

RESEARCH

Open Access



Age- and sex-associated alterations in hypothalamic mitochondrial bioenergetics and inflammatory-associated signaling in the 3xTg mouse model of Alzheimer's disease

Aida Adlimoghaddam^{1,2,3*}, Kyle M. Fontaine¹ and Benedict C. Albeni^{4,5,6*}

Abstract

Mitochondrial dysfunction and associated inflammatory signaling are pivotal in both aging and in Alzheimer's disease (AD). Studies have also shown that hypothalamic function is affected in AD. The hypothalamus may be a target for AD drugs given that mitochondrial alterations are observed in the hypothalamus. This study investigated how age and sex affect mitochondrial bioenergetics and inflammatory signaling in the hypothalamic mitochondria of 3xTg and control mice at 2, 6, and 13 months, aiming to enhance our understanding of these processes in aging and AD. Parameters included oxygen consumption rates, expression levels of subunits comprising mitochondrial complexes I-V, the enzymatic activity of cytochrome c oxidase (COX), transcription factors associated with inflammation such as NF- κ B, plkB- α , Nrf2, and other inflammatory biomarkers. Hypothalamic mitochondrial dysfunction was observed in 3xTg females as early as 2 months, but no changes were detected in 3xTg males until 6 months of age. In 3xTg mice, subunit expression levels for mitochondrial complexes I-II were significantly reduced in both sexes. Significant sex-based differences in COX activity were also observed at 13 months of age, with levels being lower in females compared to males. In addition, significant sex differences were indicated in NF- κ B, plkB- α , Nrf2, and other inflammatory biomarkers at different age groups during normal aging and AD progression. These findings highlight important sex differences in hypothalamic bioenergetics and inflammation, offering insights into potential new targets for preventing and/or treating AD.

Plain English summary

Alzheimer's disease (AD) is a gradual onset disorder, characterized by memory impairments. The greatest risk factor is age. In addition, almost two-thirds of Americans with AD are women, and reasons for this health disparity are unclear. While it's true that women live longer than men, longevity alone does not explain this disparity. Interestingly, studies have also shown that hypothalamic function is affected in AD, but it appears that men are affected differently than women. The hypothalamus regulates numerous functions, including sex hormone regulation, sleep/wake cycles, and energy expenditure, to name a few. In this study, we examined how changes

*Correspondence:

Aida Adlimoghaddam
aadlimoghaddam72@siu.edu
Benedict C. Albeni
balbeni@nova.edu

Full list of author information is available at the end of the article



© The Author(s) 2024. **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/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

in energy metabolism may be different in the hypothalamus in male vs. female genetically engineered mice that model AD pathology.

Highlights

- Hypothalamic mitochondrial dysfunction was observed in 3xTg females as early as 2 months, but no changes were detected in 3xTg males until 6 months of age.
- In 3xTg mice, sex-based differences were observed in the expression levels of subunits for mitochondrial complexes II and III.
- Significant sex-based differences in COX activity were also observed at 13 months of age, with levels being lower in females compared to males.
- Significant sex differences were indicated in NF- κ B, plkB- α , Nrf2, and other inflammatory biomarkers at different age groups during normal aging and AD progression.

Keywords Alzheimer's disease, 3xTg, Hypothalamus, NF- κ B, Nrf2, Bioenergetic profiling, Mitochondria, Inflammation

Introduction

Alzheimer's disease (AD) is a gradual onset, multifactorial, neurodegenerative disorder, characterized by progressive cognitive impairments [1, 2]. Emerging evidence now demonstrates that the earliest deficits in the pathological progression of AD are associated with impaired energy metabolism [3]. Energy metabolism is regulated by the central nervous system (CNS) [4]. Within the CNS, in particular, the hypothalamus regulates numerous metabolic functions, including energy expenditure [5], sex hormone regulation [6] sleep/wake cycles [7], and circadian rhythms [8], to name a few. Neuroimaging research suggests that atrophy in the hypothalamus, marked by a decrease in volume, is linked to AD pathology (A β 42, tau, and phosphorylated tau) and signals structural damage in this vital brain area, underscoring its significance in the disease's progression [9, 10]. Previous studies have demonstrated that the total cell population in the suprachiasmatic nucleus (SCN), located in the hypothalamus, declines with aging and is significantly reduced in AD [11, 12]. The decrease in SCN cell numbers may be linked to functional differences among the hypothalamic nuclei in AD [13]. Other studies using immunohistochemistry in AD brains confirmed the presence of neuronal loss, in the hypothalamus [9]. Also, abnormalities in hypothalamic metabolism and cerebral blood flow have been observed in the APP/PS1 mouse model of AD, occurring before the onset of cognitive decline [14]. In the Tg2476 mouse model of AD, neurons producing neuropeptide Y (NPY) neurons in the hypothalamus exhibited reduced responsiveness to appetite-regulating hormones leptin and ghrelin, a change also seen with A β treatment in normal mice [9, 15]. The interplay between hypothalamic dysfunction and AD pathology underscores the complex relationship between metabolic regulation, hormonal signaling, and neurodegenerative disease [16]. Interestingly, alterations in brain bioenergetics also intersect with inflammatory processes [17] and the hypothalamus is involved in regulating the

body's response to inflammation due to its role in regulating the immune system and coordinating various physiological processes [18]. The inflammatory responses vary between males and females [19], and sex differences exist in the prevalence of diseases, however; sex- and age-associated hypothalamic inflammation in AD is not completely understood. Emerging evidence suggests that nuclear factor kappa B (NF- κ B), which is a master switch and central regulator of inflammatory processes, is also functioning as a critical regulator of energy homeostasis and metabolic adaptation through upregulating mitochondrial respiration metabolism [20–24]. Another study found that the NF- κ B signaling pathway can negatively regulate mitochondrial Complex IV which is associated with mitochondrial oxygen consumption [25, 26]. However, whether there are hypothalamic NF- κ B-associated alterations during aging and the progression of AD remains unexplored. The consequence of sex-based differences on hypothalamic NF- κ B levels in AD also has not been examined.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is another key transcription factor that regulates oxidative stress that drives age-related pathologies [27]. In addition, it has been reported that Nrf2 declines with age and Nrf2 deficiency leads to mitochondrial dysfunction [28]. However, sex-specific transcriptional regulation of Nrf2 during normal aging and AD has not been studied completely.

Emerging evidence has also emphasized the potential of pro-inflammatory cytokines such as interleukin-1 β (IL- β), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6), as biomarkers for AD [29]. Interestingly, these cytokines are also influenced by the female sex hormone estrogen, which suppresses inflammation at high levels and promotes it at low levels [19]. Therefore, focusing on inflammatory markers provides several mechanistic approaches to better comprehend sex-based differences in AD. Further, cytokines known as interferons exhibit a major role in AD pathology [30]. For

instance, pre-treatment of human neuroblastoma cells with interferon (IFN- γ) significantly increased the sensitivity of neurons to the toxic effects of amyloid beta (A β) [31]. This suggests that IFN- γ may enhance neuronal degeneration in AD. Other studies observed an association between elevated IFN- γ levels and reduced cognitive decline, independent of A β levels [30, 32]. Additionally, higher IL-12p70 levels were linked with reduced tau pathology and neurodegeneration in participants with higher A β [33]. Monocyte chemoattractant protein-1 (MCP-1) is one of the key chemokines that mediate inflammation in AD [34]. A two-year clinical study indicated that greater severity and faster cognitive decline in AD are associated with higher levels of plasma MCP-1 [35]. Other cytokines and inflammatory markers such as IL-2, IL-4, IL-17 A, and IL-10 are major cytokines associated with the occurrence of AD [36–39]. However, sex- and age-related inflammatory changes in the hypothalamus have not been investigated.

In this study, we measured the age- and sex-associated differences in mitochondrial bioenergetic profiling and inflammatory-associated signaling in hypothalamic mitochondria from 3xTg and their controls at 2, 6, and 13 months of age. Parameters measured included mitochondrial oxygen consumption rates (OCR), alterations in OXPHOS, cytochrome c oxidase (COX) activity, transcription factors such as NF- κ B and its associated subunits (NF- κ B p50, p105, p65, p75), pI κ B- α , Nrf2, inflammatory biomarkers such as TNF- α , IFN- γ , IL-1 α , IL- β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p70, IL-13, IL-17 A, MCP-1, MIP-2, KC, LIX, and GM-CSF were evaluated in both males and females in 3xTg mice vs. age-matched male and female control mice at 2, 6, and 13 months old.

Materials and methods

Animals

In this study, we employed the triple-transgenic (3xTg #033930-JAX; Jackson Laboratories; Bar Harbor, Maine) mouse model along with the C57BL/6J-congenic background [40, 41]. The 3xTg mouse model of AD develops age-related cognitive impairments and amyloid-beta (A β) pathology by 6 months of age [33]. By 12 months, it also develops neuritic plaques and neurofibrillary tau tangles. To investigate the effect of age and sex-associated differences in mitochondrial deficits and also NF κ B signaling within the hypothalamus, a total of 60 mice (2 strains of animals (3xTg and C57BL/6) X 2 sexes of animals (males and females) X 5 biological replicates=60) were utilized. Both male and female 3xTg (AD) and C57BL/6 (control) mice were euthanized at 2 months, 6 months, and 13 months of age. Hypothalamic tissue was then dissected from the brain following decapitation. All animal procedures followed the guidelines of the Institutional Animal

Care and Use Committee (IACUC) standards. Groups of mice were given *ad libitum* access to food and water and housed on a 12-hour light/12-h dark schedule and 40% humidity.

Preparation of isolated mitochondria from hypothalamic tissue

The mice were humanely euthanized by performing decapitation, which was conducted while they were under isoflurane anesthesia. Hypothalamic tissues were then removed. Mitochondria derived from both 3xTg and control mice were extracted using specialized commercial isolation kits (110170, Abcam, Cambridge, Massachusetts, USA) as reported previously [24, 26, 42]. Briefly, tissues were initially rinsed with 1x Phosphate-buffered saline (PBS). Following this, they were homogenized in a mitochondrial isolation buffer (MIP) designed to preserve mitochondrial integrity. The resulting homogenates underwent centrifugation at 1,000 $\times g$ for 5 min at a temperature of 4 $^{\circ}C$. Then supernatants obtained from this step were then subjected to a second round of centrifugation at 10,000 $\times g$ for 20 min at 4 $^{\circ}C$. Pellets to isolate the mitochondrial fraction). The resultant pellets, which contained the isolated mitochondria, were resuspended in MIP. Subsequently, the protein concentrations in these samples were determined using a colorimetric DC protein assay kit (BioRad, Hercules, California, USA) following the protocol described previously [43].

Measurement of mitochondrial respiration rates

Real-time measurements of oxygen consumption rates (OCR) were conducted on the isolated hypothalamic mitochondria employing the Seahorse XF Analyzer (Agilent Technologies, Santa Clara, CA, USA) [24, 26, 44]. To assess mitochondrial respiration, freshly isolated mitochondrial protein (15 μg /well) was prepared in mitochondrial assay solution (MAS). This solution comprised 70 mM sucrose, 220 mM mannitol, 10 mM KH₂PO₄, 5 mM MgCl₂, 2 mM HEPES, 1 mM EGTA, and 0.2% BSA along with 2 mM malate and 10 mM pyruvate. The prepared mixture was then carefully transferred onto a Bioscience plate for analysis. Initial measurements of basal respiration were measured in the presence of malate (2 mM) and pyruvate (10 mM). Following this, coupled respiration was assessed by adding adenosine-diphosphate (ADP) at a concentration of 4 mM, which serves as an ATP synthase substrate. Subsequently, to evaluate coupled respiration ligomycin (2 μM), an ATP synthase inhibitor was then added. To evaluate maximal respiration, the protonophore carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP) as a respiratory chain uncoupler was added at 4 μM . To complete the analysis, rotenone (2 μM) as a specific inhibitor of complex I, and antimycin A (2 μM) which inhibits complex III

were added to effectively block mitochondrial respiration. All OCR data were then analysed with subtracting the non-mitochondrial respiration values. OCR readings were captured and analyzed using the Seahorse XF-24 software (Seahorse Biosciences, Billerica, MA, USA). To ensure accuracy, the OCR level was normalized based on total protein concentrations (36). These protein concentrations were determined using the DC Protein Assay (Bio-Rad, Hercules, California, USA). The final protein quantification was performed utilizing the ChemiDoc™ MP (Stain-Free™).

Western blot analysis

Initially, hypothalamic tissues were mixed with 4X Laemmli buffer and heated at 55 °C for eight minutes. Following this preparation, 15 µg per protein from each sample was carefully loaded into the wells of sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gels (Bio-Rad, Hercules, CA, USA). The proteins were separated by electrophoresis, which was conducted at 200 volts for a duration of 60 min. Once the electrophoresis was complete, the gels were activated using a ChemiDoc imaging system (Bio-Rad, Hercules, California, USA). Next, the proteins were transferred from gels into the nitrocellulose membranes (Bio-Rad, Hercules, California, USA) using the Trans-Blot Turbo Transfer System (Bio-Rad, Hercules, California, USA). Following a transfer, the total proteins present on the membranes were detected and visualized using the ChemiDoc imager. Following the imaging process, the nitrocellulose membranes were incubated in TBS-T buffer containing 5% bovine serum albumin (BSA) and incubated for one hour at room temperature to minimize non-specific binding. Subsequently, the membranes were treated with a blocking buffer, which consisted of 5% bovine serum albumin (BSA) dissolved in Tris-buffered saline with 0.1% Tween-20 (TBS-T). The membranes were immersed in this blocking buffer for one hour at room temperature to prevent any unintended bindings of antibodies to the membrane. Following blocking, the membranes were incubated with primary antibodies: Total OXPHOS Rodent WB Antibody Cocktail (ab110413, Abcam, Cambridge, Massachusetts, USA, 1:1000 dilution), NF-κB p50/p105 (ab32360, Abcam, Cambridge, UK; 1:1000), NF-κB p65 (ab16502, Abcam, Cambridge, UK; 1:1000), NF-κB p75 (ab108299, Abcam, Cambridge, UK; 1:1000), and pIκB-α (cs2859, Cell Signaling, Danvers, USA; 1:500). Following primary antibody incubation, the membranes were thoroughly washed with 1X TBS-T buffer. This washing process involved three separate washes, each lasting 15 min, to ensure the removal of any unbound primary antibodies and reduce background signals. Following the washes, the membranes were then incubated with HRP-conjugated secondary antibodies (Jackson ImmunoResearch

Laboratories, West Grove, PA, USA, 1:2000 dilution) prepared in TBS-T buffer containing 5% BSA for a duration of one hour at room temperature. Subsequent to the secondary antibody incubation, the membranes underwent another series of three washes with 1X TBS-T buffer each lasting 15 min. To visualize the protein bands, the membranes were treated with enhanced chemiluminescence (ECL) using the Bio-Rad Clarity Western ECL blotting kit (Bio-Rad, Hercules, California, USA). The chemiluminescent signals were then captured and analyzed using the ChemiDoc. Finally, to assess the relative levels of proteins, the quantification was normalized to the total protein content as previously described [45].

Measurement of cytochrome c oxidase activity

To evaluate the enzymatic activity of mitochondrial cytochrome c oxidase (COX) a spectrophotometric analysis was performed using Ultrospec 2100 pro; GE Healthcare. This assessment was conducted on mitochondrial preparations isolated from the hypothalamus and utilized an assay kit provided by Abcam (ab239711). The specific activity of COX was assessed using a colorimetric method. The involved measuring the decrease in absorbance at 550 nm, which reflects the oxidation of reduced cytochrome c, as detailed in prior studies [24, 26, 42].

Multiplex cytokines analysis

Hypothalamic tissues were harvested at three different time points (2, 6, and 13 months) from 3xTg and C57 mice and then stored at -80 °C. Further, tissues were homogenized in PBS with protease and phosphatase inhibitors. Homogenates were centrifuged at 10,000 × g for 10 min at 4 °C. Next, the protein concentrations of samples were measured using a colorimetric DC protein assay kit (Bio-Rad, Hercules, California, USA). Multiplex assay was performed at Eve Technologies by using the Bio-Plex 200 system (Bio-Rad Laboratories, Inc.), and a Milliplex Mouse Cytokine/Chemokine kit (Millipore) according to their protocol [46]. The 18-plex panel consisted of TNF-α, IFN-γ, IL-1α, IL-β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p70, IL-13, IL-17 A, MCP-1, MIP-2, KC, LIX, and GM-CSF. All samples were analyzed in triplicates. The standard curve regression was used to calculate the concentration of each target cytokine.

Statistical analysis

We used R version 4.4.1 (R Core Team 2024) and the following R packages along with the following R packages to conduct a full functional three-way ANOVA (age x genotype x sex): easystats v. 0.7.2 (Lüdtke et al. 2022), export v. 0.3.0 (Wenseleers and Vanderaa 2022), ggpubr v. 0.6.0 (Kassambara 2023a), ggstatsplot v. 0.12.4 (Patil 2021), gt v. 0.10.1 (Iannone et al. 2024), openxlsx v. 4.2.5.2 (Schauberger and Walker 2023), quarto v. 1.4

(Allaire and Dervieux 2024), rmarkdown v. 2.27 (Xie, Allaire, and Grolemond 2018; Xie, Dervieux, and Riederer 2020; Allaire et al. 2024), rstatix v. 0.7.2 (Kassambara 2023b), shiny v. 1.8.1.1 (Chang et al. 2024), tidyverse v. 2.0.0 (Wickham et al. 2019). Some of the data were subjected to statistical analysis using a two-way ANOVA, with subsequent Tukey *post-hoc* tests (GraphPad Prism 9, GraphPad Software) to assess the statistical significance of differences between the various groups. The results are presented as mean \pm standard deviation (SD) to reflect the variability within the data. A difference between or among groups was considered statistically significant if * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and **** $P \leq 0.0001$.

Results

Mitochondrial respiration rates

To assess alterations in bioenergetics, we conducted measurements of d OCR in freshly isolated mitochondria derived from the hypothalamus of female and male 3xTg at various age groups: 2, 6, and 13 months-old females (refer to Fig. 1A-D for females and Fig. 2A-D for males) alongside their respective controls. Our analysis revealed no significant differences in the baseline OCR of mitochondria from 3xTg mice across the different ages (2, 6,

and 13 months). We observed a notable reduction in coupled respiration was significantly decreased in the hypothalamic tissues of 2-month 3xTg females as illustrated in Fig. 1B. This significant decrease in coupled respiration was maintained through subsequent ages, including 6 and 13 months old as shown in Fig. 1C). A significant decline in maximal respiration was observed in the hypothalamic mitochondria of 3xTg females at 2, 6, and 13 months of age when compared to their control counterparts (Fig. 1B and C). In addition to basal, coupled, and maximal respiration, we also assessed spare respiratory. The data revealed that spare respiratory capacity in female 3xTg mice was significantly reduced throughout the progression of AD at 2, 6, and 13 months old relative to age-matched control females (as shown in Fig. 1A-C). This reduction suggests that the hypothalamus in 3xTg females is less able to meet increased energy demands associated with disease progression. Despite these findings, no significant differences were detected in coupling efficiency defined as coupled respiration relative to basal levels- between 3xTg and control female mice (Table D). In 3xTg males, mitochondrial bioenergetics parameters at basal, coupled, and maximal respiration, as well as spare respiratory capacity levels, showed no significant

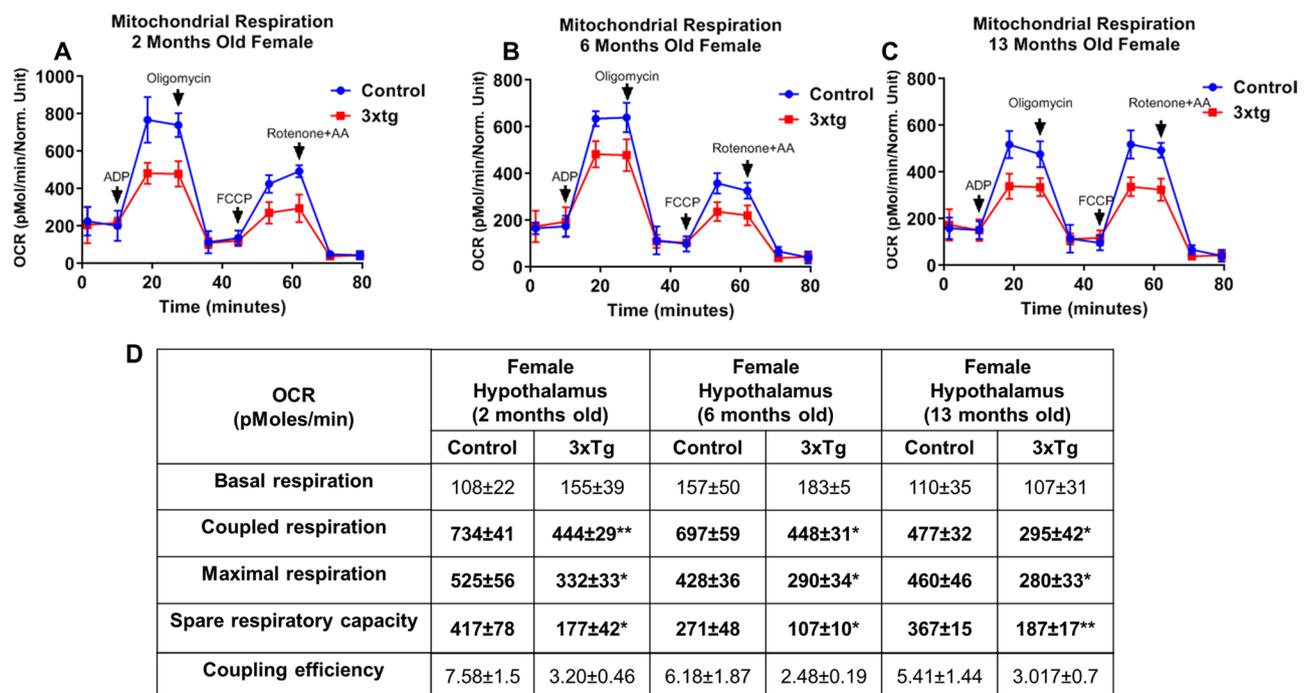


Fig. 1 Oxygen consumption rates (OCR) in hypothalamic mitochondria, comparing 3xTg females with control females. Representative measurements of OCR in isolated hypothalamic mitochondria from 3xTg and age-matched control females at three different ages: 2 months (A), 6 months (B) and 13 months of age (C). The data included real-time kinetics, which were recorded at baseline and following the addition of ADP, oligomycin, FCCP, and rotenone/antimycin A. Table D provides a detailed summary of the bioenergetic parameters, including basal, coupled, maximal respiration, spare respiratory capacity, and coupling efficiency for both 3xTg and aged-matched control females at the ages of 2, 6 and 13 months. Significant reductions were observed in the coupled, maximal respiration, and spare respiratory capacity in the 3xTg females compared to the aged-matched control group at 6 and 13 months of age. The results are reported as OCR values in pmoles/min, with all data expressed as mean \pm SD of $n=5$ replicates (* $P \leq 0.05$, ** $P \leq 0.01$) analyzed by two-way ANOVA

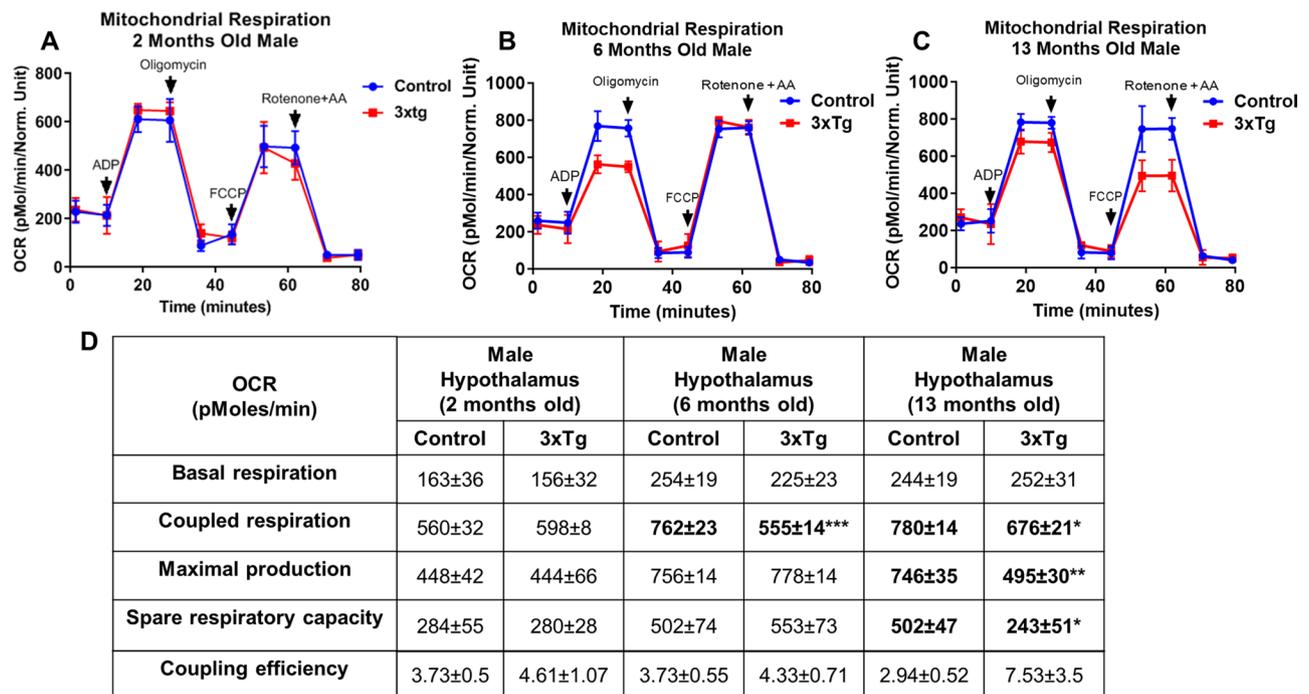


Fig. 2 Oxygen consumption rates (OCR) in hypothalamic mitochondria, comparing 3xTg males with control males. Representative measurements of OCR in isolated hypothalamic mitochondria from 3xTg and age-matched control males at three different ages: 2 months (A), 6 months (B), and 13 months of age (C). Calculated parameters, which were recorded at baseline and following the addition of ADP, oligomycin, FCCP, and rotenone/antimycin A. Table D provides a detailed summary of the bioenergetic parameters, including basal, coupled, maximal respiration, spare respiratory capacity, and coupling efficiency for both 3xTg and aged-matched control males at the ages of 2, 6 and 13 months. No significant variations were found in the bioenergetic profiles across different ages or between the 3xTg and control strains. All data are expressed as OCR in pmoles/min, and values are expressed as mean \pm SD of $n=5$ replicates (* $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$) analyzed by two-way ANOVA

changes at 2 months of age compared to control males (Fig. 2A). However, by 6 months, there was a significant decrease in coupled respiration. At 13 months, reductions were observed in coupled respiration, maximal respiration, and spare respiratory capacity compared to age-matched control males (Fig. 2B-C). All measurements are reported as OCR in pmoles/min, with values expressed as mean \pm SD of $n=4-5$ replicates. To further investigate molecular mechanisms potentially responsible for the diminished mitochondrial function in 3xTg, we conducted a Western blot analysis on various proteins in the hypothalamus.

Mitochondrial proteins

In an effort to investigate how oxidative phosphorylation (OXPHOS) machinery is affected by sex and age during normal aging and the progression of AD, we examined several mitochondrial-associated proteins in the hypothalamus. We conducted immunoblotting and relative quantification for protein levels for the subunits of OXPHOS complexes I through V, with normalization to total protein content (Fig. 3A-F). The immunoblotting results indicated that there were significant increases in the level of OXPHOS protein subunits specifically within complexes II, III, and V in the hypothalamus of

3xTg mice (Fig. 3B, C, E). The expression of complex II was significantly reduced in female 3xTg mice compared to males. No sex-based differences were found in control mice across any age group (Fig. 3B). Complex III expression varied significantly by sex, with female 3xTg mice displaying higher levels at 2 and 6 months, whereas control mice showed an increase in this protein only at 13 months (Fig. 3C). No significant differences in complex V expression were observed in the 3xTg mice. However, at 2 months of age, control mice exhibited sex-based differences, with higher levels in females, while no notable differences were found in other age groups (Fig. 3E). Furthermore, our analysis revealed that there were no statistically significant variations in the levels of protein subunits for complexes I and IV, irrespective of genotype or sex, across all age groups examined (Fig. 3A and D) (Fig. 4).

Cytochrome C oxidase (COX) activity

Mitochondrial function was evaluated by measuring the enzymatic activity of COX in both female and male 3xTg mice and age-matched control mice. At 13 months of age, female 3xTg mice exhibited significantly lower COX activity compared to males (Fig. 4). No significant

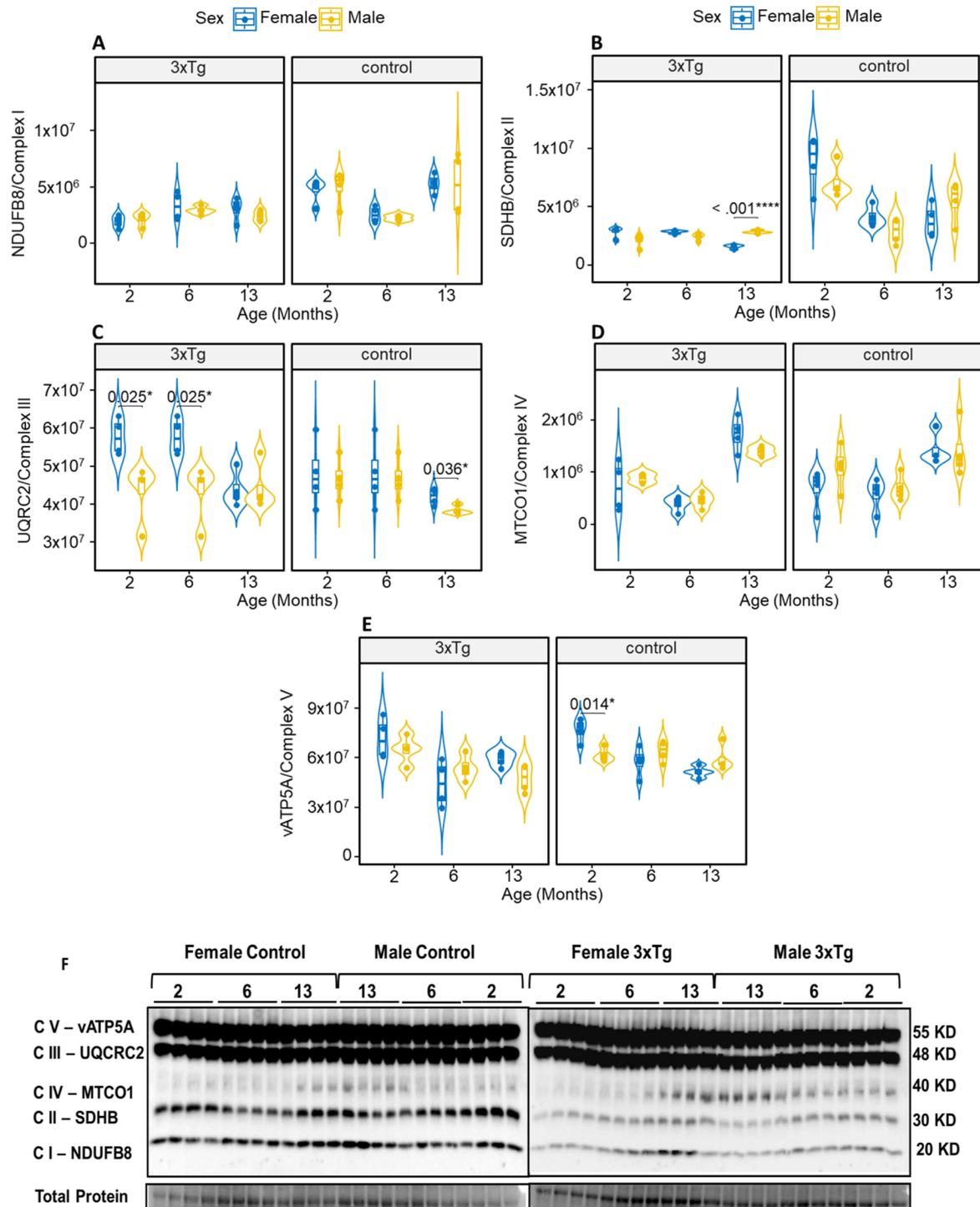


Fig. 3 OXPHOS levels in hypothalamic mitochondria of 3xTg male and female mice compared to controls. Western blot images show representative bands for NADH dehydrogenase beta subcomplex subunit 8 of Complex I (NDUFB8; **A**), succinate dehydrogenase subunit B of complex II (SDHB; **B**), cytochrome b-c1 complex subunit 2 of complex III (UQCRC2; **C**), cytochrome c oxidase subunit 1 of complex IV (MTCO1; **D**), and ATP synthase subunit alpha of complex V (ATP5A; **E**). Quantification of these proteins, normalized to total protein (**F**) for 3xTg female and male mice and their respective controls at 2, 6, and 13 months of age. Asterisks indicate statistical significance compared to corresponding control groups. Results are presented as mean \pm SD of $n=4$ per group, with significance levels denoted as $*P \leq 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$, analyzed using functional three-way ANOVA. In female 3xTg mice, complex II expression was significantly reduced compared to males, while no sex-based differences were observed in control mice across all age groups (**A**). Complex III levels were higher in female 3xTg mice at 2 and 6 months, whereas control mice showed an increase only at 13 months (**B**). No significant differences in complex V expression were noted in 3xTg mice; however, at 2 months, control mice exhibited higher levels in females, with no notable differences in other age groups (**C**). Additionally, there were no significant variations in the levels of protein subunits for complexes I and IV across all age groups, regardless of genotype or sex (**D**)

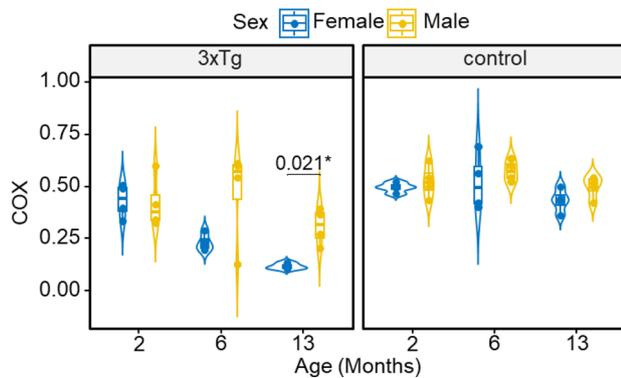


Fig. 4 Cytochrome c oxidase (COX) activity in 3xTg vs. control mice. The activity of COX was in hypothalamic mitochondria from 3xTg female and male mice, alongside their control counterparts, at 2, 6, and 13 months of age. Statistical significance is indicated by asterisks, with results shown as mean \pm SD of $n=5$ per group ($*P \leq 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$). At 13 months, COX activity was significantly reduced in females 3xTg relative to males

differences in COX activity were observed in control mice with respect to age or sex.

Inflammatory proteins

To examine the presence of potential age-related sex differences on transcriptional regulation during normal aging and AD, immunoblots of several key transcription factor proteins or their subunits (NF- κ B p50, p105, p65, pI κ B- α , and Nrf2) implicated in neuroinflammatory signaling were measured in isolated hypothalamic mitochondria from 3xTg female and male mice vs. their respective controls at 2, 6, and 13 months of age (Fig. 5A-E). Relative levels of NF- κ B subunits and Nrf2 (Fig. 5A-C, upper panel) were normalized to total protein (Fig. 5A-C, lower panel). Significant sex differences were detected in 3xTg NF- κ B p50 in all age groups, where levels were elevated in females vs. males (Fig. 5A). We noted similar findings in control mice, but these were only evident at 6 and 13 months of age. Protein levels of NF- κ B p105 were significantly different as a function of sex in 2 months old 3xTg mice, where levels were elevated in females vs. males (Fig. 5B). This increase was not sustained at later stages, as we found no sex differences in protein level of NF- κ B p105 in older 3xTg female mice (Fig. 5B). Also, no significant sex differences in NF- κ B p105 levels were observed in control mice across any age group (Fig. 5B). Protein levels of NF- κ B p65 differed significantly by sex in all age groups studied, showing a reduction in male 3xTg mice compared to female 3xTg mice (Fig. 5C). However, no significant sex differences in NF- κ B p65 were found in control mice in any age group (Fig. 5C). Significant sex differences were detected in 3xTg pI κ B- α at 6 and 13 months, where levels were elevated in females vs. males (Fig. 5D). The expression of pI κ B- α decreased in control mice, with a reduction observed in males only

at 13 months (Fig. 5D). Significant sex differences in Nrf2 levels were observed in both control and 3xTg mice at 2 months, with levels downregulated in females compared to males (Fig. 5E). However, the expression of Nrf2 increased in female 3xTg mice by 13 months, while levels in control mice remained unchanged at that age (Fig. 5E).

Cytokine/Chemokine assay

Hypothalamic tissues were collected from both female and male control and 3xTg mice at 2, 6, and 13 months of age (Fig. 6A-C). These samples were homogenized and analyzed for the concentrations of various cytokines/chemokines using the multiplex array (Eve Technologies, Calgary, Alberta, Canada). At 6 months of age, hypothalamic IL-1 α levels were significantly lower in female controls compared to males. IL-2 levels were notably decreased in male 3xTg mice compared to male controls at 2 months of age. Conversely, female 3xTg mice exhibited a significant increase in IL-2 expression compared to female controls at both 2 and 6 months of age. Significant sex-based differences in IL-2 levels were observed only in the control group at these time points. No significant differences in IL-2 levels were found at 13 months of age. At 6 months of age, IL-6 levels were significantly lower in female controls compared to males. No significant differences in IL-6 level were found at 2 or 13 months of age. Female 3xTg mice showed a significant increase in IL-17 A levels compared to male controls, while male 3xTg mice also had significantly higher IL-17 A levels compared to their male controls. No significant differences in IL-17 A levels were found at 2 or 13 months of age. The expression of KC was found to be increased in the female 3xTg as compared to female controls at 2, 6, and 13 months of age. Also, sex-based differences were observed in KC expression, with higher levels in female 3xTg mice compared to males at 6 months. LIX levels were significantly elevated in male 3xTg mice compared to male controls at 2 months of age. No significant differences in LIX level were found at 6 or 13 months of age. No statistically significant differences, as a function of genotype or sex, were detected in expression levels of GM-CSE, IFN- γ , IL- β , IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p70, IL-13, IL-17 A, MCP-1, MIP-2, and TNF- α in any age group (Fig. 6A-C).

Discussion

Changes in hypothalamic structure and function in AD are increasingly recognized, particularly regarding how early alterations may impact energy metabolism. Evidence suggests that mitochondrial dysfunction and inflammation are key factors in the onset and progression of AD, potentially resulting in pathology, synaptic dysfunction, and neuronal damage [47, 48]. An increasing body of evidence suggests that sex differences may

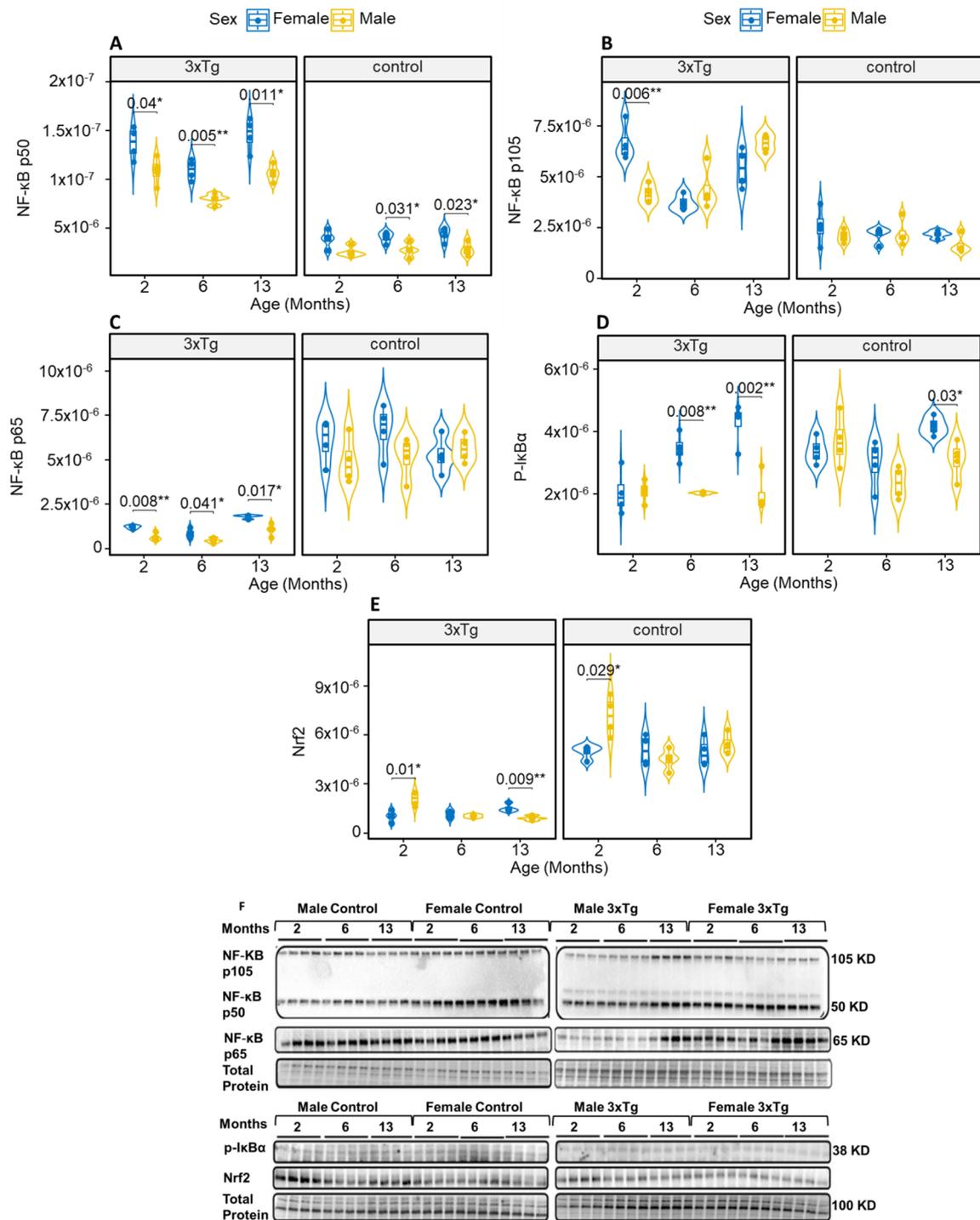


Fig. 5 Levels of NF-κB subunits, plkB-α, and Nrf2 in the hypothalamus of 3xTg mice vs. control mice. Western blot analysis shows the levels of NF-κB subunits (p50 (A), p105 (B), p65(C), plkB-α (D), Nrf2 (E) in hypothalamic tissues from 3xTg female and male mice compared to their control counterparts at 2, 6, and 13 months of age. Quantification of these proteins was normalized to total protein (F). Asterisks indicate statistical significance compared to corresponding control groups. Results are presented as mean ± SD of $n=4$ per group with significance levels marked as $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, and $****P \leq 0.0001$, and analyzed by three-way ANOVA. Significant sex differences in NF-κB and Nrf2 protein levels were observed in 3xTg mice across various ages. NF-κB p50 levels were elevated in females compared to males in all age groups (A). For NF-κB p105, females exhibited higher levels than males at 2 months, but no differences were found in older mice (B). NF-κB p65 showed reduced levels in male 3xTg mice across all ages, with no differences in control mice (C). Elevated plkB-α levels were noted in females at 6 and 13 months, while control males only showed a reduction at 13 months (D). Nrf2 levels were downregulated in females at 2 months but increased in female 3xTg mice by 13 months, with control levels remaining stable (E)

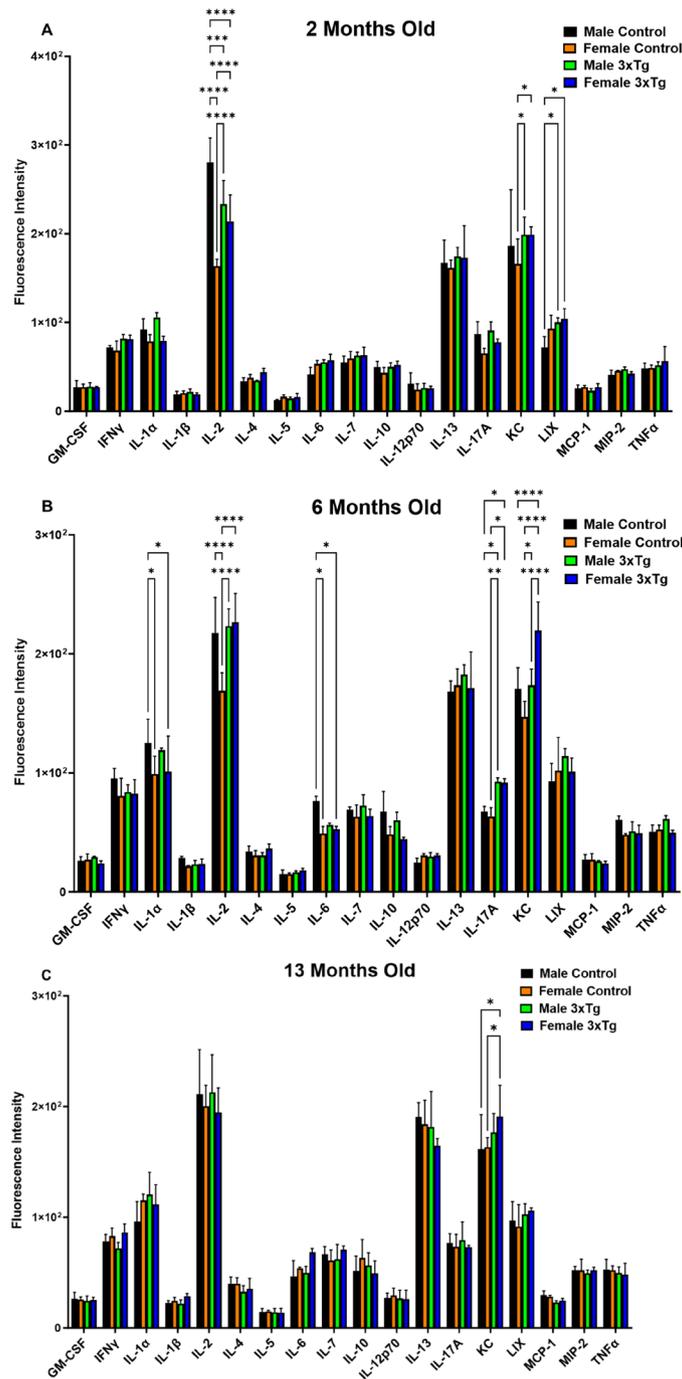


Fig. 6 Levels of hypothalamic inflammatory biomarkers in 3xTg vs. control mice. Levels of GM-CSF, IFN- γ , IL-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p70, IL-13, IL-17 A, KC, LIX, MCP-1, MIP-2, and TNF- α were assessed in hypothalamic tissues from both male and female 3xTg mice and their age-matched control counterparts at 2 (A), 6 (B), and 13 (C) months of age. Asterisks vs. corresponding control groups represent the statistical significance. Results are expressed as mean \pm SD of $n=3$ replicates (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and **** $P \leq 0.0001$) analyzed by two-way ANOVA. At 6 months of age, hypothalamic IL-1 α levels were significantly lower in female controls compared to males. IL-2 levels were notably decreased in male 3xTg mice compared to male controls at 2 months of age. Conversely, female 3xTg mice exhibited a significant increase in IL-2 expression compared to female controls at both 2 and 6 months of age. Significant sex-based differences in IL-2 levels were observed only in the control group at these time points. At 6 months of age, IL-6 levels were significantly lower in female controls compared to males. No significant differences in IL-6 levels were found at 2 or 13 months of age. Female 3xTg mice showed a significant increase in IL-17 A levels compared to male controls, while male 3xTg mice also had significantly higher IL-17 A levels compared to their male controls. KC expression was higher in female 3xTg mice compared to female controls at 2, 6, and 13 months, with increased levels also observed in females relative to males at 6 months. LIX levels were significantly elevated in male 3xTg mice compared to male controls at 2 months. No significant differences in the expression levels of other inflammatory markers were observed based on genotype or sex in any of the age groups studied

significantly influence the variations in AD prevalence including behaviour, cognitive performance, disease progression, prognosis, and underlying pathology [49–51]. For example, a previous study from our lab revealed sex differences in memory performance, showing that 3xTg females experienced memory impairments earlier than males [44]. In the current study, we observed sex-based differences in mitochondrial function. In particular, we found that mitochondrial dysfunction in female 3xTg mice occurs as early as 2 months of age, whereas males experience this dysfunction at a much later stage. Consistent with our findings, a recent study also identified sex-specific metabolic phenotypes in 3xTg mice. Notably, female 3xTg mice displayed metabolic disturbances opposite to those observed in males, characterized by increased body and fat mass as well as impaired glucose tolerance, while the study reported that although male and female 3xTg mice were metabolically similar at 3 months of age, by 6 months, male mice with AD exhibited decreased weight gain, lower adiposity, and reduced blood glucose levels after a glucose challenge, in contrast to the females [52]. Other studies have shown that A β load increases with age in 3xTg mice, beginning at 6–8 months, with females consistently exhibiting higher A β levels [53]. Furthermore, neuroimaging research has indicated that brain atrophy rates are higher in female AD patients than their male counterparts, correlating with A β and tau changes and ApoE4 allele status [54]. Research also shows that, at 12 months, females show more tau tangles in their brains than males [54]. Another study reported that glucose intolerance and A β pathology worsen with age in 3xTg mice, especially in females [55]. Further, sex differences in mitochondrial outcomes were noted in the rodent model of traumatic brain injury (TBI) [56]. Specifically, female TBI mice exhibited early impairment at 12 h after severe controlled cortical impact (CCI), whereas mitochondria from injured male mice showed impairment at 24 h post-CCI [56]. These findings indicate that mitochondria in male and female brains can exhibit distinct profiles, highlighting various functional and structural differences. Overall, early mitochondrial deficits in females could accelerate A β accumulation, leading to earlier cognitive impairments and increased tau tangles. This could suggest a connection between impaired mitochondrial function and the increased vulnerability of females to neurodegenerative pathology, implying that sex-specific biological factors may influence the timing and progression of mitochondrial impairment in AD. By highlighting these differences, particularly the sex-based disparities in mitochondrial dysfunction, our study advances the field by emphasizing the need for tailored research strategies and potential interventions that take sex into account in the context of neurodegenerative diseases.

Mitochondrial dysfunction can trigger and sustain inflammatory responses through interconnected pathways, making it a key factor in numerous diseases and conditions [42, 57]. For instance, mitochondrial dysfunction can lead to increased production of reactive oxygen species (ROS), activating inflammatory pathways [58]. Additionally, disrupted cellular metabolism due to mitochondrial malfunction alters metabolite balances and signaling pathways, such as NF- κ B, which regulates the expression of numerous inflammatory genes [59]. The correlative connection between mitochondrial components and inflammation becomes evident by identifying various metabolic by-products and molecules such as damage-associated molecular patterns (DAMPs) [60]. These molecules include mtDNA, mitochondrial ROS, mitochondrial calcium, ATP, cytochrome c, TFAM, cardiolipin, and iron [61]. The release of these substances into the cytosol or extracellular space triggers inflammation, illustrating how mitochondrial dysfunction contributes to inflammatory responses. Proteomics, genomics, and bioinformatics analysis demonstrated the disruption of mitochondrial oxidative phosphorylation machinery as hallmarks of AD [62, 63]. Additionally, the incidence of AD is higher in women compared to men, underscoring the need for further research into sex-specific differences in the development of the disease.

The current study examined mitochondrial bioenergetic profiles, along with levels of OXPHOS proteins, and COX activity in male and female control and 3xTg mice during normal aging and AD progression. We observed significant reductions in basal respiration, coupled respiration, maximal respiration, and spare respiratory capacity in female 3xTg mice compared to controls as early as 2 months of age. In contrast, declines in mitochondrial bioenergetic parameters in males were not observed until 6 months of age. Early metabolic deficits in mitochondrial function, particularly observed in females, suggest that there may be sex-specific differences in how mitochondrial function develops or is maintained during the early stages of life. Another study shows that alterations in hypothalamic bioenergetics may be attributed to reduced energy demands, potentially stemming from decreased hypothalamic neurogenesis reported in 3xTg mice [64].

In alignment with our OCR findings, we found significant differences in the expression of mitochondrial complex II and III in 3xTg mice. It has been suggested that levels of ETC subunit proteins can serve as indicators of OXPHOS, and therefore also serve as an index of mitochondrial respiration. Other studies showed a decrease in complex II, which leads to decreased ATP content in the APP/PS1 mouse model [65, 66]. Human studies have also demonstrated that patients with mild cognitive impairment (MCI) and early AD exhibit changes in mitochondrial gene expression and function [67]. Another study

demonstrated that mitochondrial genes related to complex I and II of the OXPHOS pathways are downregulated in both early and advanced stages of AD and PD which can substantially impair ATP production and mitochondrial respiration in synaptic regions [68–70]. This impairment contributes to the synaptic dysfunctions commonly associated with these diseases. We observed a trend of reduced COX activity in female 3xTg mice compared to males across all age groups. At 13 months, COX activity was significantly lower in female 3xTg mice than in their male counterparts. This sex-based difference suggests that the reduced COX function in the ETC may be more pronounced in females, potentially leading to a greater decline in mitochondrial respiration and more severe disruptions in energy metabolism. In line with our findings, a study on Parkinson's disease (PD) has also reported sex-specific effects on COX in astrocytes [71]. Another study has shown deficiencies in both Complex I and COX, in the substantia nigra neurons of patients with PD [72]. They proposed that COX deficiency appears only in the presence of Complex I deficiency and that this specific molecular pattern might be linked to changes in mitochondrial DNA (mtDNA) [72]. Also, another research has shown that a reduction in mtDNA content in the skeletal muscle of women at the onset of menopause may provide evidence for sexual dimorphism in bioenergetic and metabolic processes [73]. Quantitative proteomics studies have revealed significant alterations in mitochondrial proteomes, redox proteins, ATP synthase, and COX in females [74]. These findings suggest that females may be at greater risk for AD progression compared to men, potentially due to their distinct metabolic profiles and hormonal influences that affect mitochondrial function and oxidative stress responses.

Metabolic signals originating from mitochondria can regulate the inflammatory response [75]. In both normal aging and neurodegenerative diseases, certain inflammatory factors can be broadly neurotoxic. These responses are driven by positive feedback loops between microglia and astrocytes. When neurons are damaged, they release ATP and other DAMPs that activate glial cells, leading to the further release of inflammatory factors by astrocytes [76]. This cycle exacerbates the neurotoxic environment, accelerating neuronal damage and disease progression.

Inflammaging is a persistent inflammatory state associated with neurodegenerative diseases and cognitive decline in older adults [3, 6, 7]. Additionally, sex-specific variations in inflammasome proteins have been observed in different disease models [22, 34, 35]. However, a comprehensive comparison of inflammaging between male and female subjects has been missing.

In this study, we explored how sex and age influence the expression of transcription factors, inflammation-related proteins, cytokines, and chemokines in the

hypothalamic tissue of both young and aged male and female 3xTg mice, as well as controls, throughout normal aging and neurodegeneration. One of the transcriptional factors we measured was NF- κ B, a central inflammatory regulator with diverse effects upon activation. NF- κ B can exert neuroprotective effects in neurons while promoting inflammatory responses in glial cells [77]. By assessing NF κ B expression, we aimed to understand its dual role in modulating inflammation and its impact on normal aging and neurodegeneration in our AD mouse model. To this end, we found a robust upregulation of NF- κ B p50 and 105 in the 3xTg as compared to controls, which could contribute to a greater inflammatory response and/or more rapid disease progression. Interestingly, the overexpression of NF- κ B p50 occurred in a sex-dependent manner. NF- κ B is known to regulate energy metabolism by balancing glycolysis and mitochondrial respiration [23]. In neurodegenerative diseases, its activation can be triggered by a cascade of signals from damaged neurons and glial cells. Confocal studies from our group and others have confirmed elevated levels of NF- κ B p50 in AD astroglia compared to controls [24, 78]. Additionally, human studies have also demonstrated increased NF- κ B p50 levels in the cerebral cortex of individuals with AD [79]. Another human study reported an increase in the synthesis of the NF- κ B precursor, p105, along with the activation of NF- κ B in AD brains [80]. Further, we found a reduced level of NF- κ B p65 in 3xTg compared to controls, indicating widespread alterations in this AD model that are sex-independent. This reduction in NF- κ B p65 is significant as it can influence the expression of mitochondrial genes critical for energy production and cellular function. According to Cogswell et al. (2003), the NF- κ B pathway can regulate mitochondrial gene expression, including the COX III subunit, which is essential for complex IV of the mitochondrial ETC [25]. Additional research has highlighted the role of NF- κ B in regulating various mitochondrial genes, including COX I and cytochrome b [81]. Specifically, studies have shown that the NF- κ B p65 subunit can reduce levels of mtDNA-encoded cytochrome b mRNA in human cells in the absence of p53 [82]. Collectively, these findings suggest that NF- κ B signaling plays a crucial role in modulating the enzymatic activity of respiratory ETC complexes. Interestingly, we found that female 3xTg mice exhibited higher levels of NF- κ B subunits at a later stage compared to male 3xTg. This sex-based difference in NF- κ B subunit levels was possibly associated with more pronounced neuroinflammation in females. Additionally, we found downregulation of the NF- κ B inhibitor, I κ B, in 3xTg mice which might be a regulatory mechanism to maintain balanced NF- κ B activity. We observed a significant downregulation of the pI κ B- α in 3xTg, which corresponds with decreased activity of the NF- κ B p65 subunit. The decreases might

reflect a compensatory or initially protective response to AD. Similar to NF- κ B p65, pI κ B- α levels were found to be significantly lower as a function of sex at 6 and 13 months of age. These findings highlight potential sex-specific variations in the inflammatory response in AD.

Another transcription factor we examined was Nrf2, which plays a crucial role in neurodegeneration and interacts with NF- κ B [28]. In this study, we observed sex differences in Nrf2 expression in the 3xTg and control groups at 2 months of age, where females showed a reduction. In contrast, at 13 months of age, 3xTg females exhibit higher Nrf2 levels compared to males. Accumulating evidence on AD, PD, Huntington's disease (HD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) have demonstrated that the loss of Nrf2 exacerbates neurodegenerative symptoms [83]. This exacerbation includes elevated ROS, increased inflammation, decreased OXPHOS, synaptic plasticity, memory function, and proteotoxicity, all of which negatively impact neuronal and glial function and viability. Previous research has reported contrasting expression patterns between NF- κ B p65 and Nrf2 [84, 85]. In contrast, our findings show decreases in hypothalamic NF- κ B p65 and Nrf2 levels. Our results suggest a dynamic regulation of NF- κ B p65 and Nrf2, where these transcription factors exhibit similar expression patterns under physiological and pathological conditions.

Several lines of research demonstrate that inflammation not only plays a critical role in accelerating the progression of neurodegenerative diseases, but also serves as a key initiator of these conditions. Both male and female immune responses undergo significant changes throughout the lifespan [19]. We observed a significant increase in chemokine KC (Keratinocyte-derived Chemokine, also known as C-X-C motif chemokine 1 (CXCL1) in female 3xTg mice compared to female controls at 2, 6, and 13 months of age. We also observed that the differences in KC chemokine expression at 6 months are sex-dependent, with higher levels found in female 3xTg mice compared to 3xTg males. Another study found that the expression of this chemokine is markedly increased in APP/PS1 transgenic mice. Additionally, treatment with an anti-CXCL1 antibody significantly impairs the accumulation of CD11b+CD45hi cells recruited from peripheral blood in the brains of these APP/PS1 mice. These results suggest that elevated CXCL1 levels in monocytes might contribute to the heightened presence of these cells in the brains of transgenic mice with AD. Interestingly, CXCL1 has been shown to facilitate the migration of monocytes from the bloodstream into the brain in AD, particularly in response to A β deposition [86]. This process can intensify inflammation and may play a role in accelerating the progression of AD.

Our analysis also showed that there was an increased upregulation of LIX (Lymphotactin, also known as CCL1) in 3xTg groups compared to controls at 2 months of age. There is substantial evidence linking disruptions in CXCL1 signaling to the pathomechanism of AD. For example, a previous study showed an increase in CCL1 mRNA and protein expression in the brains of APP/PS1 mice vs. controls [87]. Monocytes collected from AD patients overexpress CXCL1. When human brain microvascular endothelial cells are treated with A β , they show increased expression of CXCR2 and this interaction with CXCL1 contributes to the disruption of the blood-brain barrier (BBB) and enhances the migration of monocytes into the brain in AD patients [86]. Likewise, CXCL1 was found to trigger the phosphorylation of tau protein, leading to the formation of varicosities or bead-like structures along the neurites. This tau cleavage was also observed after intrahippocampal microinjection of lentiviral CXCL1 in aged mice [88]. Importantly, the expression of chemokines such as CXCL1 and CCL1 is modulated by NF- κ B in many diseases such as cancer, diabetes, and neurological disorders [89–93]. Therefore, it is suggested that the overexpression of CXCL1 and CCL1 in AD may further drive NF- κ B activation by intensifying inflammation, establishing a feedback loop that could worsen the inflammatory response. Our analysis showed that there were sex-based differences with IL-2 levels being lower in female controls than in males at 2 and 6 months of age. Additionally, the expression of this interleukin decreased in male 3xTg compared to male controls at these time points.

These findings align with earlier observations of reduced IL-2 levels in hippocampal tissues derived from AD patients and APP/PS1 mice [94, 95]. These findings suggested that a decline in IL-2 is linked to a diminished immune response. Other investigations have shown that IL-2 knockout mice experience difficulties in learning and memory formation, along with altered hippocampal development [96]. Other investigations show that IL-2 treatment improves AD pathology in APP/PS1 mice by enhancing Treg activation and increasing astrocytic activity, which helps clear A β and reduce plaque formation. It also reduces the A β 42/A β 40 ratio, potentially protecting neurons [97]. In APP/PS1 mice, IL-2 activates microglia, further aiding in disease management [98]. IL-2 knockout mice exhibit learning and memory deficits and altered hippocampal development [99]. Another study reported that IL-2 treatment in APP/PS1 mice supports recovery from memory deficits by promoting brain tissue remodelling, including improved synaptic plasticity [36]. These findings suggest an association among inflammation, A β pathology, and cognitive functions.

In our study, we observed a decrease in IL-1 α and IL-6 levels specifically in 6-month-old female 3xTg mice and

controls, with no such change in males. This finding contrasts with clinical studies, which report increased levels of these interleukins in the brains of patients with MCI and AD [100, 101]. The reduction in IL-1 α and IL-6 in females may reflect a compensatory or initial neuroprotective response by the affected cells. This altered balance in IL-1 α could suggest unique regulatory mechanisms or feedback responses in females, potentially influencing the neuroinflammatory environment.

At 6 months of age, female and male 3xTg mice showed a significant increase in IL-17 A levels compared to their prospective controls. This finding aligns with other studies that report elevated IL-17 A levels in the APP/PS1 mouse model. These studies also show that increased IL-17 A enhances TNF- α secretion by microglia through the NF- κ B signaling pathway, thereby worsening neuroinflammation [102, 103]. Pre-clinical and clinical data suggest that IL-17 A promotes the formation of A β plaques and tau, impairs microglial phagocytosis, and triggers cognitive and synaptic deficits in both AD models and patients [39, 102]. Further, inhibiting IL-17 A has been shown to reduce AD pathology markers in the brains of AD patients, mitigate neuroinflammation, and slow the progression of the disease. These repeated observations of elevated IL-17 across various models and studies underscore its potential importance as a target for both understanding and potentially modulating AD progression and associated mechanisms.

Overall, the distinctive sex-based variations in cytokine and chemokine profiles observed throughout normal aging and across the stages of AD (early, middle, and late) highlight the potential of these factors as insightful biomarkers for monitoring disease progression and development.

Conclusions

As we age, the hypothalamus, vital for regulating metabolism and maintaining balance, experiences significant changes. Early mitochondrial dysfunction and chronic inflammation in this region disrupt its normal function, which, coupled with the broader metabolic shifts and inflammatory processes of aging, plays a crucial role in the development and progression of AD. This study indicates that females may experience more pronounced mitochondrial and metabolic changes early in the disease, along with sex-based differences in inflammation during AD progression. Integrating sex and age into AD research is essential for accurately assessing potential treatments and improving the relevance of preclinical results. This approach ensures a more comprehensive understanding of AD and supports the development of personalized therapies, ultimately enhancing patient care by tailoring treatments to individual needs and improving overall outcomes.

Acknowledgements

Special thanks given to Dr. Michael Bernstein, Sean McGinity, and Cade Meiss for assisting with data analysis.

Author contributions

AA co-created the idea, designed the project, performed all the experiments, wrote the main manuscript, and co-supervised its execution. KMF assisted with writing and editing the manuscript and assisted with the data analyses. BCA co-designed the project and co-supervised the work. All authors reviewed and approved the final version of the manuscript.

Funding

Funding was received from the following sources that supported this study: Alzheimer's Association (AARF-22-967198) and Research Manitoba award to Dr. Adlimoghaddam. Support also from the St. Boniface Hospital Research Foundation (Grant Nos.1406–3216 and 1410–3216), CIHR (PJT-162144), NIH R16 (1R16NS134540-01), the Alzheimer's Society of Manitoba, and the Honourable Douglas and Patricia Everett, Royal Canadian Properties Limited Endowment Fund (Grant No. 1403–3131) to Dr. Albenis.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

No humans were involved in this study. All animal procedures were approved by an animal protocol and also followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) standards.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Department of Neurology, Dale and Deborah Smith Center for Alzheimer's Research and Treatment, Neuroscience Institute, Southern Illinois University School of Medicine, Springfield, IL, USA

²Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL, USA

³Department of Medical Microbiology, Immunology and Cell Biology, Southern Illinois University School of Medicine, Springfield, IL, USA

⁴Department of Pharmaceutical Sciences, Barry & Judy Silverman College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL, USA

⁵Division of Neurodegenerative Disorders, St. Boniface Hospital Albrechtsen Research Centre, Winnipeg, MB, Canada

⁶Department of Pharmacology & Therapeutics, Max Rady College of Medicine, University of Manitoba, Winnipeg, MB, Canada

Received: 28 August 2024 / Accepted: 7 November 2024

Published online: 25 November 2024

References

- McKeith I, Cummings J. Behavioural changes and psychological symptoms in dementia disorders. *Lancet Neurol*. 2005;4(11):735–42.
- Adlimoghaddam A, Roy B, Albenis BC. Future trends and the Economic Burden of Dementia in Manitoba: comparison with the Rest of Canada and the World. *Neuroepidemiology*. 2018;51(1–2):71–81.
- Yan X, et al. Metabolic dysregulation contributes to the progression of Alzheimer's Disease. *Front Neurosci*. 2020;14:530219.
- Roh E, Kim MS. Brain regulation of Energy Metabolism. *Endocrinol Metab (Seoul)*. 2016;31(4):519–24.
- Jin S, Diano S. Mitochondrial dynamics and Hypothalamic Regulation of Metabolism. *Endocrinology*. 2018;159(10):3596–604.
- Hillier-Sturmhofel S, Bartke A. The endocrine system: an overview. *Alcohol Health Res World*. 1998;22(3):153–64.

7. Ahima RS, Antwi DA. Brain regulation of appetite and satiety. *Endocrinol Metab Clin North Am.* 2008;37(4):811–23.
8. Van Druenen R, Eckel-Mahan K. Circadian rhythms of the Hypothalamus: from function to Physiology. *Clocks Sleep.* 2021;3(1):189–226.
9. Ishii M, Iadecola C. Metabolic and non-cognitive manifestations of Alzheimer's Disease: the Hypothalamus as both culprit and target of Pathology. *Cell Metab.* 2015;22(5):761–76.
10. Verduyck P, et al. Hypothalamic alterations in neurodegenerative diseases and their relation to abnormal energy metabolism. *Front Mol Neurosci.* 2018;11:2.
11. Logan RW, McClung CA. Rhythms of life: circadian disruption and brain disorders across the lifespan. *Nat Rev Neurosci.* 2019;20(1):49–65.
12. Verma AK, Singh S, Rizvi SI. Aging, circadian disruption and neurodegeneration: interesting interplay. *Exp Gerontol.* 2023;172:112076.
13. Baloyannis SJ, et al. The hypothalamus in Alzheimer's disease: a golgi and electron microscope study. *Am J Alzheimers Dis Other Dement.* 2015;30(5):478–87.
14. Zheng H, et al. The hypothalamus as the primary brain region of metabolic abnormalities in APP/PS1 transgenic mouse model of Alzheimer's disease. *Biochim Biophys Acta Mol Basis Dis.* 2018;1864(1):263–73.
15. Robison LS, et al. Role of sex and high-fat diet in metabolic and hypothalamic disturbances in the 3xTg-AD mouse model of Alzheimer's disease. *J Neuroinflammation.* 2020;17(1):285.
16. Semjonous NM, et al. Coordinated changes in energy intake and expenditure following hypothalamic administration of neuropeptides involved in energy balance. *Int J Obes (Lond).* 2009;33(7):775–85.
17. Cherry AD, Piantadosi CA. Regulation of mitochondrial biogenesis and its intersection with inflammatory responses. *Antioxid Redox Signal.* 2015;22(12):965–76.
18. Akiyama H, et al. Inflammation and Alzheimer's disease. *Neurobiol Aging.* 2000;21(3):383–421.
19. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol.* 2016;16(10):626–38.
20. Lamas O, et al. NF-kappa B-binding activity in an animal diet-induced overweightness model and the impact of subsequent energy restriction. *Biochem Biophys Res Commun.* 2003;311(2):533–9.
21. Moretti M, et al. Cancer: NF-kappaB regulates energy metabolism. *Int J Biochem Cell Biol.* 2012;44(12):2238–43.
22. Morandi B, et al. Dendritic cell editing by activated natural killer cells results in a more protective cancer-specific immune response. *PLoS ONE.* 2012;7(6):e39170.
23. Mauro C, et al. NF-kappaB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration. *Nat Cell Biol.* 2011;13(10):1272–9.
24. Adlimoghaddam A, et al. Nilotinib improves Bioenergetic Profiling in Brain Astroglia in the 3xTg Mouse Model of Alzheimer's Disease. *Aging Dis.* 2021;12(2):441–65.
25. Cogswell PC, et al. NF-kappa B and I kappa B alpha are found in the mitochondria. Evidence for regulation of mitochondrial gene expression by NF-kappa B. *J Biol Chem.* 2003;278(5):2963–8.
26. Adlimoghaddam A, Albeni BC. The nuclear factor kappa B (NF-kappaB) signaling pathway is involved in ammonia-induced mitochondrial dysfunction. *Mitochondrion.* 2021;57:63–75.
27. Dinkova-Kostova AT, Abramov AY. The emerging role of Nrf2 in mitochondrial function. *Free Radic Biol Med.* 2015;88(Pt B):179–88.
28. Davies DA, Adlimoghaddam A, Albeni BC. Role of Nrf2 in Synaptic Plasticity and Memory in Alzheimer's Disease. *Cells.* 2021. 10(8).
29. Park JC, Han SH, Mook-Jung I. Peripheral inflammatory biomarkers in Alzheimer's disease: a brief review. *BMB Rep.* 2020;53(1):10–9.
30. Deczkowska A, Baruch K, Schwartz M. Type I/II Interferon Balance in the regulation of Brain Physiology and Pathology. *Trends Immunol.* 2016;37(3):181–92.
31. Bate C, et al. Interferon-gamma increases neuronal death in response to amyloid-beta1–42. *J Neuroinflammation.* 2006;3:7.
32. Denver P, McClean PL. Distinguishing normal brain aging from the development of Alzheimer's disease: inflammation, insulin signaling and cognition. *Neural Regen Res.* 2018;13(10):1719–30.
33. Yang HS, et al. Plasma IL-12/IFN-gamma axis predicts cognitive trajectories in cognitively unimpaired older adults. *Alzheimers Dement.* 2022;18(4):645–53.
34. Sokolova A, et al. Monocyte chemoattractant protein-1 plays a dominant role in the chronic inflammation observed in Alzheimer's disease. *Brain Pathol.* 2009;19(3):392–8.
35. Lee WJ, et al. Plasma MCP-1 and Cognitive decline in patients with Alzheimer's disease and mild cognitive impairment: a two-year follow-up study. *Sci Rep.* 2018;8(1):1280.
36. Alves S, et al. Interleukin-2 improves amyloid pathology, synaptic failure and memory in Alzheimer's disease mice. *Brain.* 2017;140(3):826–42.
37. Porro C, Cianciulli A, Panaro MA. The Regulatory Role of IL-10 in neurodegenerative diseases. *Biomolecules.* 2020. 10(7).
38. Dionisio-Santos DA, et al. Evaluating the Effect of Interleukin-4 in the 3xTg Mouse Model of Alzheimer's Disease. *Front Neurosci.* 2020;14:441.
39. Yan XZ, et al. Interleukin-17A in Alzheimer's Disease: recent advances and controversies. *Curr Neuropharmacol.* 2022;20(2):372–83.
40. Javonillo DI, et al. Systematic phenotyping and characterization of the 3xTg-AD mouse model of Alzheimer's Disease. *Front Neurosci.* 2021;15:785276.
41. Oddo S, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular abeta and synaptic dysfunction. *Neuron.* 2003;39(3):409–21.
42. Adlimoghaddam A, et al. Regional hypometabolism in the 3xTg mouse model of Alzheimer's disease. *Neurobiol Dis.* 2019;127:264–77.
43. Fontaine KM, et al. Photobiont relationships and phylogenetic history of *Dermatocarpon luridum* var. *luridum* and related *dermatocarpon* species. *Plants (Basel).* 2012;1(2):39–60.
44. Snow WM et al. Sex-Specific Effects of Chronic Creatine Supplementation on Hippocampal-Mediated Spatial Cognition in the 3xTg Mouse Model of Alzheimer's Disease. *Nutrients.* 2020. 12(11).
45. Mostafazar M et al. Challenges with methods for detecting and studying the transcription factor nuclear factor Kappa B (NF-kappaB) in the Central Nervous System. *Cells.* 2021. 10(6).
46. Lim SY, et al. Evaluation of two high-throughput proteomic technologies for plasma biomarker discovery in immunotherapy-treated melanoma patients. *Biomark Res.* 2017;5:32.
47. Ke J, et al. Mitochondrial dysfunction: a potential target for Alzheimer's disease intervention and treatment. *Drug Discov Today.* 2021;26(8):1991–2002.
48. Walker KA, Ficek BN, Westbrook R. Understanding the role of systemic inflammation in Alzheimer's Disease. *ACS Chem Neurosci.* 2019;10(8):3340–2.
49. Toro CA, et al. Sex differences in Alzheimer's disease: understanding the molecular impact. *Brain Res.* 2019;1719:194–207.
50. Hagg S, Jylhava J. Sex differences in biological aging with a focus on human studies. *Elife.* 2021; Vol. 10.
51. Burmistrov DE et al. Sex as a determinant of age-related changes in the brain. *Int J Mol Sci.* 2024. 25(13).
52. Robison LS, et al. Sex differences in metabolic phenotype and hypothalamic inflammation in the 3xTg-AD mouse model of Alzheimer's disease. *Biol Sex Differ.* 2023;14(1):51.
53. Carroll JC, et al. Sex differences in beta-amyloid accumulation in 3xTg-AD mice: role of neonatal sex steroid hormone exposure. *Brain Res.* 2010;1366:233–45.
54. Hua X, et al. Sex and age differences in atrophic rates: an ADNI study with n = 1368 MRI scans. *Neurobiol Aging.* 2010;31(8):1463–80.
55. Vandal M, et al. Age-dependent impairment of glucose tolerance in the 3xTg-AD mouse model of Alzheimer's disease. *FASEB J.* 2015;29(10):4273–84.
56. Kalimon OJ, et al. Characterizing sex differences in mitochondrial dysfunction after severe traumatic brain injury in mice. *Neurotrauma Rep.* 2023;4(1):627–42.
57. Adlimoghaddam A, Benson T, Albeni BC. Mitochondrial transfusion improves mitochondrial function through Up-regulation of mitochondrial complex II protein subunit SDHB in the Hippocampus of aged mice. *Mol Neurobiol.* 2022;59(10):6009–17.
58. Kowalczyk P et al. Mitochondrial oxidative Stress-A causative factor and therapeutic target in many diseases. *Int J Mol Sci.* 2021. 22(24).
59. Ramanathan R, Ali AH, Ibdah JA. Mitochondrial dysfunction plays central role in nonalcoholic fatty liver disease. *Int J Mol Sci.* 2022. 23(13).
60. Marchi S, et al. Mitochondrial control of inflammation. *Nat Rev Immunol.* 2023;23(3):159–73.
61. Lake NJ, et al. Leigh syndrome: one disorder, more than 75 monogenic causes. *Ann Neurol.* 2016;79(2):190–203.
62. Butterfield DA, Palmieri EM, Castegna A. Clinical implications from proteomic studies in neurodegenerative diseases: lessons from mitochondrial proteins. *Expert Rev Proteom.* 2016;13(3):259–74.
63. Zhang L, et al. Potential hippocampal genes and pathways involved in Alzheimer's disease: a bioinformatic analysis. *Genet Mol Res.* 2015;14(2):7218–32.

64. Do K, et al. The effects of exercise on hypothalamic neurodegeneration of Alzheimer's disease mouse model. *PLoS ONE*. 2018;13(1):e0190205.
65. Zhang C, Rissman RA, Feng J. Characterization of ATP alternations in an Alzheimer's disease transgenic mouse model. *J Alzheimers Dis*. 2015;44(2):375–8.
66. Emmerzaal TL, et al. Age-dependent decrease of mitochondrial complex II activity in a familial mouse model for Alzheimer's Disease. *J Alzheimers Dis*. 2018;66(1):75–82.
67. Lunnon K, et al. Mitochondrial genes are altered in blood early in Alzheimer's disease. *Neurobiol Aging*. 2017;53:36–47.
68. Telford JE, Kilbride SM, Davey GP. Complex I is rate-limiting for oxygen consumption in the nerve terminal. *J Biol Chem*. 2009;284(14):9109–14.
69. Manczak M, et al. Differential expression of oxidative phosphorylation genes in patients with Alzheimer's disease: implications for early mitochondrial dysfunction and oxidative damage. *Neuromolecular Med*. 2004;5(2):147–62.
70. Goetzman E et al. *Complex II Biology in Aging, Health, and Disease*. Antioxid (Basel). 2023. 12(7).
71. Sundar Boyalla S, et al. Sex- and brain region-specific role of cytochrome c oxidase in 1-methyl-4-phenylpyridinium-mediated astrocyte vulnerability. *J Neurosci Res*. 2011;89(12):2068–82.
72. Grunewald A, et al. Mitochondrial DNA depletion in respiratory chain-deficient Parkinson Disease neurons. *Ann Neurol*. 2016;79(3):366–78.
73. Garrabou G, et al. Relevance of sex-differenced analyses in bioenergetics and nutritional studies. *Front Nutr*. 2022;9:936929.
74. Gallart-Palau X, et al. Gender differences in white matter pathology and mitochondrial dysfunction in Alzheimer's disease with cerebrovascular disease. *Mol Brain*. 2016;9:27.
75. Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death. *Nat Rev Mol Cell Biol*. 2020;21(2):85–100.
76. Zhang W, et al. Role of neuroinflammation in neurodegeneration development. *Signal Transduct Target Ther*. 2023;8(1):267.
77. Dresselhaus EC, Meffert MK. Cellular specificity of NF- κ B function in the nervous system. *Front Immunol*. 2019;10:1043.
78. Jong Huat T, et al. The impact of astrocytic NF- κ B on healthy and Alzheimer's disease brains. *Sci Rep*. 2024;14(1):14305.
79. Sun E et al. The Pivotal Role of NF- κ B in the Pathogenesis and Therapeutics of Alzheimer's Disease. *Int J Mol Sci*. 2022. 23(16).
80. Huang Y, et al. NF- κ B precursor, p105, and NF- κ B inhibitor, I κ B γ , are both elevated in Alzheimer disease brain. *Neurosci Lett*. 2005;373(2):115–8.
81. Barshad G, et al. Mitochondrial DNA transcription and its regulation: an evolutionary perspective. *Trends Genet*. 2018;34(9):682–92.
82. Johnson RF, Witzel ND II, Perkins. p53-dependent regulation of mitochondrial energy production by the RelA subunit of NF- κ B. *Cancer Res*. 2011;71(16):5588–97.
83. Chen WT, Dodson M. The untapped potential of targeting NRF2 in neurodegenerative disease. *Front Aging*. 2023;4:1270838.
84. Permpoonputtana K, Govitrapong P. The anti-inflammatory effect of melatonin on methamphetamine-induced proinflammatory mediators in human neuroblastoma dopamine SH-SY5Y cell lines. *Neurotox Res*. 2013;23(2):189–99.
85. Ashabi G, et al. Reduction of hippocampal apoptosis by intracerebroventricular administration of extracellular signal-regulated protein kinase and/or p38 inhibitors in amyloid beta rat model of Alzheimer's disease: involvement of nuclear-related factor-2 and nuclear factor- κ B. *Basic Clin Pharmacol Toxicol*. 2013;112(3):145–55.
86. Zhang K, et al. CXCL1 contributes to beta-amyloid-induced transendothelial migration of monocytes in Alzheimer's disease. *PLoS ONE*. 2013;8(8):e72744.
87. Zhu X, et al. Causes of oxidative stress in Alzheimer disease. *Cell Mol Life Sci*. 2007;64(17):2202–10.
88. Zhang XF, et al. CXCL1 triggers Caspase-3 dependent tau cleavage in long-term neuronal cultures and in the Hippocampus of aged mice: implications in Alzheimer's Disease. *J Alzheimers Dis*. 2015;48(1):89–104.
89. Burke SJ, et al. NF- κ B and STAT1 control CXCL1 and CXCL2 gene transcription. *Am J Physiol Endocrinol Metab*. 2014;306(2):E131–49.
90. Lazennec G, Richmond A. Chemokines and chemokine receptors: new insights into cancer-related inflammation. *Trends Mol Med*. 2010;16(3):133–44.
91. Tourniaire F, et al. Chemokine expression in Inflamed Adipose tissue is mainly mediated by NF- κ B. *PLoS ONE*. 2013;8(6):e66515.
92. Richmond A. NF- κ B, chemokine gene transcription and tumour growth. *Nat Rev Immunol*. 2002;2(9):664–74.
93. Jiang S, et al. The role of CXCL1/CXCR2 axis in neurological diseases. *Int Immunopharmacol*. 2023;120:110330.
94. Ibarra-Meneses AV, et al. Interleukin-2 as a marker for detecting asymptomatic individuals in areas where Leishmania infantum is endemic. *Clin Microbiol Infect*. 2016;22(8):e7391–4.
95. Beloesesky Y, et al. Cytokine levels and phagocytic activity in patients with Alzheimer's disease. *Gerontology*. 2002;48(3):128–32.
96. Petitto JM, et al. Impaired learning and memory and altered hippocampal neurodevelopment resulting from interleukin-2 gene deletion. *J Neurosci Res*. 1999;56(4):441–6.
97. Murray PS, et al. beta-amyloid 42/40 ratio and kalirin expression in Alzheimer disease with psychosis. *Neurobiol Aging*. 2012;33(12):2807–16.
98. Dansokho C, et al. Regulatory T cells delay disease progression in Alzheimer-like pathology. *Brain*. 2016;139(Pt 4):1237–51.
99. Beck RD Jr, et al. IL-2 deficiency results in altered septal and hippocampal cytoarchitecture: relation to development and neurotrophins. *J Neuroimmunol*. 2005;160(1–2):146–53.
100. Lyra ESNM, et al. Pro-inflammatory interleukin-6 signaling links cognitive impairments and peripheral metabolic alterations in Alzheimer's disease. *Transl Psychiatry*. 2021;11(1):251.
101. Italiani P, et al. Circulating levels of IL-1 family cytokines and receptors in Alzheimer's disease: new markers of disease progression? *J Neuroinflammation*. 2018;15(1):342.
102. Cao M, et al. IL-17A promotes the progression of Alzheimer's disease in APP/PS1 mice. *Immun Ageing*. 2023;20(1):74.
103. Brigas HC, et al. IL-17 triggers the onset of cognitive and synaptic deficits in early stages of Alzheimer's disease. *Cell Rep*. 2021;36(9):109574.

Publisher's note

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