

Article

Body-Wide Glycolytic Shift, Oxidative Stress, and Sex-Specific Effect of Caloric Restriction in a Mouse Model of Alzheimer's Disease

Myroslava V. Vatachuk¹, Viktoriia V. Hurza¹ , Kuang Pan², Maria M. Bayliak¹ , Dmytro V. Gospodaryov^{1,*}, Volodymyr I. Lushchak^{1,3}  and Olga Garaschuk^{2,*} 

¹ Department of Biochemistry and Biotechnology, Vasyl Stefanyk Carpathian National University, 57 Shevchenko Str., 76018 Ivano-Frankivsk, Ukraine; mariia.bailiak@cnu.edu.ua (M.M.B.); volodymyr.lushchak@cnu.edu.ua (V.I.L.)

² Department of Neurophysiology, Institute of Physiology, Eberhard Karls University of Tübingen, 15 Keplerstraße, 72074 Tübingen, Germany

³ Research and Development University, 13a Shota Rustaveli Str., 76018 Ivano-Frankivsk, Ukraine

* Correspondence: dmytro.hospodarov@cnu.edu.ua (D.V.G.); olga.garaschuk@uni-tuebingen.de (O.G.)

Abstract

Alzheimer's disease (AD) is regarded as a disease of the brain. Cumulative evidence increasingly supports a full-body view on this disorder, with the liver and kidneys playing an important role in amyloid clearance. The latter is likely potentiated by caloric restriction (CR), whose impact on the metabolism of amyloid-handling tissues is poorly understood. We studied the sex-specific effects of amyloidosis and CR on oxidative and metabolic processes in APPPS1 mice that express amyloidogenic proteins. Wild-type (WT) and APPPS1 mice were either fed ad libitum (AL) or received 70% of their AL caloric intake (CR). Compared to age-matched WT controls, the brain, liver, and kidney of 9-month-old AL APPPS1 mice exhibited higher levels of oxidative stress markers, higher superoxide dismutase, and lower catalase activities. These differences were sex- and tissue-specific, with kidneys showing the largest AD-induced differences between sexes. In addition, APPPS1 mice possessed higher pyruvate kinase activity than WT mice in all organs and higher hexokinase and phosphofructokinase activities in the brain, with stronger effects in males. CR intensified the accumulation of lipid peroxides in the liver and the female brain but decreased it in the female kidney. CR potentiated glycolysis, predominantly in females and modulated glutathione-dependent enzymes, in a sex-dependent manner.

Keywords: Alzheimer's disease; sex; caloric restriction; antioxidant defense; glycolysis



Academic Editor: Carlo Cervellati

Received: 15 December 2025

Revised: 26 January 2026

Accepted: 30 January 2026

Published: 2 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Alzheimer's disease (AD) is the most widespread neurodegenerative disorder, characterized by progressive memory loss, cognitive decline, and changes in behavior and personality. A predominant sporadic form of the disease (i.e., late-onset AD) is likely caused by a combination of genetic, environmental, and lifestyle factors and typically develops after the age of 65. The early-onset AD comprises about 2–10% of all AD cases and is typically associated with autosomal-dominant mutations in genes encoding amyloid precursor protein (APP) or presenilins 1 (PS1) and 2 (PS2) [1,2]. Nearly two-thirds of individuals diagnosed with AD are women, with AD ranking as the fifth leading cause of death for women (6.1% of deaths) and seventh (2.6% of deaths) for men [3].

Histologically, AD is defined by the presence of extracellular amyloid deposits (senile plaques built of amyloid β ($A\beta$)) in the brain parenchyma and intracellular accumulations of neurofibrillary tangles containing hyperphosphorylated microtubule-associated protein tau. The functional hallmarks of the disease include synaptic vulnerability, metabolic dyshomeostasis, impaired neuro-glial Ca^{2+} signaling, endosomal/lysosomal dysfunction, impairment of autophagy, activation of the brain's innate immune system, oxidative stress/damage, and impaired lipid metabolism [2,4–6].

Interestingly, APP expression is not restricted to the brain but is also found in the peripheral tissues like skin, intestine, and liver [7]. The same applies to amyloid plaques, which, for example, have been detected in the gastrointestinal tracts of AD patients and mouse models of AD. Moreover, peripherally generated $A\beta$ is capable of entering the brain and aggregating therein, as documented by a parabiosis model between AD (APP/PS1) and WT mice or a mouse model selectively overexpressing human APP with Swedish (KM670/671NL) and Indiana (V717F) AD mutations in hepatocytes [8,9]. In addition, peripheral organs such as the liver, kidney, spleen, and gut are important for $A\beta$ clearance [7,10].

The progression of AD is accompanied by the impairment of energy metabolism [5]. In fact, inefficient glucose utilization represents one of the early and well-known hallmarks of AD, which often comes hand-in-hand with peripheral (and likely also central) insulin resistance [11]. One possible mechanism promoting insulin resistance is the AD-driven hyperactivation of the mechanistic target of rapamycin (mTOR). Activation of the mTOR pathway is known to impact protein biosynthesis, mitochondrial function, lipo- and ketogenesis, and to inhibit autophagy and insulin signaling [5]. This activation is tightly linked to glucose metabolism and was shown to increase the production of reactive oxygen species (ROS), tau hyperphosphorylation, cerebrovascular dysfunction, and likely also neuroinflammation [12]. Sirtuin-3 (SIRT3), a mitochondrial nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylase, is another key player impacting mitochondrial function, lipid metabolism, ROS production, inflammation, and antioxidant defense. SIRT3 is widely expressed in metabolically active tissues, including the heart, liver, kidney, and brain, and SIRT3 expression is downregulated in the brain tissue of AD patients and mouse models of AD [13]. Cumulative evidence suggests that increasing SIRT3 activity might alleviate AD progression by positively influencing mitochondrial homeostasis and neuroinflammation, activating antioxidant enzymes, and decreasing levels of tau and $A\beta$.

Several studies have shown that caloric restriction (CR) can attenuate the severity of AD symptoms by reducing the density of amyloid plaques and inhibiting mTOR signaling, generation of ROS, neuroinflammation, and tau hyperphosphorylation, as well as increasing SIRT3 expression in the liver and brain [14–19]. Yet, the opposite effects were also reported [20–22]. For example, the every-other-day feeding (EODF) of 5XFAD transgenic female mice during the plaque deposition period (2–6 months of age) failed to decrease plaque size or density, brain $A\beta_{42}$ levels, and the AD-induced permeability of the blood–brain barrier [21]. Moreover, it exacerbated the activation of microglia and astrocytes, increased the brain level of proinflammatory cytokine tumor necrosis factor α , further reduced the density of synaptic marker synaptophysin, and promoted neuronal death. Similarly, no effect of CR on amyloid plaque density was found in the aged rhesus monkeys that were fed 30% less than individual sex-, age-, and weight-matched controls for up to 15 years (till death [22]), in line with the relatively low WT mouse brain responsiveness to CR [23,24].

In the current study, we explored the amyloidosis-induced response of key amyloid-handling organs (brain, liver, and kidney) in a widely used mouse model of AD (APPPS1 mice [25]) and tested the ability of CR to mitigate oxidative stress caused by the accumulation of $A\beta$. In calorie-restricted and ad libitum-fed APPPS1 (hereafter AD) mice,

as well as in age-matched WT littermates, we measured markers of oxidative stress and the activities of antioxidant enzymes, as well as activities of key glycolytic enzymes and lactate dehydrogenase (Figure 1A) to infer metabolic changes caused by both amyloid accumulation and CR. The analyses considered sex as a biological variable, thus deepening our understanding of possible mechanisms underlying the increased incidence rates for Alzheimer's disease in women [26].

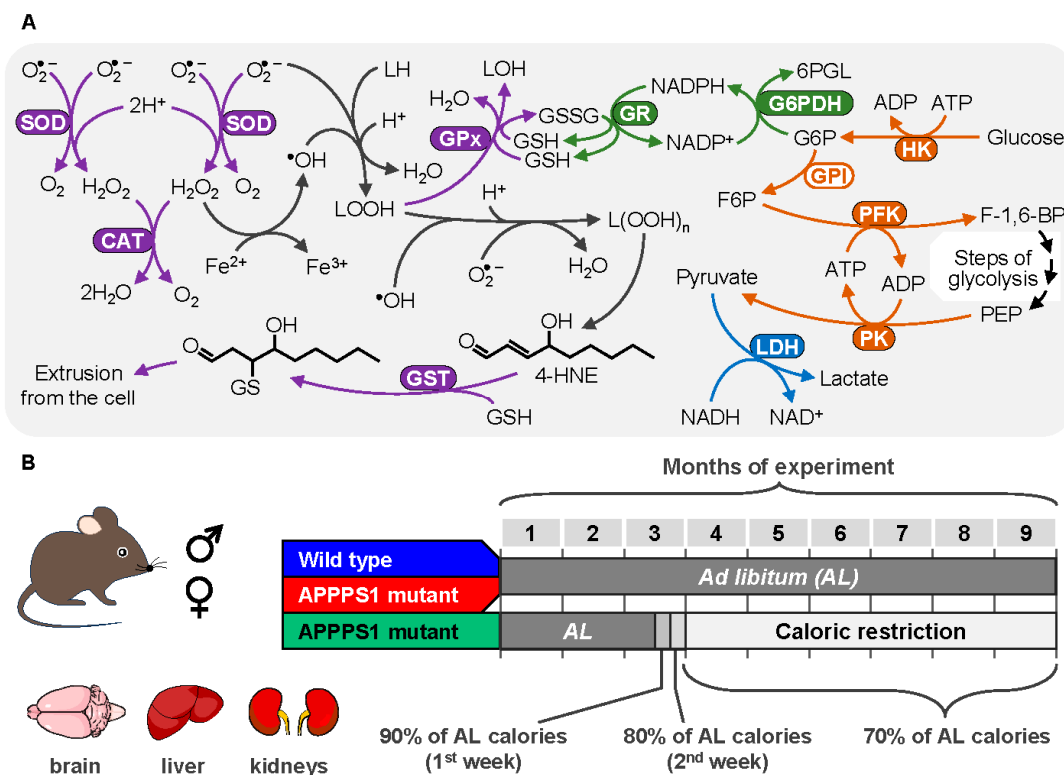


Figure 1. Experimental settings. (A) Scheme illustrating the relationship between oxidative damage, antioxidant defense enzymes, and glycolytic enzymes. Activities of enzymes whose names are shown in a white font on a colored background were measured in the present study. Reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), are metabolized by superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), with the support of glutathione reductase (GR) and NADPH. The latter is produced in the glucose-6-phosphate dehydrogenase (G6PDH) reaction during the oxidation of glucose-6-phosphate (G6P) to 6-phosphogluconolactone (6PGL). G6P is formed in the reaction of glucose phosphorylation catalyzed by hexokinase (HK) and can enter glycolysis. Hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK) are key enzymes of glycolysis, which catalyze irreversible reactions. Lactate dehydrogenase (LDH) converts pyruvate to lactate to maintain redox balance. If the activity of antioxidant enzymes and the pool of reduced glutathione (GSH) are insufficient, oxidative damage to proteins and lipids increases. The scheme shows how ROS initiate lipid peroxidation and emphasizes the importance of GSH in forming primary and relatively stable products of lipid oxidation: lipid hydroperoxides (LOOH, $L(OOH)_n$). Glutathione S-transferase (GST) detoxifies lipid peroxidation products such as 4-hydroxy-2-nonenal (4-HNE) by conjugating them with GSH, and the resulting conjugates are extruded from the cell. Metabolite abbreviations are as follows: F6P—fructose-6-phosphate, F-1,6-BP—fructose-1,6-biphosphate, and PEP—phosphoenolpyruvate. Arrows and enzymes shown in purple denote reactions directed to antioxidant defense; gray arrows mark reactions that promote oxidative damage in the form of lipid peroxides and products of their breakdown. Green arrows and enzymes mark reactions directed to glutathione reduction, orange-colored enzymes and arrows—key glycolytic reactions, and the cyan arrow—the lactate dehydrogenase reaction. (B) Experimental arrangement as detailed in Materials and Methods. Here and hereafter, Mars and Venus symbols denote males and females, respectively.

2. Materials and Methods

2.1. Animals and Experimental Conditions

C57BL/6N (wild type, WT) and APPPS1 (Thy1-APP^{Sw}, Thy1-PSEN1*^{L166P})21Jckr/J) mice of both sexes were used. APPPS1 mice overexpress human APP with the Swedish mutation (KM670/671NL) and presenilin 1 with the L166P mutation, under the control of the neuronal Thy-1 promoter. In these mice, both the PS1 and the APP constructs are expressed only in the postnatal brain, and cerebral amyloidosis starts at 6–8 weeks of age [25]. Subsequently, however, the amyloidosis spreads via the entire body, including the liver and kidney, as shown in another mouse model of AD, using the same promoter [27,28].

WT mice (brain: 3 males and 3 females; liver and kidney: 3 males and 4 females) and one group of APPPS1 mice (AL group, 4 males and 5 females) had unlimited access to food and water during 9 months of the experiment (Figure 1B). In the CR group (4 males and 4 females), APPPS1 mice had unrestricted access to food for 2.5 months and thereafter were switched to caloric restriction for 7 months, until the end of the experiment. In the CR group, each mouse received 90% of its individual ad libitum food intake during the first week and 80% during the second week of the CR regimen. Starting from the third week and continuing until the end of the experiment, the CR animals received 70% of their respective ad libitum food intake (Figure 1B). Under all experimental conditions, the diet (Ssniff® V1534-703, Ssniff® Spezialdiäten GmbH, Soest, Germany) provided a sufficient amount of vitamins and micronutrients [29,30].

All animals were kept under standard conditions with a 12 h light/dark cycle. Ad libitum-fed mice stayed in groups of 3–5 mice, and CR mice were conventionally housed in individual neighboring cages with all olfactory, visual, and acoustic stimuli preserved. All mice were provided with cellulose bedding and a wooden tube as cage enrichment. All procedures complied with the ARRIVE guidelines, were carried out following the EU Directive 2010/63/EU for animal experiments, and were approved by the state government of Baden-Württemberg, Germany, on 2 November 2021.

2.2. Tissue Sampling

Mice were deeply anesthetized (ketamine 200 mg/kg body weight; xylazine 20 mg/kg body weight), tested for the surgical depth of anesthesia by checking the interphalangeal reflex, and subjected to the transcardial perfusion with cold phosphate-buffered saline (PBS) until their blood vessels and organs were largely bloodless [31]. After collection, the liver, kidneys, and brain (without olfactory bulb and cerebellum) were immediately frozen on dry ice and stored in a freezer at −80 °C.

2.3. Tissue Homogenization and Determination of Oxidative Stress Markers

Lipid peroxides and protein carbonyls were measured as markers of oxidative stress. Note that the use of frozen tissues or tissue homogenates does not allow for reliable measurement of ROS using probes for either H₂O₂ or O₂^{•−}, since these species are very short-lived and require intact membranes [32].

Lipid peroxides (LOOH) from frozen tissues were extracted with ethanol, as described previously [24], and measured with the Fe²⁺/xylenol orange method [33,34]. Cumene hydroperoxide was used to build the standard calibration curve [33,34].

To determine the levels of carbonyl groups in proteins, frozen tissue samples were homogenized in 50 mM potassium phosphate buffer (KPi, pH 7.0), containing 0.5 mM N,N,N',N'-ethylenediaminetetraacetic acid (EDTA) and 1 mM phenylmethylsulfonyl fluoride at a ratio of 1:10 (milligram of tissue : microliter of homogenization medium). The homogenates were centrifuged at 21,000 × g for 15 min at 4 °C in a Thermo Fisher Scientific Multifuge X1R centrifuge (Waltham, MA, USA). The supernatants were then mixed with

20% (final concentration) trichloroacetic acid to precipitate proteins. The content of carbonyl groups in proteins was determined by their reaction with 2,4-dinitrophenylhydrazine, resulting in the formation of colored dinitrophenylhydrazones, and recorded spectrophotometrically at 370 nm [35].

2.4. Tissue Homogenization and Determination of the Activities of Antioxidant Enzymes

Supernatants were prepared as described above for the protein carbonyls assay. Superoxide dismutase (SOD) activity was measured based on the inhibition of quercetin oxidation by superoxide anion radicals, which were generated in an alkaline medium by TEMED (*N,N,N',N'*-tetramethylethylenediamine) in the presence of oxygen. The rate of the quercetin oxidation reaction was monitored spectrophotometrically at a wavelength of 406 nm [36]. The reaction was run in a mixture containing 30 mM Tris-HCl buffer (pH 10.0), 0.5 mM EDTA, 0.8 mM TEMED, 0.05 mM quercetin, and 0.3–100 μ L supernatant; the change in absorbance with time was tested for 6–8 different volumes of supernatant. One unit of SOD activity was defined as the amount of enzyme (per mg protein) that inhibits the quercetin oxidation reaction by 50% of the maximum. Calculations were performed using KINETICS software [37].

The activities of catalase, glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and glucose-6-phosphate dehydrogenase (G6PDH) were measured spectrophotometrically, as described in our previous studies [23]. Briefly, all reaction mixtures contained 50 mM KPi (pH 7.0) and 0.5 mM EDTA, and additional components as follows: for catalase—10 mM H_2O_2^* , and 10 μ L supernatant; for GPx—0.25 mM NADPH, 4 mM sodium azide, 1 unit (U) GR, 15 mM reduced glutathione (GSH), 0.2 mM H_2O_2 , and 20 μ L supernatant*; for GST—5 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene, and 2–5 μ L supernatant*; for GR—0.25 mM NADPH, 1 mM oxidized glutathione (GSSG)*, and 2–50 μ L supernatant; and for G6PDH—5 mM MgCl_2 , 0.2 mM NADP^+ , 2 mM G6P* with 20 μ L supernatant. Catalase activity was measured by following the rate of H_2O_2 decomposition at 240 nm (molar extinction coefficient $\epsilon = 39.4 \text{ M}^{-1} \text{ cm}^{-1}$), GST activity was monitored at 340 nm by the formation of 1-(S-glutathionyl)-2,4-dinitrobenzene ($\epsilon = 9600 \text{ M}^{-1} \text{ cm}^{-1}$), an adduct between GSH and 1-chloro-2,4-dinitrobenzene. The activities of GPx, GR, and G6PDH were assayed at 340 nm by monitoring NADPH ($\epsilon = 6220 \text{ M}^{-1} \text{ cm}^{-1}$), consumed (GPx, GR), or formed (G6PDH) in these reactions. Asterisks mark components that were omitted in blank reactions. One unit of catalase, GPx, GST, GR, and G6PDH activity is defined as the amount of the enzyme consuming 1 μ mol substrate or generating 1 μ mol product per minute.

2.5. Tissue Homogenization and Determination of the Activity of Glycolytic Enzymes

To determine the activity of glycolytic enzymes, frozen tissue samples were homogenized in a lysis medium containing 50 mM imidazole buffer (pH 7.5), 0.5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 20 mM NaF, and 150 mM KCl. The resulting homogenates were centrifuged at $21,000 \times g$ for 15 min at 4 °C. After centrifugation, the supernatants were collected and stored on ice for several hours to determine biochemical parameters.

The activities of hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK) were measured spectrophotometrically in coupled enzyme reactions by registering NADP^+ reduction (for HK) or NADH oxidation (for PFK and PK) at 340 nm [38]. All assays except LDH activity were run in 50 mM imidazole buffer (pH 7.5) at 25 °C with a final volume of 1.0 mL, including the supernatant. Blank activities were assayed in mixtures without a specific substrate, indicated by the asterisk. LDH activity was measured in 50 mM KPi (pH 7.5). Briefly, reaction buffers contained the following: for HK—10 mM glucose*,

0.2 mM NADP⁺, 2 mM ATP, 5 mM MgCl₂, 0.5 units (U) G6PDH, and 30 µL supernatant; for PFK—5 mM fructose 6-phosphate*, 5 mM MgCl₂, 5 mM ATP, 0.16 mM NADH, 50 mM KCl, 0.5 U aldolase, 0.5 U triose-phosphate isomerase, 2 U glyceraldehyde-3-phosphate dehydrogenase, and 30 µL supernatant; and for PK—1 mM 2-phosphoenolpyruvate*, 5 mM MgCl₂, 50 mM KCl, 2.5 mM ADP, 0.16 mM NADH, 2.5 U LDH, and 5 µL supernatant. The reaction mixture for lactate dehydrogenase contained 0.5 mM EDTA, 0.2 mM NADH, 1 mM pyruvate*, and 10 µL supernatant. The total protein concentration in the samples was determined by the Bradford method [39] with bovine serum albumin as a standard.

2.6. Analyses of the Plaque Load

Motor cortex areas of the previously fixed and embedded brains from our data bank were cut into 50-µm-thick slices on a cryostat (Leica CM1950, Leica Biosystems Nussloch GmbH, Nussloch, Germany). The staining was performed on free-floating sections in 24-well plates (Greiner Bio-One GmbH, Kremsmünster, Austria) at room temperature. The plaques were stained with $2 \times 10^{-6}\%$ Thioflavin S for 8 min in the dark and then washed 5 times in PBS. Finally, the slices were mounted on the fluorescence-free Superfrost Plus microscope slides (R. Langenbrinck GmbH, Emmendingen, Germany) with the antifade mounting medium (Vectashield, Vector Laboratories, Inc., Newark, CA, USA).

Images were acquired at an excitation wavelength of 800 nm with a custom-made two-photon laser-scanning microscope, based on the FV300 confocal system (Olympus Optical Co., Ltd., Tokyo, Japan), attached to the upright microscope (BX51WI, Olympus Optical Co., Ltd., Tokyo, Japan) with a 40× water-immersion objective (0.80 NA, Nikon Europe B.V., Amstelveen, Netherlands) and a mode-locked laser (MaiTai, MKS Inc., Andover, MA, USA). A 515 dichroic mirror and a 512 nm short-pass filter were used to separate the Thioflavin S signal. The plaque load (6 images from 3 to 4 slices per mouse) was measured with the following settings: 512 × 512 pixels field of view, 1 µm step size, 50 µm depth, 40× objective, and 1× zoom.

Image processing and analyses were performed using Imaris 9.8.0 (Oxford Instruments, Abingdon, UK) software. To calculate the plaque load, the volume of Thioflavin S-positive Aβ deposits was reconstructed using the surface function of Imaris. A fluorescence threshold of mean plus one standard deviation was used, which was read from the corresponding fluorescence intensity histogram of the entire imaged volume. Using the volume filter, reconstructed entities smaller than 65.4 µm³ (the volume of a sphere with a diameter of 5 µm) were excluded.

2.7. Statistical Analysis

The sample size for the study was estimated in R version 4.3.1 (package ‘pwr’, version 1.3-0), using the function `pwr.anova.test` with the following settings: 3 groups, effect size of 1.25, significance level of 0.05, and power of 0.8. This yielded a sample size of 3.29 animals per group. We have also used Robin Ristl’s sample size calculator for analysis of variance (<https://homepage.univie.ac.at/robin.ristl/samplesize.php?test=anova>, URL accessed on 30 January 2026) with the following settings: 3 groups with means of 1.5, 2.5, and 1.5; standard deviation of 0.5, significance level of 0.05, power of 0.8, and unequal sample sizes (1, 0.8, 0.6). This calculation yielded a sample size of 3–5 mice.

The normality of the datasets was tested using the Jarque–Bera test, and the homoscedasticity was tested using the Brown–Forsythe test, implemented in the R package ‘DescTools’ (version 0.99.49). To satisfy the normality and homoscedasticity assumptions, the data on activities of G6PDH and PFK in the brain, HK in the liver, and GR in the kidney were logarithmically transformed for model testing and multiple comparisons. The data were analyzed using generalized linear models (Gaussian distribution and identity link

function), followed by the analysis of deviance and multiple comparisons, using estimated marginal means (Tukey adjustment for p -values obtained from pairwise multiple comparisons and computed using Studentized range distribution). The analyses were conducted in R using packages ‘car’ (version 3.1-2, for analysis of deviance) and ‘emmeans’ (version 1.8.8, for multiple comparisons). A combined factor, mutation plus diet, was chosen as a predictor variable. The sex of animals was chosen as another factor. Interaction between the combined factor and sex was included in the model.

Data are represented as box-and-whisker plots with marked median, first, and third quartiles (box floor and ceiling, respectively). The whiskers are extended to the most extreme data points, which do not exceed the 1.5 interquartile range, and other data points are shown as outliers. Differences between the groups for which the p -value < 0.05 were considered statistically significant. The boxplots were made using GraphPad Prism version 8.0.2 for Windows (GraphPad Software, Boston, MA, USA).

Principal component analysis (PCA) was conducted and visualized using R packages ‘ggplot2’ (version 3.5.1), ‘ggpubr’ (version 3.5.1), ‘ggfortify’ (version 0.6.0), ‘ggrepel’ (version 0.9.3), and ‘gridExtra’ (version 2.3). The principal components were estimated using an algorithm of singular value decomposition, implemented in the ‘base’ function `prcomp`.

3. Results

At 2–3 months of age, all experimental animals had a roughly similar body weight (20–24 g) and food intake (Figure S1A,B), with small but significant sex-specific differences in body weight between WT and AD mice (Figure S1C,E). According to the literature, the food intake [40] and blood glucose levels [41] do not differ between these mouse strains at midlife (8 months of age). Towards midlife, however, ad libitum-fed WT mice gained 37.6% and ad libitum-fed AD mice gained 29.2% more weight, whereas the weight of calorically restricted AD mice remained stable (Figure S1). Note that none of the experimental animals ever showed any weight loss during the entire experiment. The age-related weight increase in ad libitum-fed WT mice was higher in males (42.3%) than in females (33.2%), whereas in the ad libitum-fed AD mice, the weight increases in both sexes were more homogeneous.

3.1. Lipid Peroxide Levels and Activity of Antioxidant and Glycolytic Enzymes in the Brain

At midlife, approximately 3% of the brain volume in AD mice was occupied by amyloid β , accumulating in Thioflavin S-positive dense core plaques (Figure 2). Consistent with the prior literature data [16,18], CR caused a strong reduction in the brain plaque load in both sexes, with significantly less amyloid found in CR male compared to CR female brains (Figure 2B,C).

In the brains of male and female ad libitum-fed AD mice (AL group), the levels of LOOH, a marker of lipid peroxidation intensity, were 3.4- and 2.2-fold (here and below, the median values per group are reported) higher compared to those measured in WT littermates, whereas no difference in lipid peroxides was observed between AL and CR groups of AD mice (Figure 3A). SOD activity was 2.8- and 2.5-fold higher in male and 3.1- and 3.9-fold higher in female AD mice on the AL and CR regimens compared to the values measured in the respective WT groups (Figure 3B). Under the AL regimen, the catalase activity was 56% lower in female AD mice compared to the respective WT mice, with no difference observed between the AL and CR groups of AD mice (Figure 3C).

Male WT and AD mice fed ad libitum did not differ in the activities of GPx, GR, GST, and G6PDH (Figure 3D–G). CR regimen, however, resulted in 2.4-fold higher GR (Figure 3E) and 1.5-fold higher GST (Figure 3F) activities in AD males compared to the respective WT groups. In contrast, the GPx (Figure 3D) and G6PDH (Figure 3G) activities were significantly lower in the brains of AD compared to WT females, while the GR

(Figure 3E) and GST (Figure 3F) activities were similar. Under caloric restriction, AD females had 1.3- and 1.6-fold higher activities of SOD and GPx, respectively, compared to males (Figure 3B,D), while the activities of GR and GST were 30% and 42% lower in AD females compared to males (Figure 3E,F). Note that only in females, CR was able to reverse the AD-mediated decrease in the GPx activity (Figure 3D).

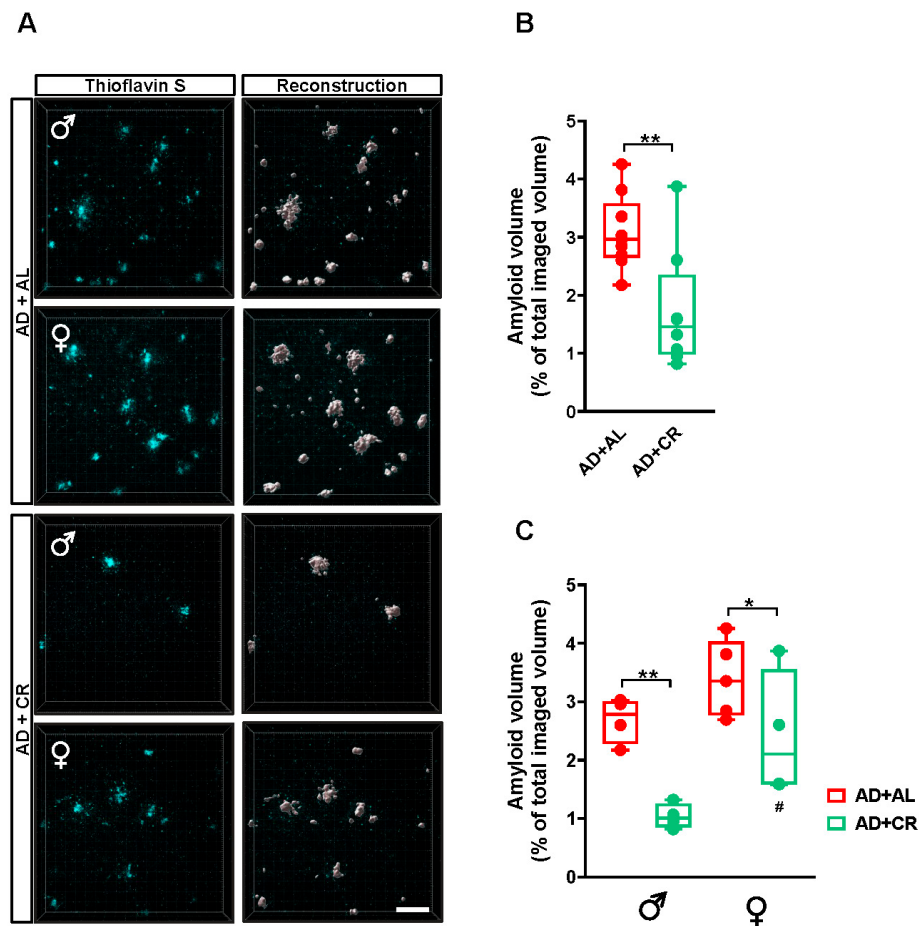


Figure 2. The effect of caloric restriction on the abundance of dense core amyloid deposits. (A) Sample maximum intensity projection images (50 μm depth, 1 μm step size) of Thioflavin S-stained cortical amyloid plaques (left) and their 3D reconstructions, using the Imaris surface function (right) in both sexes. Scale bar 60 μm . (B) Quantification of amyloid burden across AD groups ($n = 9$ for AD+AL and 8 for AD+CR mice). ** $p < 0.01$, unpaired unequal variance t -test. (C) Comparison of amyloid burden between sexes ($n = 4$ –5 mice per group). Significantly different among sexes, # $p < 0.05$, and among diets, * $p < 0.05$, ** $p < 0.01$; pairwise comparisons with Tukey adjustment for p -values computed using Studentized range distribution.

Ad libitum-fed AD males had significantly (3.4-, 6.3-, 34-, and 1.5-fold, respectively) higher HK, PFK, PK, and LDH (Figure 4A–D) activities compared to the respective WT male group. In AD males, caloric restriction did not affect HK (Figure 4A), PFK (Figure 4B), and LDH (Figure 4D) activities. However, it significantly reduced the AD-mediated increase in PK activity (Figure 4C). Compared to WT females, AD females in the AL group also exhibited significantly (4.3-, 6.2-, 60-, and 1.4-fold, respectively) higher activities of HK (Figure 4A), PFK (Figure 4B), PK (Figure 4C), and LDH (Figure 4D). Caloric restriction further increased the PFK activity in AD females, leading to a significantly different level of PFK activity in males and females, with little impact on the activities of other enzymes tested (Figure 4A–D).

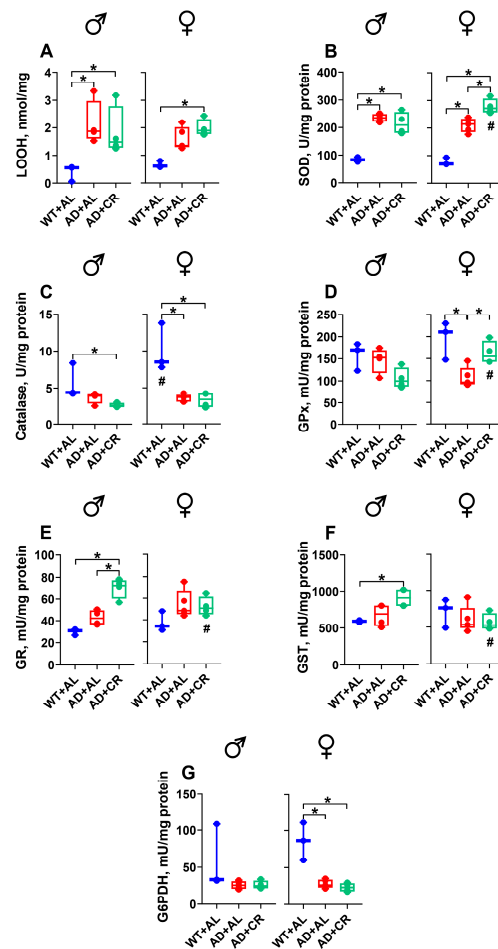


Figure 3. Markers of oxidative stress and activities of antioxidant enzymes in the brain. Effects of amyloidosis (AD phenotype) and caloric restriction (CR) on the APPPS1 (AD) and C57BL/6N (WT) mice of both sexes. Here and below: AL—mice fed ad libitum. (A)—levels of lipid peroxides, (B)—superoxide dismutase activity, (C)—catalase activity, (D)—glutathione peroxidase activity, (E)—glutathione reductase activity, (F)—glutathione S-transferase activity, and (G)—glucose-6-phosphate dehydrogenase activity. Here and below, $n = 7$ WT mice (3 males and 4 females), 9 AL-fed APPPS1 mice (4 males and 5 females), and 8 CR APPPS1 mice (4 males and 4 females). * Significantly different between genotype+diet groups, $p < 0.05$. # Significantly different among sexes, $p < 0.05$.

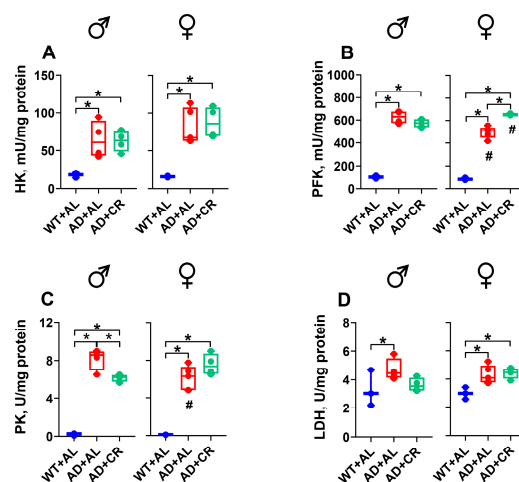


Figure 4. The activities of glycolytic enzymes in the brain. (A)—hexokinase activity, (B)—phosphofructokinase activity, (C)—pyruvate kinase activity, and (D)—lactate dehydrogenase. * Significantly different between genotype+diet groups, $p < 0.05$. # Significantly different among sexes, $p < 0.05$.

3.2. Oxidative Stress Markers and Activities of Antioxidant and Glycolytic Enzymes in the Liver

Male AD mice on the AL and CR regimens had 3.7- and 6.7-fold higher levels of LOOH in the liver, compared to the WT group (Figure 5A). Of note, the levels of lipid peroxides in the liver of AD males increased further (1.8-fold compared to the respective AL group) under caloric restriction (Figure 5A). A similar observation was made in AD females. The livers of AL and CR AD females showed 3.7- and 5.2-fold higher LOOH levels than those measured in the WT group. AD+CR females exhibited 41% higher LOOH levels than the AD+AL counterparts (Figure 5A). The content of protein carbonyl groups (PC), a marker of protein oxidation, in the male liver was not affected by the amyloidosis or dietary regimen (Figure 5B). However, AL-fed AD females had 60% lower PC levels compared to the WT counterparts, likely reflecting the protective effect of A β on the liver [42]. SOD activity was significantly (2.2-fold) higher in AL-fed AD compared to WT males (Figure 5C), and this AD-induced increase was reversed by CR. In contrast, no significant changes in the activity of this enzyme were observed in females. Catalase activity was significantly higher in male compared to female WT mice and decreased in both sexes of AD mice, with the difference becoming significant in male mice only (Figure 5D).

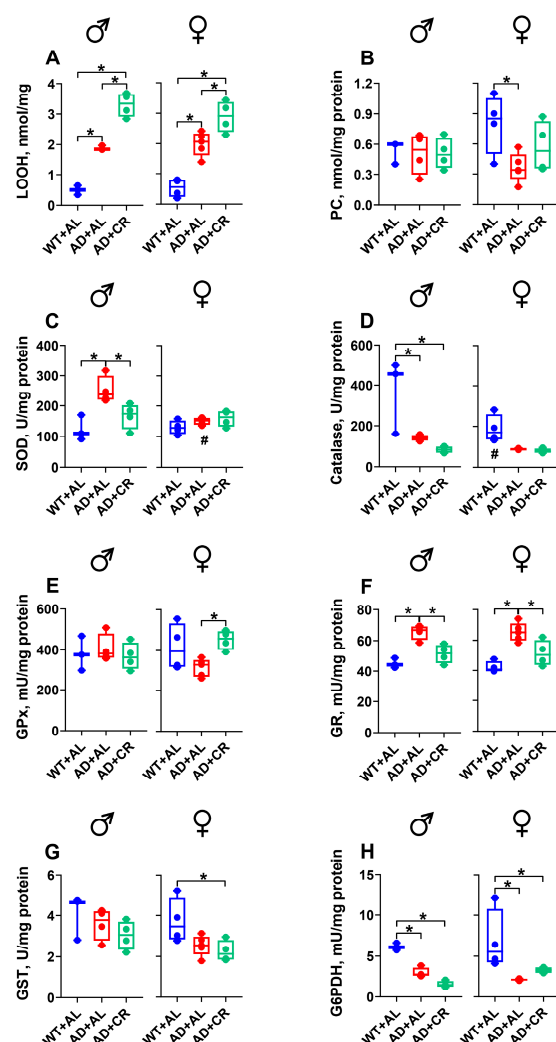


Figure 5. Levels of the oxidative stress markers and activities of antioxidant enzymes in the liver. (A)—levels of lipid peroxides, (B)—levels of protein carbonyls, (C)—superoxide dismutase activity, (D)—catalase activity, (E)—glutathione peroxidase activity, (F)—glutathione reductase activity, (G)—glutathione S-transferase activity, and (H)—glucose-6-phosphate dehydrogenase activity. * Significantly different between genotype+diet groups, $p < 0.05$. # Significantly different among sexes, $p < 0.05$.

AL-fed AD males did not differ from the WT males in GPx (Figure 5E) and GST (Figure 5G) activities. The caloric restriction did not affect the activity of these enzymes either. In contrast, GR activity was significantly (1.5-fold) higher in AD and significantly (by 23%) lower in AD+CR male mice (Figure 5F). Compared to the WT group, liver G6PDH activity was 55% and 76% lower in the AL and CR groups of AD males, respectively (Figure 5H). Compared to WT littermates, AL-fed AD females showed a trend towards reduced activity of GPx and GST and had a significantly (1.6-fold) higher GR, as well as a significantly lower (by 62%) G6PDH activity (Figure 5E–H). Importantly, CR changed the GPx and GR activities significantly, bringing them much closer to control values measured in female WT mice (Figure 5E,F). No differences between dietary regimens were found for GST and G6PDH activities in female AD mice.

Compared to the respective WT group, AL-fed AD mice of both sexes had significantly (4.8-fold in males and 2.6-fold in females) elevated glucose levels, with comparable glucose levels in AL and CR groups of AD mice (Figure 6A). Ad libitum-fed AD males showed similar hepatic HK and LDH activities but a significantly (57%) lower PFK activity and a significantly (2.3-fold) higher PK activity compared to WT males (Figure 6B–E). AD with or without CR affected neither HK activity (Figure 6B) in males nor LDH activity (Figure 6E) in both sexes. In the livers of AD+AL females, the activity of HK was slightly (1.3-fold, Figure 6B) and of PK significantly (4.4-fold, Figure 6D) higher compared to that measured in WT females. Hepatic PFK activity was significantly (43%) lower in the AL-fed AD females compared to the WT group (Figure 6C). The only difference between the dietary regimens was observed for PK, which was significantly higher in CR compared to AL+AD females. Moreover, sex-specific differences were observed for HK in the AD+CR group and for LDH in the WT group of mice (Figure 6B,E).

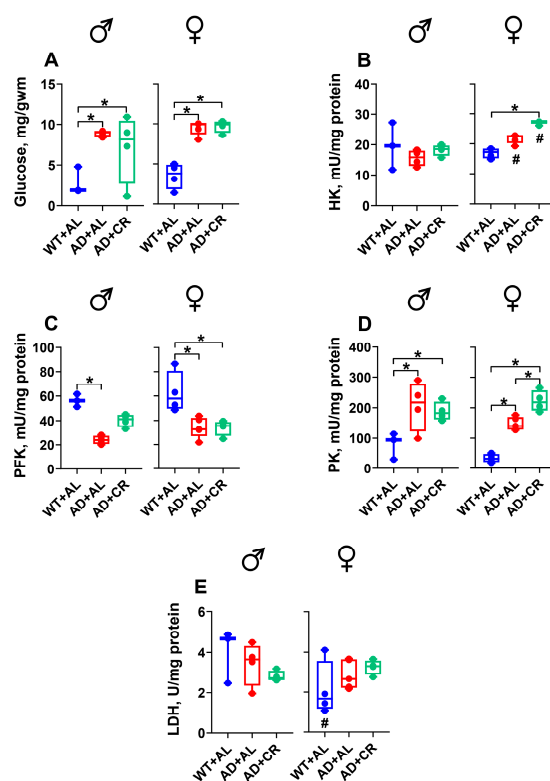


Figure 6. Levels of glucose and activity of glycolytic enzymes in the liver. (A)—levels of glucose, (B)—hexokinase activity, (C)—phosphofructokinase activity, (D)—pyruvate kinase activity, and (E)—lactate dehydrogenase activity. * Significantly different between genotype+diet groups, $p < 0.05$. # Significantly different among sexes, $p < 0.05$.

3.3. Oxidative Stress Markers, Activities of Antioxidant and Glycolytic Enzymes in the Kidneys

Under the AL regimen, kidney LOOH levels in AD mice of both sexes were significantly higher (1.7-fold in males, 2.2-fold in females) than those in the respective WT groups (Figure 7A). The CR regimen brought the LOOH levels closer to the WT values in both sexes, causing a slight reduction in the CR compared to AL males and a significant reduction in the CR compared to AL females. In AD+AL mice, the levels of PC slightly decreased in males and significantly increased in females, resulting in a profound sex-specific difference (Figure 7B). The CR regimen normalized the level of protein carbonyls in AD mice of both sexes.

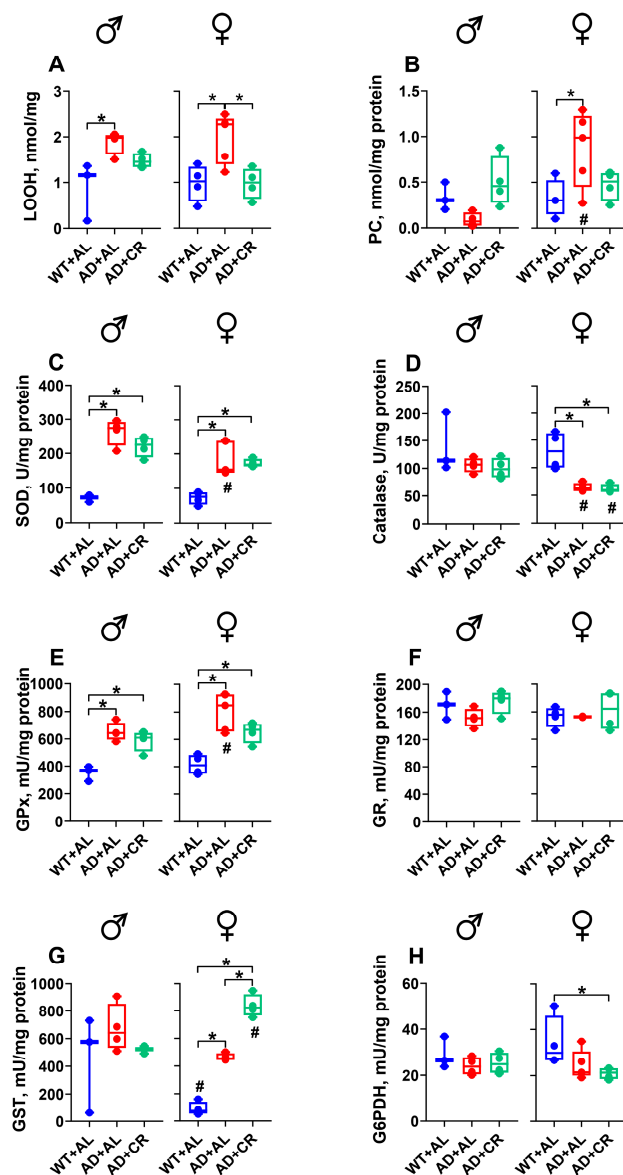


Figure 7. Levels of the oxidative stress markers and activities of antioxidant enzymes in the kidney. (A)—levels of lipid peroxides, (B)—levels of protein carbonyls, (C)—superoxide dismutase activity, (D)—catalase activity, (E)—glutathione peroxidase activity, (F)—glutathione reductase activity, (G)—glutathione S-transferase activity, and (H)—glucose-6-phosphate dehydrogenase activity. * Significantly different between genotype+diet groups, $p < 0.05$. # Significantly different among sexes, $p < 0.05$.

The AL and CR groups of AD males and females had significantly higher kidney SOD activity compared to the respective WT groups (Figure 7C). Still, the AD-mediated SOD

activity in males was higher than that in females, resulting in a significant sex-specific difference. Compared to the respective WT groups, the catalase activity did not differ in males but was significantly lower in AL (51%) and CR (54%) AD females, again revealing significant sex-specific differences. Compared to the respective WT controls, GPx activity was significantly higher in AD+AL and AD+CR groups of both male and female mice (Figure 7E). Still, GPx activity in AL females was significantly higher than that observed in AL males. Irrespective of dietary regimen, male AD mice showed no significant differences in kidney GR, GST, or G6PDH activities compared to WT mice (Figure 7F–H). Similarly, AD females did not differ from WT mice in the activity of kidney GR (Figure 7F). Surprisingly, in the WT mice, the GST activity was significantly lower (87%) in females compared to males (Figure 7G). Compared to this control, the GST activity in the kidneys of AD females was 6-fold higher in the AL group and 10.9-fold in the CR group (Figure 7G). Moreover, GST activity was significantly (81%) higher in AD females on the CR regimen compared to the AL group (Figure 7G). For G6PDH, the only significant difference observed in females was between the WT and AD+CR groups (Figure 7H).

As in the liver (Figure 6A), glucose levels were significantly higher in the kidneys of male and female AD compared to the respective WT mice (Figure 8A). AD+AL male mice had significantly (3-fold) higher kidney glucose levels than their female counterparts. Moreover, while CR caused a significant (46%) reduction (i.e., normalization) of glucose levels in males, it caused a significant (2.2-fold) further increase in glucose levels in females (Figure 8A).

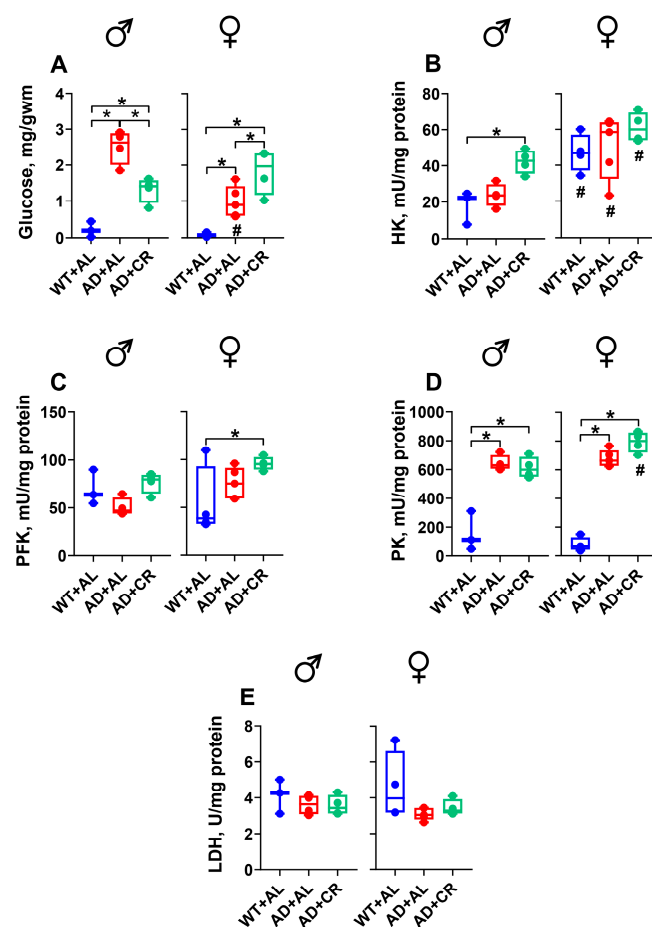


Figure 8. Levels of glucose and activity of glycolytic enzymes in the kidney. (A)—levels of glucose, (B)—hexokinase activity, (C)—phosphofructokinase activity, (D)—pyruvate kinase activity, and (E)—lactate dehydrogenase activity. * Significantly different between genotype+diet groups, $p < 0.05$. # Significantly different among sexes, $p < 0.05$.

AL-fed AD males and females had similar levels of HK, PFK, and LDH activities to WT mice (Figure 8B,C,E). Surprisingly, however, HK activity was significantly higher in females compared to males for all groups tested, and the CR regimen selectively increased HK activity in males (Figure 8B). Compared to the WT mice, a small but significant CR-induced increase in PFK activity was observed in AD females (Figure 8C), while LDH activity remained stable throughout the experiment in both sexes (Figure 8E). Finally, the kidneys of AD males had significantly higher PK activity in the AL (5.6-fold) and CR (5.3-fold) groups compared to the WT group (Figure 8D). Similar effects were also observed in the kidneys of female AD mice, with a small but significant sex-specific difference in the AD+CR group.

3.4. Principal Component Analyses

Principal component analyses revealed substantial differences between WT and AD mice in all three tested organs, with male and female data mapping to the overlapping clusters (Figure 9).

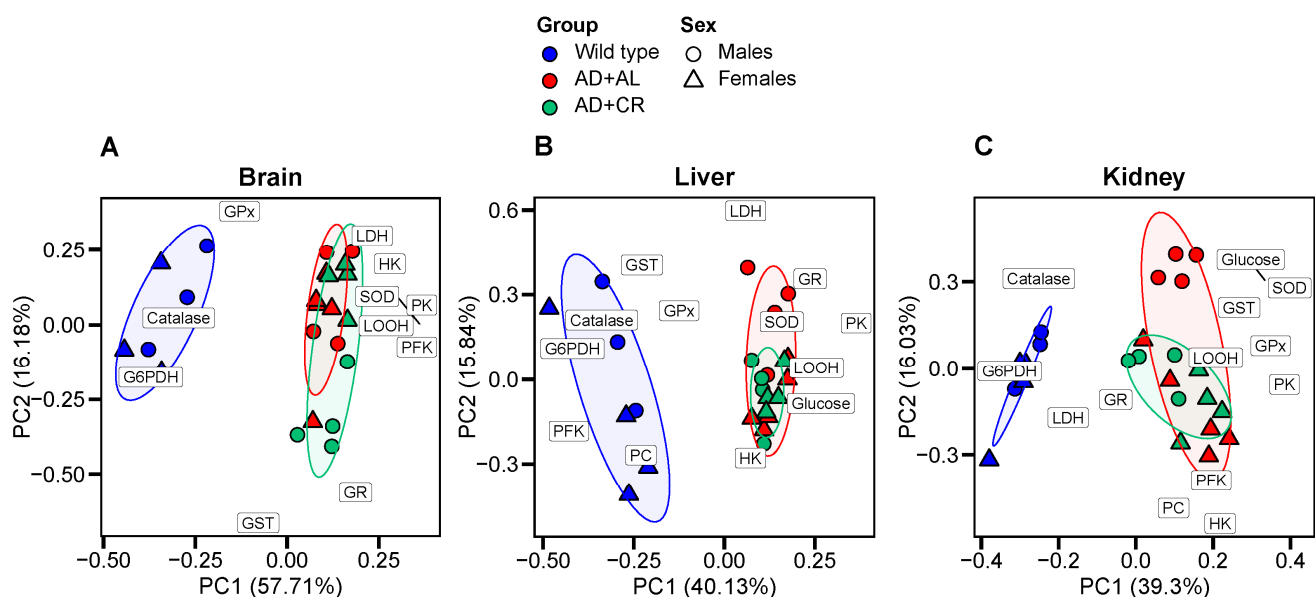


Figure 9. Principal component analysis of all parameters measured in this study as biplots, demonstrating the contribution of each measured parameter to the total variance of the dataset. The distance of each parameter label from the center of the figure indicates the amount of variance along each principal component. The data used for the PCA were taken from measurements conducted on samples from the brain (A), liver (B), and kidney (C) of WT, AD+AL, and AD+CR mice of both sexes. Note that liver and kidney datasets also comprise measurements of glucose and protein carbonyls that are not present in the brain dataset.

In the liver and brain, the effect of caloric restriction was modest. In all studied organs, parameters explaining the largest proportion of variance along PC1 were LOOH, SOD, G6PDH, catalase, and PK. The contribution of several parameters was tissue-specific. Thus, in the brain, along with LOOH, SOD, and PK, AD development was associated with large changes in the activity of PFK and HK. There was also a strong association of amyloidosis with the tissue glucose levels, where measured. In the liver, high PFK activity was associated with the WT phenotype (Figures 6C and 9B). In the kidney, GPx and GST, enzymes able to detoxify lipid peroxides, also contributed to the PC1 variance, whereas in the brain and liver, these two enzymes did not show strong associations with either the WT or AD phenotype. In the brain and liver, LDH activity explains the largest proportion of variance along PC2, likely indicating the particular importance of this enzyme for these two organs.

4. Discussion

4.1. AD-Induced Body-Wide Metabolic Changes

4.1.1. Oxidative Stress

Recent cumulative evidence refutes the purely central-nervous-system-centered etiology of AD and positions it as a metabolic disease of the entire body [9,10,43–45]. By analyzing back-to-back AD-induced metabolic changes in the brain, liver, and kidney, i.e., the three key organs for A β generation (brain) and clearance (liver and kidney), we observed a strong and consistent increase in oxidative stress markers (Figures 3A, 5A, 7A,B, and 9). The increase in LOOH levels is consistent with previous brain data from humans and rodent AD models [5,46]. Neurotoxic byproducts of lipid peroxidation, 4-HNE (Figure 1) and acrolein, were elevated in the hippocampus, cortex, and cerebellum of subjects with mild cognitive impairment and early AD [47,48]. High levels of thiobarbiturate-reactive substances and F4-isoprostanes were found in the postmortem brains of AD patients [49,50]. Cortical data from 3 to 5-month-old female 3 $\times$ Tg-AD mice suggest that oxidative stress intensifies early, before the development of senile plaques or tau pathology, and is likely triggered by intracellular accumulation of A β oligomers [51]. If so, similar changes are expected in our AD mice.

Intracellular A β can induce oxidative stress in several ways. In the brain mitochondria of AD patients and mAPP mice, it complexes with the A β -binding alcohol dehydrogenase at nanomolar concentrations [52], causing mitochondrial dysfunction, ROS overproduction, and cytochrome c release from mitochondria. A β also binds transition metals, favoring hydroxyl radical formation through the Fenton reaction [53]. Finally, A β -induced ROS may impair antioxidant or glycolytic enzymes [5]. As the disease progresses, extracellular A β oligomers bind pattern recognition receptors on microglia [6,54], activating NF- κ B. This upregulates inducible NO-synthase and NADPH oxidase 2; increases production of nitric oxide, superoxide anion radical, hydrogen peroxide, and peroxynitrite; and increases levels of proinflammatory cytokines like interleukin 1 β (IL-1 β), which further amplify NF- κ B signaling [6]. Furthermore, extracellular A β oligomers induce neuronal hyperactivity and enhance intracellular Ca²⁺ signaling [55], raising the mitochondrial Ca²⁺ content [56]. Elevated Ca²⁺ stimulates the oxidative phosphorylation (OXPHOS) system and dehydrogenase complexes (e.g., α -ketoglutarate and pyruvate dehydrogenase) to generate ROS [57]. Note that in AD mice, and likely also in patients, amyloid accumulations are found not only in the brain but also in the liver and kidney [10,27,28].

The three organs under study reacted to the above challenges with an activation of the antioxidant defense, but the activation patterns were organ-specific: the activity of (i) SOD was higher in all tissues except the female liver; (ii) catalase was lower in the female brain and kidney, as well as in the male liver; (iii) GPx was lower in the female brain and higher in the kidney of both sexes, with no change in the liver; and (iv) GST selectively increased in the female kidney. The AD-mediated decrease in catalase activity is likely due to depletion of active heme, the prosthetic group of catalases, as intracellular A β may complex with free heme, reducing its bioavailability [58]. In the brain, liver, and kidney, the AD-induced G6PDH changes paralleled changes in catalase activity, in line with our previous studies [24,35,59]. G6PDH is vulnerable to ROS, especially when the catalase activity is decreased [35,60]. Thus, the ubiquitous AD-mediated decrease in G6PDH activity (see also Figure 9) may represent an additional oxidative stress marker, along with increased LOOH levels and SOD activity, as a possible compensatory response. G6PDH lies at the crossroads of antioxidant defense (as an enzyme producing an important reductant NADPH) and carbohydrate metabolism. With reduced G6PDH activity in the liver of both sexes and all female tissues, carbohydrate metabolism likely shifts to glycolysis.

This reduction may also reflect the secondary consequences of altered energy metabolism, given the central role of G6PDH in NADPH production and carbohydrate flux.

Together, the observed enzymatic changes may reflect both organ-specific AD-induced intensification of oxidative burden and compensatory responses. Decreases in catalase and G6PDH activity, along with increased LOOH levels, are consistent with increased oxidative burden. The increase in SOD (and in some tissues GST) activities suggests compensatory antioxidant responses aimed at neutralizing superoxide and electrophilic metabolites. Parallel changes in glycolytic enzymes point to the secondary consequences of an altered energy metabolism, where shifts in carbohydrate flux may indirectly modulate redox homeostasis.

4.1.2. Enhancement of Glycolytic Capacity

In our study, the strongest amyloid-induced effect on glycolytic enzymes was observed in the brain, with a selective increase in the activities of HK and PFK, accompanied by a ubiquitous increase in PK activity. Previously, a higher abundance of pyruvate kinase M1 was found in cortical extracts of 6-month-old APP^{swe}/PSEN1 Δ E9 male AD mice [61] and in brain and cerebrospinal fluid samples of AD patients [62–64]. Higher activities of glycolytic enzymes may reflect the amyloidosis-driven inflammation and metabolic reprogramming of immune cells, shifting from OXPHOS to glycolysis, even in the presence of oxygen. In peripheral and central immune cells, glycolysis and its metabolites (i) activate proinflammatory signaling pathways, such as NFAT (nuclear factor of activated T-cells), NF- κ B, and mTOR complex 1; (ii) enhance production of IL-1 β and interferon; and (iii) increase intracellular Ca²⁺ levels and trigger post-transcriptional, post-translational, and epigenetic changes [65]. In microglia, this shift supports proliferation, phagocytosis, and production of pro-inflammatory cytokines [66]. As glycolytic enzymes are abundantly expressed in the postsynaptic density [67], the higher activities of these enzymes might reflect the ubiquitous AD-induced hyperactivity of neural networks [68,69]. While in neurons elevated glycolysis potentially promotes neurodegeneration, in astrocytes it is mostly beneficial, sparing the blood-borne glucose for neurons and protecting them against oxidative stress [70,71].

Increased activities of glycolytic enzymes in our study may indicate both a compensatory role of glycolysis in providing cells with ATP and a metabolic reprogramming that indirectly modulates redox balance. Of note, the activity of PK, which provides ATP in the glycolysis pay-off phase, increased in all three organs of AD mice. CR may exacerbate the energy deficit in AD mice, forcing cells to activate energy-producing pathways, for example, by inducing AMP-activated protein kinase and its downstream targets, including glycolytic enzymes [72].

PCA indicates the opposite trend for PFK activity in the liver, with the lowest levels being in AD animals (Figures 6C and 9B). This might reflect predominant gluconeogenesis in the liver and implies that gluconeogenesis in the liver and glycolysis in the brain and kidney maximize the flux of specific metabolites (e.g., glycerol-3-phosphate). In glycolytic tissues, PFK directs the flow to the production of trioses, whereas in the liver, trioses can be formed in gluconeogenesis, in reverse reactions of the glycolytic pay-off phase. Among trioses, dihydroxyacetone phosphate can be converted into glycerol-3-phosphate, which may contribute to mitochondrial ATP production through the glycerol-3-phosphate shuttle, bypassing complex I of the electron-transport system. This shuttle may provide energy for brain cells [73] and was recently shown to support neuronal metabolic flexibility [74]. Increased levels of glycerol-3-phosphate were also found in the liver and kidney of APP^{swe}/PSEN1 Δ E9 AD mice [75].

Collectively, these data show that AD induces significant body-wide metabolic changes (Figure 9), accompanied by peripheral (and likely also central [76]) hyperglycemia, ox-

oxidative damage, and enhanced glycolysis and antioxidant defense. The latter could be interpreted as a multidirectional organ-specific functional adaptation to the metabolic shifts entailed by amyloid deposits and soluble oligomers. In the brain, enhanced glycolysis may support ATP production, whereas in the liver, decreased PFK activity may prioritize systemic glucose supply at the expense of local antioxidant defense. Thus, the enzymatic changes we report likely represent an integrated response to both oxidative stress and metabolic shifts, with distinct functional outcomes across organs.

4.2. Sex-Specificity of the AD-Induced Changes

Two-thirds of AD patients are women, and at the age of 65, women are twice as likely to develop AD as men (Women and Alzheimer's | Alzheimer's Association). While the reasons also include longevity, pay gap, and stress from unpaid caregiving, sex-specific biological differences are crucial [3,77,78]. We observed sex-specific differences already in middle-aged WT mice: the catalase activity was higher in the female brain and the male liver. In addition, LDH activity was higher in the male liver, and HK in the female kidney. All but one (kidney HK activity) differences disappeared in the AD mice, showing that in disease, similarities between sexes may have different etiologies. Compared to AD+AL males, PK and PFK activities were lower in the brains of females, along with lower SOD and higher HK activities in the liver and kidney. In addition, the G6PDH activity was consistently lower in AD than in WT females, leading to decreased NADPH production. In AD mice, kidneys were the most sex-sensitive organ, with lower glucose and higher protein carbonyls, as well as lower catalase activities in females versus males. Note, however, that our findings represent phenotypic associations, rather than mechanistic explanations.

Together, these data point to less efficient AD-induced glycolysis in the female brain, as well as lower antioxidant defense in the female liver and kidney (likely impacting A β clearance), thus providing yet another facet to the complex picture of female vulnerability in AD.

4.3. Impact of Caloric Restriction

Obesity, insulin resistance, and type 2 diabetes are well-known AD comorbidities, sharing markers like oxidative damage; neuroinflammation; Ca²⁺ dyshomeostasis; impaired autophagy; neural network activity; adult neurogenesis; and learning and memory [72]. At the same time, caloric restriction extends lifespan across species (from yeast to humans), enhances autophagy and DNA repair, reduces oxidative damage and neuroinflammation, stabilizes Ca²⁺ homeostasis and neural activity, and promotes mitochondrial biogenesis and neurogenesis [72,79]. Moreover, CR decreases amyloid deposition in AD mice (this study and [16,18]) and improves the performance of elderly subjects in auditory verbal learning [19]. Therefore, we expected CR to counteract AD-induced metabolic dysfunction.

To our surprise, the LOOH levels were only lowered by CR in female kidneys. In the brain, LOOH levels remained unchanged, whereas in the liver, they were significantly higher compared to those in AD+AL mice, likely because the CR-mediated enhancement of mitochondrial biogenesis was not matched by a corresponding increase in antioxidant defense. This may explain why CR was insufficient to counteract the oxidative damage. Although in the brain CR sex-specifically increased the activity of antioxidant enzymes (Figure 3), this increase was probably insufficient to suppress lipid peroxidation.

A β can bind transition metals [53] and stimulate hydroxyl or superoxide radical formation from hydrogen peroxide near phospholipid membranes (see above). This complicates scavenging of oxygen-containing radicals and prevents fast ROS detection by signaling systems such as Keap1 [6]. While CR decreased the density of Thioflavin S-positive dense core plaques (Figure 2), this may not apply to the intracellular A β oligomers. It is therefore

not surprising that the catalase activity did not increase under CR. Additionally, CR may limit dietary precursors for heme biosynthesis (glycine, succinyl-CoA), constraining catalase function. Our previous study [24] showed a trend toward reduced catalase activity in WT+CR mice, supporting the notion that CR alone may suppress catalase independently of A β load, consistent with the modest CR outcomes we observed.

The only CR effect in the kidneys was a further increase in female GST activity. As GST detoxifies LOOH breakdown products (Figure 1), the increase in GST activity is consistent with a selective LOOH decrease in females (Figure 7A,G). For glycolytic enzymes, CR increased female PFK and decreased male PK in the brain and increased female PK in the liver. Compared to males, AD+CR females had higher PFK activity in the brain, HK activity in the liver and kidneys, and PK activity in the kidneys. Thus, in females, glycolysis is more responsive to CR than in males, so CR-induced glycolysis may help regulate AD-induced inflammation [65]. In general, however, all observed differences between the AD+AL and AD+CR mice were modest compared to those between the WT and AD mice (Figure 9).

5. Strengths and Limitations

In our study, group-housed AL-fed animals were compared with CR mice housed individually, but with the olfactory, visual, and acoustic stimuli preserved (see Materials and Methods). This might be seen as a limitation confounding data interpretation. To counteract single housing effects [80–84], all animals received cage enrichment [85–87]. For both sexes, long-term housing under similar conditions yielded burrowing, social interaction, anxiety, exploration, body weight, and stress hormone levels that were comparable to group-housed conspecifics [88–90]. Note that EODF, the only dietary intervention permitting group housing, does not limit the total amount of calories consumed per gram of body weight and thus is not a CR [29,91–93]. Consistently, in 5XFAD female mice, the EODF failed to decrease the brain's A β 42 levels or the AD-induced blood–brain barrier permeability [22]. Moreover, unlike our (Figure 2) and other data [16,18], this regimen failed to reduce amyloid deposits. Of note, our animals never showed weight loss under CR (Figure S1), and in the WT brains, the same CR protocol [24] did not change the studied parameters (Figure S2). In the liver and kidney, the control experiments [24] revealed only a few differences, with the signs of changes being mostly opposite to those reported here. However, our study uses relatively small sample sizes. Therefore, some conclusions, especially those related to subtle differences between dietary conditions, should be interpreted with caution and may require confirmation in larger cohorts.

The strength of our study lies in the sex-specific investigation of the systemic metabolic effects of amyloidosis and CR, and in a back-to-back biochemical analysis of central (brain) and peripheral (liver and kidney) tissues. While biomarker accessibility was not directly assessed, consistent AD-induced shifts in enzymes, such as pyruvate kinase, hexokinase, and glutathione S-transferase, across the organs suggest that peripheral metabolic signatures mirror amyloid-induced changes in the brain. These findings warrant further investigation into whether such enzymatic changes, or their upstream regulators, could serve as surrogate markers of AD progression or treatment response. Moreover, sex-dependent responsiveness to CR, especially in female kidneys and liver, supports the rationale for personalized dietary interventions to enhance peripheral metabolic resilience in AD. Together, our results provide a foundation for future translational studies targeting peripheral metabolism in AD diagnostics and therapy.

6. Conclusions

The current study shows that APPPS1 mice overexpressing mutant human amyloid precursor protein and presenilin 1 display body-wide adverse changes, with increased

levels of lipid peroxides in the brain, liver, and kidney, and altered activities of the first-line antioxidant enzymes, such as superoxide dismutase and catalase (Figure 9). Compared to the WT mice, the activity of pyruvate kinase was always higher, while differences in activities of glutathione-related enzymes, and enzymes of glucose catabolism (glucose 6-phosphate dehydrogenase, hexokinase, and phosphofructokinase) were tissue- and sex-specific. Under CR, female mice had more effective glucose catabolism, and their kidneys showed the greatest decrease in oxidative stress markers among all studied tissues. In general, however, the impact of caloric restriction on key glycolytic, antioxidant, and related enzymes was rather modest.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antiox15020191/s1>, Figure S1: Body weight and food intake in experimental mice; Figure S2: Effects of amyloidosis and caloric restriction (CR) on the APPPS1 (AD) and C57BL/6N (WT) mice.

Author Contributions: M.V.V., V.V.H. and K.P.: investigation; M.V.V. and K.P.: visualization; M.V.V. and D.V.G.: writing—original draft; M.M.B., D.V.G., V.I.L. and O.G.: writing—review and editing; M.M.B.: validation, data curation; D.V.G.: formal analysis; V.I.L. and O.G.: conceptualization, methodology; O.G.: supervision, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This work was mainly supported by the grant from the Volkswagen Foundation (VolkswagenStiftung, #90233), Germany, to V.I.L. and O.G., and the grant from the German Academic Exchange Service (DAAD; German: Deutscher Akademischer Austauschdienst, # 57722940) within the framework “Ukraine digital: Ensuring academic success in times of crisis 2024”.

Institutional Review Board Statement: All procedures for animal care and use complied with the ARRIVE guidelines, were carried out following the EU Directive 2010/63/EU for animal experiments, and were approved by the state government of Baden-Württemberg, Germany on the 2nd of November 2021.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

Acknowledgments: We thank E. Zirdum and K. Schmidt for their technical assistance, and E. Zirdum and A. Hahn for their help with caloric restriction and tissue sampling.

Conflicts of Interest: The authors declare no competing interests.

Abbreviations

The following abbreviations are used in this manuscript:

AD	Alzheimer’s disease
AL	Ad libitum
APP	Amyloid precursor protein
ARRIVE	Animal Research: Reporting of In Vivo Experiments
A β	Amyloid β
CR	Caloric restriction
EDTA	<i>N,N,N',N'</i> -Ethylene diamine tetraacetic acid
EODF	Every-other-day-feeding
G6PDH	Glucose-6-phosphate dehydrogenase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH/GSSG	Glutathione reduced/oxidized
GST	Glutathione S-transferase

HK	Hexokinase
4-HNE	4-hydroxy-2-nonenal
IL-1 β	Interleukin 1 β
Keap1	Kelch-like ECH-associated protein 1
KPi	Potassium phosphate buffer
LDH	Lactate dehydrogenase
LOOH	Lipid peroxides
mTOR	Mechanistic target of rapamycin
NAD ⁺ /NADH	Nicotinamide adenine dinucleotide oxidized/reduced
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NFAT	Nuclear factor of activated T-cells
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
OXPHOS	Oxidative phosphorylation
PC	Protein carbonyls
PCA	Principal component analysis
PFK	Phosphofructokinase
PK	Pyruvate kinase
PS1	Presenilin 1
ROS	Reactive oxygen species
SIRT3	Sirtuin-3
SOD	Superoxide dismutase
TEMED	<i>N,N,N',N'</i> -Tetramethylethylenediamine
WT	Wild-type
Mouse models of AD	3 $\times$ Tg-AD, 5XFAD, mAPP, APP ^{swe} /PSEN1 Δ E9 mice

References

1. Cuyvers, E.; Sleegers, K. Genetic Variations Underlying Alzheimer's Disease: Evidence from Genome-Wide Association Studies and Beyond. *Lancet Neurol.* **2016**, *15*, 857–868. [\[CrossRef\]](#)
2. Young-Pearse, T.L.; Lee, H.; Hsieh, Y.-C.; Chou, V.; Selkoe, D.J. Moving beyond Amyloid and Tau to Capture the Biological Heterogeneity of Alzheimer's Disease. *Trends Neurosci.* **2023**, *46*, 426–444. [\[CrossRef\]](#)
3. Mauvais-Jarvis, F.; Bairey Merz, N.; Barnes, P.J.; Brinton, R.D.; Carrero, J.-J.; DeMeo, D.L.; De Vries, G.J.; Epperson, C.N.; Govindan, R.; Klein, S.L.; et al. Sex and Gender: Modifiers of Health, Disease, and Medicine. *Lancet* **2020**, *396*, 565–582. [\[CrossRef\]](#)
4. Brawek, B.; Garaschuk, O. Network-Wide Dysregulation of Calcium Homeostasis in Alzheimer's Disease. *Cell Tissue Res.* **2014**, *357*, 427–438. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Butterfield, D.A.; Halliwell, B. Oxidative Stress, Dysfunctional Glucose Metabolism and Alzheimer's Disease. *Nat. Rev. Neurosci.* **2019**, *20*, 148–160. [\[CrossRef\]](#)
6. Lushchak, V.I.; Duszenko, M.; Gospodaryov, D.V.; Garaschuk, O. Oxidative Stress and Energy Metabolism in the Brain: Midlife as a Turning Point. *Antioxidants* **2021**, *10*, 1715. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Liu, N.; Haziyyihan, A.; Zhao, W.; Chen, Y.; Chao, H. Trajectory of Brain-Derived Amyloid Beta in Alzheimer's Disease: Where Is It Coming from and Where Is It Going? *Transl. Neurodegener.* **2024**, *13*, 42. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Bu, X.-L.; Xiang, Y.; Jin, W.-S.; Wang, J.; Shen, L.-L.; Huang, Z.-L.; Zhang, K.; Liu, Y.-H.; Zeng, F.; Liu, J.-H.; et al. Blood-Derived Amyloid- β Protein Induces Alzheimer's Disease Pathologies. *Mol. Psychiatry* **2018**, *23*, 1948–1956. [\[CrossRef\]](#)
9. Lam, V.; Takechi, R.; Hackett, M.J.; Francis, R.; Bynevelt, M.; Celliers, L.M.; Nesbit, M.; Mamsa, S.; Arfuso, F.; Das, S.; et al. Synthesis of Human Amyloid Restricted to Liver Results in an Alzheimer's Disease-like Neurodegenerative Phenotype. *PLoS Biol.* **2021**, *19*, e3001358. [\[CrossRef\]](#)
10. Tian, D.-Y.; Cheng, Y.; Zhuang, Z.-Q.; He, C.-Y.; Pan, Q.-G.; Tang, M.-Z.; Hu, X.-L.; Shen, Y.-Y.; Wang, Y.-R.; Chen, S.-H.; et al. Physiological Clearance of Amyloid-Beta by the Kidney and Its Therapeutic Potential for Alzheimer's Disease. *Mol. Psychiatry* **2021**, *26*, 6074–6082. [\[CrossRef\]](#)
11. Kellar, D.; Craft, S. Brain Insulin Resistance in Alzheimer's Disease and Related Disorders: Mechanisms and Therapeutic Approaches. *Lancet Neurol.* **2020**, *19*, 758–766. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Davoody, S.; Asgari Taei, A.; Khodabakhsh, P.; Dargahi, L. mTOR Signaling and Alzheimer's Disease: What We Know and Where We Are? *CNS Neurosci. Ther.* **2024**, *30*, e14463. [\[CrossRef\]](#)
13. Cheng, Y.; Zhao, A.; Li, Y.; Li, C.; Miao, X.; Yang, W.; Wang, Y. Roles of SIRT3 in Cardiovascular and Neurodegenerative Diseases. *Ageing Res. Rev.* **2025**, *104*, 102654. [\[CrossRef\]](#)

14. Elias, A.; Padinjakara, N.; Lautenschlager, N.T. Effects of Intermittent Fasting on Cognitive Health and Alzheimer's Disease. *Nutr. Rev.* **2023**, *81*, 1225–1233. [\[CrossRef\]](#)
15. Kincaid, B.; Bossy-Wetzel, E. Forever Young: SIRT3 a Shield against Mitochondrial Meltdown, Aging, and Neurodegeneration. *Front. Aging Neurosci.* **2013**, *5*, 48. [\[CrossRef\]](#)
16. Patel, N.V.; Gordon, M.N.; Connor, K.E.; Good, R.A.; Engelman, R.W.; Mason, J.; Morgan, D.G.; Morgan, T.E.; Finch, C.E. Caloric Restriction Attenuates A β -Deposition in Alzheimer Transgenic Models. *Neurobiol. Aging* **2005**, *26*, 995–1000. [\[CrossRef\]](#)
17. Van Cauwenbergh, C.; Vandendriessche, C.; Libert, C.; Vandenbroucke, R.E. Caloric Restriction: Beneficial Effects on Brain Aging and Alzheimer's Disease. *Mamm. Genome* **2016**, *27*, 300–319. [\[CrossRef\]](#)
18. Wang, J.; Ho, L.; Qin, W.; Rocher, A.B.; Seror, I.; Humala, N.; Maniar, K.; Dolios, G.; Wang, R.; Hof, P.R.; et al. Caloric Restriction Attenuates β -amyloid Neuropathology in a Mouse Model of Alzheimer's Disease. *FASEB J.* **2005**, *19*, 1–18. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Witte, A.V.; Fobker, M.; Gellner, R.; Knecht, S.; Flöel, A. Caloric Restriction Improves Memory in Elderly Humans. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 1255–1260. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Chen, X.; Kondo, K.; Motoki, K.; Homma, H.; Okazawa, H. Fasting Activates Macroautophagy in Neurons of Alzheimer's Disease Mouse Model but Is Insufficient to Degrade Amyloid-Beta. *Sci. Rep.* **2015**, *5*, 12115. [\[CrossRef\]](#)
21. Lazic, D.; Tesic, V.; Jovanovic, M.; Brkic, M.; Milanovic, D.; Zlokovic, B.V.; Kanazir, S.; Perovic, M. Every-Other-Day Feeding Exacerbates Inflammation and Neuronal Deficits in 5XFAD Mouse Model of Alzheimer's Disease. *Neurobiol. Dis.* **2020**, *136*, 104745. [\[CrossRef\]](#)
22. Stonebarger, G.; Urbanski, H.; Woltjer, R.; Vaughan, K.; Ingram, D.; Schultz, P.; Calderazzo, S.; Siedeman, J.; Mattison, J.; Rosene, D.; et al. Amyloidosis Increase Is Not Attenuated by Long-Term Calorie Restriction or Related to Neuron Density in the Prefrontal Cortex of Extremely Aged Rhesus Macaques. *GeroScience* **2020**, *42*, 1733–1749. [\[CrossRef\]](#)
23. Bayliak, M.M.; Sorochnytska, O.M.; Kuzniak, O.V.; Gospodaryov, D.V.; Demianchuk, O.I.; Vasylyk, Y.V.; Mosiichuk, N.M.; Storey, K.B.; Garaschuk, O.; Lushchak, V.I. Middle Age as a Turning Point in Mouse Cerebral Cortex Energy and Redox Metabolism: Modulation by Every-Other-Day Fasting. *Exp. Gerontol.* **2021**, *145*, 111182. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Vatachuk, M.V.; Hurza, V.V.; Stefanyshyn, N.; Bayliak, M.M.; Gospodaryov, D.V.; Garaschuk, O.; Lushchak, V.I. Impact of Caloric Restriction on Oxidative Stress and Key Glycolytic Enzymes in the Cerebral Cortex, Liver and Kidney of Old and Middle-Aged Mice. *Neuropharmacology* **2024**, *247*, 109859. [\[CrossRef\]](#)
25. Radde, R.; Bolmont, T.; Kaeser, S.A.; Coomaraswamy, J.; Lindau, D.; Stoltze, L.; Calhoun, M.E.; Jäggi, F.; Wolburg, H.; Gengler, S.; et al. A β 42-driven Cerebral Amyloidosis in Transgenic Mice Reveals Early and Robust Pathology. *EMBO Rep.* **2006**, *7*, 940–946. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Lopez-Lee, C.; Torres, E.R.S.; Carling, G.; Gan, L. Mechanisms of Sex Differences in Alzheimer's Disease. *Neuron* **2024**, *112*, 1208–1221. [\[CrossRef\]](#)
27. Fraile-Ramos, J.; Garrit, A.; Reig-Vilallonga, J.; Giménez-Llort, L. Hepatic Oxi-Inflammation and Neophobia as Potential Liver-Brain Axis Targets for Alzheimer's Disease and Aging, with Strong Sensitivity to Sex, Isolation, and Obesity. *Cells* **2023**, *12*, 1517. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Fraile-Ramos, J.; Reig-Vilallonga, J.; Giménez-Llort, L. Glomerular Hypertrophy and Splenic Red Pulp Degeneration Concurrent with Oxidative Stress in 3xTg-AD Mice Model for Alzheimer's Disease and Its Exacerbation with Sex and Social Isolation. *Int. J. Mol. Sci.* **2024**, *25*, 6112. [\[CrossRef\]](#)
29. Cerqueira, F.M.; Kowaltowski, A.J. Commonly Adopted Caloric Restriction Protocols Often Involve Malnutrition. *Ageing Res. Rev.* **2010**, *9*, 424–430. [\[CrossRef\]](#)
30. Turturro, A.; Witt, W.W.; Lewis, S.; Hass, B.S.; Lipman, R.D.; Hart, R.W. Growth Curves and Survival Characteristics of the Animals Used in the Biomarkers of Aging Program. *J. Gerontol. Ser. A* **1999**, *54*, B492–B501. [\[CrossRef\]](#)
31. Olmedillas Del Moral, M.; Fröhlich, N.; Figarella, K.; Mojtahedi, N.; Garaschuk, O. Effect of Caloric Restriction on the in Vivo Functional Properties of Aging Microglia. *Front. Immunol.* **2020**, *11*, 750. [\[CrossRef\]](#)
32. Murphy, M.P.; Bayir, H.; Belousov, V.; Chang, C.J.; Davies, K.J.A.; Davies, M.J.; Dick, T.P.; Finkel, T.; Forman, H.J.; Janssen-Heininger, Y.; et al. Guidelines for Measuring Reactive Oxygen Species and Oxidative Damage in Cells and in Vivo. *Nat. Metab.* **2022**, *4*, 651–662. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Hermes-Lima, M. Quantification of Lipid Peroxidation in Tissue Extracts Based on Fe(III)Xylenol Orange Complex Formation. *Free Radic. Biol. Med.* **1995**, *19*, 271–280. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Lushchak, V.I.; Semchyshyn, H.M.; Lushchak, O.V. The Classic Methods to Measure Oxidative Damage: Lipid Peroxides, Thiobarbituric-Acid Reactive Substances, and Protein Carbonyls. In *Oxidative Stress in Aquatic Ecosystems*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2011; pp. 420–431. ISBN 978-1-4443-4598-8.
35. Lushchak, V.; Gospodaryov, D. Catalases Protect Cellular Proteins from Oxidative Modification in *Saccharomyces cerevisiae*. *Cell Biol. Int.* **2005**, *29*, 187–192. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Semchyshyn, H.; Lushchak, V.; Storey, K. Possible Reasons for Difference in Sensitivity to Oxygen of Two Escherichia Coli Strains. *Biochem. Mosc.* **2005**, *70*, 424–431. [\[CrossRef\]](#)

37. Brooks, S.P. A Simple Computer Program with Statistical Tests for the Analysis of Enzyme Kinetics. *BioTechniques* **1992**, *13*, 906–911.
38. Lushchak, V.I.; Bahnjukova, T.V.; Storey, K.B. Effect of Hypoxia on the Activity and Binding of Glycolytic and Associated Enzymes in Sea Scorpion Tissues. *Braz. J. Med. Biol. Res.* **1998**, *31*, 1059–1067. [\[CrossRef\]](#)
39. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. [\[CrossRef\]](#)
40. Harach, T.; Marungruang, N.; Duthilleul, N.; Cheatham, V.; Mc Coy, K.D.; Frisoni, G.; Neher, J.J.; Fåk, F.; Jucker, M.; Lasser, T.; et al. Reduction of Abeta Amyloid Pathology in APPPS1 Transgenic Mice in the Absence of Gut Microbiota. *Sci. Rep.* **2017**, *7*, 41802. [\[CrossRef\]](#)
41. Poxleitner, M.; Hoffmann, S.H.L.; Berezhnoy, G.; Ionescu, T.M.; Gonzalez-Menendez, I.; Maier, F.C.; Seyfried, D.; Ehrlichmann, W.; Quintanilla-Martinez, L.; Schmid, A.M.; et al. Western Diet Increases Brain Metabolism and Adaptive Immune Responses in a Mouse Model of Amyloidosis. *J. Neuroinflamm.* **2024**, *21*, 129. [\[CrossRef\]](#)
42. Buniatian, G.H.; Schwinghammer, U.; Tremmel, R.; Cynis, H.; Weiss, T.S.; Weiskirchen, R.; Lauschke, V.M.; Youhanna, S.; Ramos, I.; Valcarcel, M.; et al. Consequences of Amyloid- β Deficiency for the Liver. *Adv. Sci.* **2024**, *11*, e2307734. [\[CrossRef\]](#)
43. Bassendine, M.F.; Taylor-Robinson, S.D.; Fertleman, M.; Khan, M.; Neely, D. Is Alzheimer's Disease a Liver Disease of the Brain? *J. Alzheimers Dis.* **2020**, *75*, 1–14. [\[CrossRef\]](#)
44. Estrada, L.D.; Ahumada, P.; Cabrera, D.; Arab, J.P. Liver Dysfunction as a Novel Player in Alzheimer's Progression: Looking Outside the Brain. *Front. Aging Neurosci.* **2019**, *11*, 174. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Luo, Y.-X.; Yang, L.-L.; Yao, X.-Q. Gut Microbiota-Host Lipid Crosstalk in Alzheimer's Disease: Implications for Disease Progression and Therapeutics. *Mol. Neurodegener.* **2024**, *19*, 35. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Foret, M.K.; Orciani, C.; Welikovitch, L.A.; Huang, C.; Cuello, A.C.; Do Carmo, S. Early Oxidative Stress and DNA Damage in A β -Burdened Hippocampal Neurons in an Alzheimer's-like Transgenic Rat Model. *Commun. Biol.* **2024**, *7*, 861. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Reed, T.; Perluigi, M.; Sultana, R.; Pierce, W.M.; Klein, J.B.; Turner, D.M.; Coccia, R.; Markesbery, W.R.; Butterfield, D.A. Redox Proteomic Identification of 4-Hydroxy-2-Nonenal-Modified Brain Proteins in Amnesic Mild Cognitive Impairment: Insight into the Role of Lipid Peroxidation in the Progression and Pathogenesis of Alzheimer's Disease. *Neurobiol. Dis.* **2008**, *30*, 107–120. [\[CrossRef\]](#)
48. Williams, T.I.; Lynn, B.C.; Markesbery, W.R.; Lovell, M.A. Increased Levels of 4-Hydroxynonenal and Acrolein, Neurotoxic Markers of Lipid Peroxidation, in the Brain in Mild Cognitive Impairment and Early Alzheimer's Disease. *Neurobiol. Aging* **2006**, *27*, 1094–1099. [\[CrossRef\]](#)
49. Marcus, D.L.; Thomas, C.; Rodriguez, C.; Simberkoff, K.; Tsai, J.S.; Strafaci, J.A.; Freedman, M.L. Increased Peroxidation and Reduced Antioxidant Enzyme Activity in Alzheimer's Disease. *Exp. Neurol.* **1998**, *150*, 40–44. [\[CrossRef\]](#)
50. Nourooz-Zadeh, J.; Liu, E.H.C.; Yhlen, B.; Änggård, E.E.; Halliwell, B. F₄—Isoprostanes as Specific Marker of Docosaehaenoic Acid Peroxidation in Alzheimer's Disease. *J. Neurochem.* **1999**, *72*, 734–740. [\[CrossRef\]](#)
51. Resende, R.; Moreira, P.I.; Proença, T.; Deshpande, A.; Busciglio, J.; Pereira, C.; Oliveira, C.R. Brain Oxidative Stress in a Triple-Transgenic Mouse Model of Alzheimer's Disease. *Free Radic. Biol. Med.* **2008**, *44*, 2051–2057. [\[CrossRef\]](#)
52. Lustbader, J.W.; Cirilli, M.; Lin, C.; Xu, H.W.; Takuma, K.; Wang, N.; Caspersen, C.; Chen, X.; Pollak, S.; Chaney, M.; et al. ABAD Directly Links Abeta to Mitochondrial Toxicity in Alzheimer's Disease. *Science* **2004**, *304*, 448–452. [\[CrossRef\]](#)
53. Tamagno, E.; Guglielmotto, M.; Vasciaveo, V.; Tabaton, M. Oxidative Stress and Beta Amyloid in Alzheimer's Disease. Which Comes First: The Chicken or the Egg? *Antioxidants* **2021**, *10*, 1479. [\[CrossRef\]](#)
54. Liu, S.; Liu, Y.; Hao, W.; Wolf, L.; Kiliaan, A.J.; Penke, B.; Rube, C.E.; Walter, J.; Heneka, M.T.; Hartmann, T.; et al. TLR2 Is a Primary Receptor for Alzheimer's Amyloid β Peptide to Trigger Neuroinflammatory Activation. *J. Immunol.* **2012**, *188*, 1098–1107. [\[CrossRef\]](#)
55. Busche, M.A.; Chen, X.; Henning, H.A.; Reichwald, J.; Staufenbiel, M.; Sakmann, B.; Konnerth, A. Critical Role of Soluble Amyloid- β for Early Hippocampal Hyperactivity in a Mouse Model of Alzheimer's Disease. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 8740–8745. [\[CrossRef\]](#)
56. Rossi, A.; Pizzo, P.; Filadi, R. Calcium, Mitochondria and Cell Metabolism: A Functional Triangle in Bioenergetics. *Biochim. Biophys. Acta BBA—Mol. Cell Res.* **2019**, *1866*, 1068–1078. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Chinopoulos, C.; Adam-Vizi, V. Calcium, Mitochondria and Oxidative Stress in Neuronal Pathology: Novel Aspects of an Enduring Theme. *FEBS J.* **2006**, *273*, 433–450. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Atamna, H.; Frey, W.H. A Role for Heme in Alzheimer's Disease: Heme Binds Amyloid Beta and Has Altered Metabolism. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11153–11158. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Semaniuk, U.V.; Gospodaryov, D.V.; Strilbytska, O.M.; Kucharska, A.Z.; Sokół-Łętowska, A.; Burdyliuk, N.I.; Storey, K.B.; Bayliak, M.M.; Lushchak, O. Chili-Supplemented Food Decreases Glutathione-S-Transferase Activity in *Drosophila Melanogaster* Females without a Change in Other Parameters of Antioxidant System. *Redox Rep.* **2022**, *27*, 221–229. [\[CrossRef\]](#)

60. Reyes, J.S.; Fuentes-Lemus, E.; Fierro, A.; Rivero-Rodríguez, K.; Arenas, F.; Davies, M.J.; López-Alarcón, C. Inactivation of Human Glucose 6-Phosphate Dehydrogenase (G6PDH) by Peroxyl Radicals Is Strongly Modulated by Its Substrate and Cofactor. *Free Radic. Biol. Med.* **2025**, *233*, 55–69. [\[CrossRef\]](#)
61. Harris, R.A.; Tindale, L.; Lone, A.; Singh, O.; Macauley, S.L.; Stanley, M.; Holtzman, D.M.; Bartha, R.; Cumming, R.C. Aerobic Glycolysis in the Frontal Cortex Correlates with Memory Performance in Wild-Type Mice But Not the APP/PS1 Mouse Model of Cerebral Amyloidosis. *J. Neurosci.* **2016**, *36*, 1871–1878. [\[CrossRef\]](#)
62. Bader, J.M.; Geyer, P.E.; Müller, J.B.; Strauss, M.T.; Koch, M.; Leyboldt, F.; Koertvelyessy, P.; Bittner, D.; Schipke, C.G.; Incesoy, E.I.; et al. Proteome Profiling in Cerebrospinal Fluid Reveals Novel Biomarkers of Alzheimer’s Disease. *Mol. Syst. Biol.* **2020**, *16*, e9356. [\[CrossRef\]](#)
63. Bigl, M.; Brückner, M.K.; Arendt, T.; Bigl, V.; Eschrich, K. Activities of Key Glycolytic Enzymes in the Brains of Patients with Alzheimer’s Disease. *J. Neural Transm.* **1999**, *106*, 499–511. [\[CrossRef\]](#)
64. Johnson, E.C.B.; Dammer, E.B.; Duong, D.M.; Ping, L.; Zhou, M.; Yin, L.; Higginbotham, L.A.; Guajardo, A.; White, B.; Troncoso, J.C.; et al. Large-Scale Proteomic Analysis of Alzheimer’s Disease Brain and Cerebrospinal Fluid Reveals Early Changes in Energy Metabolism Associated with Microglia and Astrocyte Activation. *Nat. Med.* **2020**, *26*, 769–780. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Soto-Herederó, G.; Gómez de Las Heras, M.M.; Gabandé-Rodríguez, E.; Oller, J.; Mittelbrunn, M. Glycolysis—A Key Player in the Inflammatory Response. *FEBS J.* **2020**, *287*, 3350–3369. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Jung, E.S.; Choi, H.; Mook-Jung, I. Decoding Microglial Immunometabolism: A New Frontier in Alzheimer’s Disease Research. *Mol. Neurodegener.* **2025**, *20*, 37. [\[CrossRef\]](#)
67. Zhang, X.; Alshakhshir, N.; Zhao, L. Glycolytic Metabolism, Brain Resilience, and Alzheimer’s Disease. *Front. Neurosci.* **2021**, *15*, 662242. [\[CrossRef\]](#) [\[PubMed\]](#)
68. Busche, M.A.; Eichhoff, G.; Adelsberger, H.; Abramowski, D.; Wiederhold, K.-H.; Haass, C.; Staufenbiel, M.; Konnerth, A.; Garaschuk, O. Clusters of Hyperactive Neurons near Amyloid Plaques in a Mouse Model of Alzheimer’s Disease. *Science* **2008**, *321*, 1686–1689. [\[CrossRef\]](#)
69. Palop, J.J.; Mucke, L. Network Abnormalities and Interneuron Dysfunction in Alzheimer’s Disease. *Nat. Rev. Neurosci.* **2016**, *17*, 777–792. [\[CrossRef\]](#)
70. Sadeghdoust, M.; Das, A.; Kaushik, D.K. Fueling Neurodegeneration: Metabolic Insights into Microglia Functions. *J. Neuroinflamm.* **2024**, *21*, 300. [\[CrossRef\]](#)
71. Dienel, G.A.; Rothman, D.L.; Mangia, S. A Bird’s-Eye View of Glycolytic Upregulation in Activated Brain: The Major Fate of Lactate Is Release From Activated Tissue, Not Shuttling to Nearby Neurons. *J. Neurochem.* **2025**, *169*, e70111. [\[CrossRef\]](#)
72. Mattson, M.P.; Arumugam, T.V. Hallmarks of Brain Aging: Adaptive and Pathological Modification by Metabolic States. *Cell Metab.* **2018**, *27*, 1176–1199. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Ryu, W.-I.; Bormann, M.K.; Shen, M.; Kim, D.; Forester, B.; Park, Y.; So, J.; Seo, H.; Sonntag, K.-C.; Cohen, B.M. Brain Cells Derived from Alzheimer’s Disease Patients Have Multiple Specific Innate Abnormalities in Energy Metabolism. *Mol. Psychiatry* **2021**, *26*, 5702–5714. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Dhoundiyal, A.; Goeschl, V.; Boehm, S.; Kubista, H.; Hotka, M. Glycerol-3-Phosphate Shuttle Is a Backup System Securing Metabolic Flexibility in Neurons. *J. Neurosci. Off. J. Soc. Neurosci.* **2022**, *42*, 7339–7354. [\[CrossRef\]](#) [\[PubMed\]](#)
75. González-Domínguez, R.; García-Barrera, T.; Vitorica, J.; Gómez-Ariza, J.L. Metabolomic Investigation of Systemic Manifestations Associated with Alzheimer’s Disease in the APP/PS1 Transgenic Mouse Model. *Mol. Biosyst.* **2015**, *11*, 2429–2440. [\[CrossRef\]](#)
76. An, Y.; Varma, V.R.; Varma, S.; Casanova, R.; Dammer, E.; Pletnikova, O.; Chia, C.W.; Egan, J.M.; Ferrucci, L.; Troncoso, J.; et al. Evidence for Brain Glucose Dysregulation in Alzheimer’s Disease. *Alzheimers Dement. J. Alzheimers Assoc.* **2018**, *14*, 318–329. [\[CrossRef\]](#)
77. Kolahchi, Z.; Henkel, N.; Eladawi, M.A.; Villarreal, E.C.; Kandimalla, P.; Lundh, A.; McCullumsmith, R.E.; Cuevas, E. Sex and Gender Differences in Alzheimer’s Disease: Genetic, Hormonal, and Inflammation Impacts. *Int. J. Mol. Sci.* **2024**, *25*, 8485. [\[CrossRef\]](#)
78. Tzeng, W.-Y.; Figarella, K.; Garaschuk, O. Olfactory Impairment in Men and Mice Related to Aging and Amyloid-Induced Pathology. *Pflügers Arch.* **2021**, *473*, 805–821. [\[CrossRef\]](#)
79. Fontana, L.; Partridge, L.; Longo, V.D. Extending Healthy Life Span—from Yeast to Humans. *Science* **2010**, *328*, 321–326. [\[CrossRef\]](#)
80. Vöikar, V.; Polus, A.; Vasar, E.; Rauvala, H. Long-term Individual Housing in C57BL/6J and DBA/2 Mice: Assessment of Behavioral Consequences. *Genes Brain Behav.* **2005**, *4*, 240–252. [\[CrossRef\]](#)
81. Krohn, T.; Sørensen, D.; Ottesen, J.; Hansen, A. The Effects of Individual Housing on Mice and Rats: A Review. *Anim. Welf.* **2006**, *15*, 343–352. [\[CrossRef\]](#)
82. Sun, M.; Choi, E.Y.; Magee, D.J.; Stets, C.W.; During, M.J.; Lin, E.-J.D. Metabolic Effects of Social Isolation in Adult C57BL/6 Mice. *Int. Sch. Res. Not.* **2014**, *2014*, 690950. [\[CrossRef\]](#)
83. Schipper, L.; Harvey, L.; Van Der Beek, E.M.; Van Dijk, G. Home Alone: A Systematic Review and Meta-analysis on the Effects of Individual Housing on Body Weight, Food Intake and Visceral Fat Mass in Rodents. *Obes. Rev.* **2018**, *19*, 614–637. [\[CrossRef\]](#)

84. Balatskyi, V.; Gospodaryov, D.; Abrat, O.; Strilbytska, O.; Demianchuk, O.; Tkachyk, A.; Vasylyshyn, O.; Lushchak, O.; Lushchak, V.I.; Bayliak, M. Differential Long-Term Effects of Physical, Social, and Psychological Stressors in Male Mice: Down-Regulation of Inflammatory Markers and Unresponsiveness of the Antioxidant System. *Physiol. Behav.* **2025**, *301*, 115065. [[CrossRef](#)] [[PubMed](#)]
85. Love, C.J.; Gubert, C.; Renoir, T.; Hannan, A.J. Environmental Enrichment and Exercise Housing Protocols for Mice. *STAR Protoc.* **2022**, *3*, 101689. [[CrossRef](#)] [[PubMed](#)]
86. Domínguez-Oliva, A.; Hernández-Avalos, I.; Bueno-Nava, A.; Chávez, C.; Verduzco-Mendoza, A.; Olmos-Hernández, A.; Villanueva-García, D.; Avila-Luna, A.; Mora-Medina, P.; Martínez-Burnes, J.; et al. Environmental Enrichment for Laboratory Rats and Mice: Endocrine, Physiological, and Behavioral Benefits of Meeting Rodents' Biological Needs. *Front. Vet. Sci.* **2025**, *12*, 1622417. [[CrossRef](#)]
87. Li, S.; Wang, H.; Qin, M.; Huang, W.; Gao, H.; Song, X.; Chen, X.; Li, B. Environmental Enrichment Attenuates Social Isolation-Exacerbated Postoperative Cognitive Dysfunction in Aged Mice via Inhibition of RAGE-HMGB1 Proinflammatory Signaling. *Brain Res. Bull.* **2025**, *229*, 111462. [[CrossRef](#)] [[PubMed](#)]
88. Hohlbaum, K.; Frahm, S.; Rex, A.; Palme, R.; Thöne-Reineke, C.; Ullmann, K. Social Enrichment by Separated Pair Housing of Male C57BL/6JRj Mice. *Sci. Rep.* **2020**, *10*, 11165. [[CrossRef](#)]
89. Hohlbaum, K.; Merle, R.; Frahm, S.; Rex, A.; Palme, R.; Thöne-Reineke, C.; Ullmann, K. Effects of Separated Pair Housing of Female C57BL/6JRj Mice on Well-Being. *Sci. Rep.* **2022**, *12*, 8819. [[CrossRef](#)]
90. Buckinx, A.; Van Schuerbeek, A.; Bossuyt, J.; Allaoui, W.; Van Den Herrewegen, Y.; Smolders, I.; De Bundel, D. Exploring Refinement Strategies for Single Housing of Male C57BL/6JRj Mice: Effect of Cage Divider on Stress-Related Behavior and Hypothalamic-Pituitary-Adrenal-Axis Activity. *Front. Behav. Neurosci.* **2021**, *15*, 743959. [[CrossRef](#)]
91. Westbrook, R.; Bonkowski, M.S.; Arum, O.; Strader, A.D.; Bartke, A. Metabolic Alterations Due to Caloric Restriction and Every Other Day Feeding in Normal and Growth Hormone Receptor Knockout Mice. *J. Gerontol. A. Biol. Sci. Med. Sci.* **2014**, *69*, 25–33. [[CrossRef](#)]
92. Anson, R.M.; Guo, Z.; de Cabo, R.; Iyun, T.; Rios, M.; Hagepanos, A.; Ingram, D.K.; Lane, M.A.; Mattson, M.P. Intermittent Fasting Dissociates Beneficial Effects of Dietary Restriction on Glucose Metabolism and Neuronal Resistance to Injury from Calorie Intake. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 6216–6220. [[CrossRef](#)] [[PubMed](#)]
93. Hipkiss, A.R. Dietary Restriction, Glycolysis, Hormesis and Ageing. *Biogerontology* **2007**, *8*, 221–224. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.