. All membranes were then incubated overnight on a rocking platform at 4 °C with Total OXPHOS Rodent WB Antibody Cocktail (ab110413, Abcam, Cambridge, MA, USA, 1:1000 dilution). Following primary antibody incubation, membranes were washed with 1X TBS-T buffer (three times; 15 min each) and then incubated with goat anti-mouse IgG (H + L) antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA, 1:2000 dilution) prepared in TBS-T buffer with 5% BSA for one and half hours at room temperature. Following secondary antibody incubation, membranes were washed with 1X TBS-T buffer (three times; 15 min each). Then, membranes were treated with enhanced chemiluminescence (ECL) utilizing the Bio-Rad Clarity Weston ECL blotting kit (Bio-Rad, Hercules, CA, USA), and visualized by the ChemiDoc. Relative quantification of OXPHOS protein levels was normalized to total protein as described previously [5, 23].

**Statistical Analysis**

The data were analyzed with Student’s \( T \)-test or with one-way ANOVA, followed by a Tukey post hoc test (GraphPad Prism 6, GraphPad Software) to evaluate the statistical significance of differences between study groups. All values are expressed as the mean ± standard deviation (SD). A difference between or among groups was determined as statistically significant, if *\( P \leq 0.05 \), **\( P \leq 0.01 \), and ***\( P \leq 0.001 \).

**Results**

**Effects of Aging on the Expression of Mitochondrial-Associated Proteins in C57BL/6 Brain Tissue**

In order to evaluate the effect of aging on the mitochondrial respiratory chain, we compared the expression of mitochondrial complex proteins in hippocampal and cortical tissue derived from 1-month and 12-month-old C57BL/6 mice. Interestingly, immunoblotting results showed that the expression levels of NADH dehydrogenase beta subcomplex subunit 8 of complex I (NDUF8; \( P < 0.05 \)), succinate dehydrogenase subunit B of complex II (SDHB; \( P < 0.05 \)), cytochrome b-c\(_1\) complex subunit 2 of complex III (UQCRC2;
cytochrome c oxidase subunit 1 of complex IV (MTCO1; p < 0.05), and ATP synthase subunit alpha of complex V (ATP5A; p < 0.01) decrease significantly in these brain tissues with age (Fig. 1). Overall, both cortical and hippocampal tissues display significantly higher levels of complex I–V in younger mitochondria compared to the aged ones.

**Effects of Transfused Mitochondria in Mitochondrial-Associated Proteins in C57BL/6 Brain Tissue**

To measure the effect of mitochondrial transfusion on brain mitochondrial function, the expression levels of key mitochondrial subunit proteins were measured in brain tissue utilizing immunoblotting. Mitochondrial transfusion treatment significantly increased (p < 0.05) the level of succinate dehydrogenase subunit B of complex II (SDHB) in C57BL/6 hippocampal tissue, indicating that mitochondrial transfusion induces changes in mitochondrial function (Fig. 2). However, levels of NADH dehydrogenase beta subcomplex subunit 8 of complex I (NDUFB8), cytochrome b-c1 complex subunit 2 of complex III (UQRC2), cytochrome c oxidase subunit 1 of complex IV (MTCO1), and ATP synthase subunit alpha of complex V (ATP5A) were not significantly different as a function of mitochondrial transfusion treatment in C57BL/6 hippocampus (Fig. 2).

Immunoblotting results revealed that the expression level of key mitochondrial subunit protein (complex I-V) was not significantly different as a function of mitochondrial transfusion treatment in C57BL/6 cortical tissue (Fig. 3), indicating that transfused mitochondria did not induce changes in
Discussion

Brain adenosine triphosphate (ATP) is generated through oxidative phosphorylation in the mitochondria [24]. Mitochondria derived from aged animals, unlike young animals, cannot produce sufficient supplies of energy as a result of reduced oxidative phosphorylation [25]. Disruptions to mitochondrial oxidative phosphorylation have also been attributed to aging and neurodegeneration [26, 27]. There is still a lack of understanding of how these disruptions may be therapeutically targeted to slow aging, and also to prevent or stop disease progression and improve memory functions. In this study, however, we tested mitochondrial transfusion therapy as a strategy to target biological processes that change during aging. To this end, we looked at mitochondria from both...
The methods we utilized in this study for mitochondrial transfusion are straightforward and less expensive. Also, in order to prevent immune responses due to the delivery of exogenous mitochondria to aged mice, we purified mitochondria with several filtration steps. In addition, we monitored the health and safety of the mice during the transfusion procedure, in order to ensure that the method was appropriate and the transfusion of mitochondria did not cause inflammation or death. Likewise, the protocol was optimized to include additional filtration steps to minimize the chance of plugging the catheter with debris, and thus reducing the probability of septic infection. Lastly, the use of filtration eliminates time-consuming centrifugation steps, which offers a quick isolation of purified mitochondria. The key consideration for the efficacy of mitochondrial transfusion is preparing and delivering mitochondria in a timely manner. In this study, the isolation and preparation and measurement of the concentration of mitochondria are timely (within 40 min), which makes the mitochondrial transfusion therapy feasible for clinical practice. The current study does in fact reveal the feasibility of utilizing mitochondrial transfusion as a valued treatment for disorders associated with mitochondrial dysfunction.

The current study reports that mitochondrial transfusion from young mice to aged mice improves the expression of a key mitochondrial protein involved in oxidative phosphorylation. It is suggested that facilitating mitochondrial transfusion and exploring putative mechanisms (e.g.,

**Fig. 3** Transfused mitochondria did not alter the expression of mitochondrial complex (I-V) protein subunit in C57BL/6 cortex. Western blot experiments demonstrating relative levels of mitochondrial protein subunits in cortical tissue of 12-month-old transfused mice (dose I: 10 mg/kg; dose II 20 mg/kg). A–B Representative western blot for NADH dehydrogenase beta subcomplex subunit 8 of complex I (NDUFB8), succinate dehydrogenase subunit B of complex II (SDHB), cytochrome b-c\(_{1}\) complex subunit 2 of complex III (UQCRCC2), cytochrome c oxidase subunit 1 of complex IV (MTCO1), and ATP synthase subunit alpha of complex V (ATP5A). C–J Relative quantification for protein levels of complex I-V normalized to total protein. Results are expressed as mean ± SD of \(n=3\) per group (*\(P \leq 0.05\)) analyzed by Student's T-test.
OXPHOS) involved in aging should hasten the application of mitochondria for the treatment of age-related diseases caused by mitochondrial dysfunction.

Previous reports suggest that exogenous mitochondria, following intravenous administration, can cross the blood–brain barrier (BBB) and improve mitochondrial function potentially through the up-regulation of mitochondrial complex II protein subunits in the hippocampal tissue of aged mice. However, the mechanism(s) for penetrating the BBB remains poorly characterized. Our experiments for the first time show that young mitochondria may reverse mitochondrial dysfunction potentially by the up-regulation of specific mitochondrial complex protein subunit in aged hippocampal tissues. One recent study by Nitzan et al. [28] in an Alzheimer’s disease (AD) mouse model has also shown that amelioration of mitochondrial dysfunction in the brain was achieved with mitochondrial transfusion. Here they found mitochondrial transfusion increased citrate-synthase and cytochrome c oxidase activities relative to untreated AD mice [28]. Several studies reported that the activity of complex II decreases with age in rat heart, lung, kidney, muscle, liver, and brain [29–32]. In a study [33] involving APP/PS1 AD mice, the investigators showed age-dependent decreases in mitochondrial complex II activity starting at 9 months. Recently, our laboratory found that the expression of a complex II subunit significantly decreases in the cortical tissue of 3xTg-AD mice [3]. Other studies indicated that mutations in the subunits of complex II hasten aging and decrease lifespan in Caenorhabditis elegans [34] and Drosophila melanogaster [35]. Other studies showed age-dependent decreases in mitochondrial complex II activity in human skin fibroblasts and human skeletal muscles [36, 37]. Moreover, in a model for older and younger human skin cells (in terms of telomerase activity), complex II activity was found to be much higher in the younger than in the older cells [38]. In addition, a recent study revealed that complex II activity is lower in the skin of naturally aged mice compared to younger mice [39]. These results support our findings and taken together suggest that mitochondrial transfusion may offer a novel therapeutic approach for both aging and age-related diseases, such as AD [37].

In particular, we found that the expression of mitochondrial complex protein subunits decreases in aged brain tissue as compared to younger tissue, which suggests a potential link between complex II and aging. It could be that age-related decline in complex II activity is related to increases in reactive oxygen species (ROS) leakage, which results in oxidative stress and a decrease in normal brain function.

As a next step, future targets should aim to measure oxygen consumption rates and the activity of terminal mitochondrial respiration (cytochrome c oxidase). Furthermore, to verify that transplanted mitochondria are in fact internalized into brain cells, transmission electron microscopy (TEM) or light microscopy using fluorescent mitochondrial labels could be utilized, as previous studies have demonstrated the usefulness within cardiac cells [15, 40–42]. In order to determine the activity of mitochondria, the mitochondrial membrane potential could be measured following mitochondrial transfusion using a MitoTracker dye as carried out in ischemic injury studies [43].

In conclusion, our data demonstrate that expression of mitochondrial complex protein subunits decreases as a result of aging and that mitochondrial transfusion can improve specific parameters of mitochondrial bioenergetics in models of aging. In particular, the data indicate that mitochondrial transfusion improves the expression of mitochondrial complex II protein subunits in hippocampal tissue derived from aged mice, which may offer a new therapeutic approach for slowing processes of aging and could have implications for age-related diseases.

Acknowledgements B. C. A. previously held the Manitoba Dementia Research Chair (funded by the Alzheimer’s Soc. of Manitoba and Research Manitoba) and the Honourable Douglas and Patricia Everett, Royal Canadian Properties Limited Endowment Chair. Dr. Don Davies wrote the animal protocol.

Author Contribution Dr. Aida Adlimoghadam (AA) performed the experiments, created the outline, and wrote the paper. Dr. Benedict C. Albens (BCA) supervised the writing, edited the manuscript, and provided additional text and references when necessary. Mr. Tom Benson (TB), B. C. A., and A. A. created the original experiment design, and B. C. and T. B. directed and supervised the project.

Funding Financial support for this study comes from the Mitrix Bio Company. We also received support from the St. Boniface Hospital Research Foundation (Grant Nos. 1406–3216 and 1410–3216), the Canadian Institute of Health Research (CIHR; Grant No. PJT-162144) to B. C. A., the Alzheimer’s Society of Manitoba, the Honourable Douglas and Patricia Everett, Royal Canadian Properties Limited Endowment Fund (Grant No. 1403–3131) to B. C. B. C. A. previously held the Manitoba Dementia Research Chair (funded by the Alzheimer’s Soc. of Manitoba and Research Manitoba).

Data Availability The data and the material supporting this paper are available in the article or are available from the corresponding author upon request.

Declarations

Ethics Approval and Consent to Participate Not applicable for human data. All animal procedures followed guidelines of the University of Manitoba Animal Care Committee, the Canadian Council of Animal Care rules, and the Institutional Animal Care and Use Committee (IACUC) standards.

Consent for Publication The authors consent to publish identifiable details within the text in the journal of molecular neurobiology.

Competing Interests The authors declare no competing interests. B. C. A. is a scientific advisor for Mitrix Bio.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long
as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Yamaguchi R, Perkins G (2009) Dynamics of mitochondrial structure during apoptosis and the enigma of Opa1. Biochim Biophys Acta 1787(8):963–972. https://doi.org/10.1016/j.bbabio.2009.02.005

2. Chistiakov DA, Sobenin IA, Revin VV, Orekhov AN, Bobryshev YV (2014) Mitochondrial aging and age-related dysfunction of mitochondria. Biomed Res Int 2014:238463. https://doi.org/10.1155/2014/238463

3. Adlimoghaddam A, Snow WM, Stortz G, Perez C, Djordjevic J, Goertzen AL et al (2019) Regional hypometabolism in the 3xTg mouse model of Alzheimer’s disease. Neurobiol Dis 127:264–277. https://doi.org/10.1016/j.nbd.2019.03.008

4. Brand MD, Orr AL, Perevoshchikova IV, Quinlan CL (2013) The role of mitochondrial function and cellular bioenergetics in ageing and disease. Br J Dermatol 169(Suppl 2):1–8. https://doi.org/10.1111/bjd.12208

5. Adlimoghaddam A, Odero GG, Glazner G, Turner RS, Albenzi BC (2021) Nilotinib improves bioenergetic profiling in brain astroglia in the 3xTg mouse model of Alzheimer’s disease. Aging Dis 12(2):441–465. https://doi.org/10.14336/AD.2020.0910

6. Pacak CA, Preble JM, Kondo H, Seibel P, Levitsky S, Del Nido PJ et al (2015) Actin-dependent mitochondrial internalization in cardiomyocytes: evidence for rescue of mitochondrial function. Biol Open 4(5):622–626. https://doi.org/10.1242/bio.201511478

7. Caicedo A, Fritz V, Borodello JM, Ayala M, Dennemont I, Abdel-lauqi N et al (2015) MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell mitochondria on cancer cell metabolism and function. Sci Rep 5:9073. https://doi.org/10.1038/srep09073

8. Katrangi E, D’Souza G, Boddapati SV, Kulawiec M, Singh KK, Bigger B et al (2007) Xenogenic transfer of isolated murine mitochondrial DNA into human rho0 cells can improve respiratory function. Rejuvenation Res 10(4):561–570. https://doi.org/10.1089/rej.2007.0575

9. Clark MA, Shay JW (1982) Mitochondrial transformation of mammalian cells. Nature 295(5850):605–607. https://doi.org/10.1038/295605a0

10. Elliott RL, Jiang XP, Head JF (2012) Mitochondria organelle transplantation: introduction of normal epithelial mitochondria into human cancer cells inhibits proliferation and increases drug sensitivity. Breast Cancer Res Treat 136(2):347–354. https://doi.org/10.1007/s10549-012-2283-2

11. Shi X, Zhao M, Fu C, Fu A (2017) Intravenous administration of mitochondria for treating experimental Parkinson’s disease. Mitochondrion 34:91–100. https://doi.org/10.1016/j.mito.2017.02.005

12. Chang JC, Wu SL, Liu KH, Chen YH, Chuang CS, Cheng FC et al (2016) Allogeneic/xenogeneic transplantation of peptide-labeled mitochondria in Parkinson’s disease: restoration of mitochondrial functions and attenuation of 6-hydroxydopamine-induced neurotoxicity. Transf Sci 170(40–56):e3. https://doi.org/10.1016/j.trsl.2015.12.003

13. McCully JD, Cowan DB, Emani SM, Del Nido PJ (2017) Mitochondrial transplantation: From animal models to clinical use in humans. Mitochondrion 34:127–134. https://doi.org/10.1016/j.mito.2017.03.004

14. Lin HC, Lai IR (2013) Isolated mitochondria infusion mitigates ischemia-reperfusion injury of the liver in rats: reply. Shock 39(6):543. https://doi.org/10.1097/SHK.0000430660.63077.7f

15. McCully JD, Cowan DB, Pacak CA, Toumpoulis IK, Dayalan H, Levitsky S (2009) Injection of isolated mitochondria during early reperfusion for cardioprotection. Am J Physiol Heart Circ Physiol 296(1):H94–H105. https://doi.org/10.1152/ajpheart.00567.2008

16. Huang PJ, Kuo CC, Lee HC, Shen CI, Cheng FC, Wu SF et al (2016) Transferring xenogenic mitochondria provides neural protection against ischemic stress in ischemic rat brains. Cell Transplant 25(5):913–927. https://doi.org/10.3727/096368915X689785

17. McCully JD, Levitsky S, Del Nido PJ, Cowan DB (2016) Mitochondrial transplantation for therapeutic use. Clin Transl Med 5(1):16. https://doi.org/10.1186/s40169-016-0095-4

18. Feng X, Guo J, Sigmon HC, Sloan RP, Brickman AM, Provenzano FA et al (2020) Brain regions vulnerable and resistant to aging without Alzheimer’s disease. PLoS ONE 15(7):e234255. https://doi.org/10.1371/journal.pone.0234255

19. Liu D, Gao Y, Liu J, Huang Y, Yin J, Feng Y et al (2021) Intercellular mitochondrial transfer as a means of tissue revitalization. Signal Transduct Target Ther 6(1):65. https://doi.org/10.1038/s41392-020-00440-z

20. Fu A (2020) Mitotherapy as a novel therapeutic strategy for mitochondrial diseases. Curr Mol Pharmacol 13(1):41–49. https://doi.org/10.2174/1874472126661900920144115

21. Adlimoghaddam A, Albenzi BC (2021) The nuclear factor kappa B (NF-kappaB) signaling pathway is involved in ammonia-induced mitochondrial dysfunction. Mitochondrion 57:63–75. https://doi.org/10.1016/j.mito.2020.12.008

22. Fontaine KM, Beck A, Stocker-Worgotter E, Piercey-Normore MD (2012) Photobiont relationships and phylogenetic history of Dermatocarpon luridum var. luridum and related Dermatocarpon species. Plants (Basel) 1(2):39–60. https://doi.org/10.3390/plant1020039

23. Mostaﬁzar M, Cortes-Perez C, Snow W, Djordjevic J, Adlimoghaddam A, Albenzi BC (2021) Challenges with methods for detecting and studying the transcription factor nuclear factor kappa B (NF-kappaB) in the central nervous system. Cells 10(6). https://doi.org/10.3390/cells10061335

24. Srivastava S (2017) The mitochondrial basis of aging and age-related disorders. Genes (Basel) 8(12). https://doi.org/10.3390/genes8120398

25. Grimm A, Eckert A (2017) Brain aging and neurodegeneration: from a mitochondrial point of view. J Neurochem 143(4):418–431. https://doi.org/10.1111/jnc.14037

26. Khacho M, Clark A, Svoboda DS, MacLaurin JG, Lagace DC, Park DS et al (2017) Mitochondrial dysfunction underlies cognitive defects as a result of neural stem cell depletion and impaired neurogenesis. Hum Mol Genet 26(17):3327–3341. https://doi.org/10.1093/hmg/ddx217

27. Watts ME, Pocock R, Claudianos C (2018) Brain energy and oxygen metabolism: emerging role in normal function and disease. Front Mol Neurosci 11:216. https://doi.org/10.3389/fnmol.2018.00216

28. Nitzan K, Benhamron S, Valitsky M, Kesner EE, Lichtenstein M, Ben-Zvi A et al (2019) Mitochondrial transfer ameliorates cognitive deficits, neuronal loss, and gliosis in Alzheimer’s disease mice. J Alzheimers Dis 72(2):587–604. https://doi.org/10.3233/JAD-190853
29. Tatarkova Z, Kuka S, Racay P, Lehotsky J, Dobrota D, Mistuna D et al (2011) Effects of aging on activities of mitochondrial electron transport chain complexes and oxidative damage in rat heart. Physiol Res 60(2):281–289. https://doi.org/10.33549/physiolres.932019

30. Sandhu SK, Kaur G (2003) Mitochondrial electron transport chain complexes in aging rat brain and lymphocytes. Biogerontology 4(1):19–29. https://doi.org/10.1023/a:1022473219044

31. Kumaran S, Subathra M, Balu M, Panneerselvam C (2004) Age-associated decreased activities of mitochondrial electron transport chain complexes in heart and skeletal muscle: role of L-carnitine. Chem Biol Interact 148(1–2):11–18. https://doi.org/10.1016/j.cbi.2003.10.010

32. Cocco T, Gsobo P, Clemente M, Lopriore B, Grattagliano I, Di Paola M et al (2005) Tissue-specific changes of mitochondrial functions in aged rats: effect of a long-term dietary treatment with N-acetylcysteine. Free Radic Biol Med 38(6):796–805. https://doi.org/10.1016/j.freeradbiomed.2004.11.034

33. Emmerzaal TL, Rodenburg RJ, Tanila H, Verweij V, Kiliaan AJ, Kozicz T (2018) Age-dependent decrease of mitochondrial complex II activity in a familial mouse model for Alzheimer’s disease. J Alzheimers Dis 66(1):75–82. https://doi.org/10.3233/JAD-180337

34. Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N et al (1998) A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 394(6694):694–697. https://doi.org/10.1038/29331

35. Walker DW, Hajek P, Muffat J, Knoepfle D, Cornelison S, Attardi G et al (2006) Hypersensitivity to oxygen and shortened lifespan in a Drosophila mitochondrial complex II mutant. Proc Natl Acad Sci USA 103(44):16382–16387. https://doi.org/10.1073/pnas.0607918103

36. Boffoli D, Scacco SC, Vergari R, Solarino G, Santacroce G, Papa S (1994) Decline with age of the respiratory chain activity in human skeletal muscle. Biochim Biophys Acta 1226(1):73–82. https://doi.org/10.1016/0925-4439(94)90061-2

37. Bowman A, Birch-Machin MA (2016) Age-dependent decrease of mitochondrial complex II activity in human skin fibroblasts. J Invest Dermatol 136(5):912–919. https://doi.org/10.1016/j.jid.2016.01.017

38. Anderson A, Bowman A, Boulton SJ, Manning P, Birch-Machin MA (2014) A role for human mitochondrial complex II in the production of reactive oxygen species in human skin. Redox Biol 2:1016–1022. https://doi.org/10.1016/j.redox.2014.08.005

39. Velarde MC, Flynn JM, Day NU, Melov S, Campisi J (2012) Mitochondrial oxidative stress caused by Sod2 deficiency promotes cellular senescence and aging phenotypes in the skin. Aging (Albany NY) 4(1):3–12. https://doi.org/10.18632/aging.100423

40. Preble JM, Pacak CA, Kondo H, MacKay AA, Cowan DB, McCully JD (2014) Rapid isolation and purification of mitochondria for transplantation by tissue dissociation and differential filtration. J Vis Exp 91:e51682. https://doi.org/10.3791/51682

41. Masuzawa A, Black KM, Pacak CA, Ericsson M, Barnett RJ, Drumm C et al (2013) Transplantation of autologously derived mitochondria protects the heart from ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 304(7):H966–H982. https://doi.org/10.1152/ajpheart.00883.2012

42. Davis CH, Kim KY, Bushong EA, Mills EA, Boassa D, Shih T et al (2014) Transcellular degradation of axonal mitochondria. Proc Natl Acad Sci USA 111(26):9633–9638. https://doi.org/10.1073/pnas.1404651111

43. Kholmukhamedov A, Schwartz JM, Lemasters JJ (2013) Isolated mitochondria infusion mitigates ischemia-reperfusion injury of the liver in rats: mitotracker probes and mitochondrial membrane potential. Shock 39(6):543. https://doi.org/10.1097/SHK.0b013e318292300d

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.