PERIODICUM BIOLOGORUM
VOL. 125, No 3–4, 195–203, 2023
DOI: 10.18054/pb.v125i3-4.30257

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Original Research Article

Sex-Related Differences in Oxidant and Antioxidant Profiles of Murine Kidney and Brain: A Focus on Sirt3-Mediated Regulation

ROBERT BELUŽIĆ[1,#,*]
MARTA KALOPER[2,#]
MARIJA PINTERIĆ[1]
ENA ŠIMUNIĆ[1]
IVA I. PODGORSKI[1]
MARIJANA POPOVIĆ HADŽIJA[1]
TIHOMIR BALOG[1]
SANDRA SOBOČANEC[1]

[1] Laboratory for Metabolism and Aging, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia

[2] Department of Biology, Faculty of Science, University of Zagreb, Croatia

[#]Contributed equally.

***Correspondence:**
Robert Belužić
E-mail address: rbeluzic@irb.hr

Keywords: sirtuin 3; sex; mouse; oxidative stress; antioxidant enzymes; kidney; brain

Abbreviations:

Cat	– catalase
CuZnSOD	– copper zinc superoxide dismutase
DNPH	– 2,4-dinitrophenylhydrazine
ELISA	– enzyme–linked immunosorbent assay
LPO	– lipid peroxidation
MDA	– malondialdehyde
MnSOD	– manganese superoxide dismutase
PC	– protein carbonylation
PGC-1α	– peroxisome proliferator–activated receptor gamma coactivator 1–alpha
Sirt3	– sirtuin 3
SOD	– superoxide dismutase
TBA	– thiobarbituric acid

Received March 3, 2024
Revised March 11, 2024
Accepted March 12, 2024

Abstract

Background: *Sirt3 is a mitochondrial deacetylase with an important role in maintainance of cellular redox and metabolic homeostasis and mitochondrial function. As growing evidence support the existence of sex-specific responses to metabolic and oxidative stress, we aimed to investigate sex- and organ-specific effects of Sirt3 loss.*

Materials and methods: *Expression of Sirt3, PGC-1α, CuZnSOD, MnSOD and Cat proteins in kidneys and brains of Sirt3-wild type (Sirt3 WT) and Sirt3-knockout (Sirt3 KO) mice was assessed by Western blotting. Protein carbonylation and lipid peroxidation levels were measured using ELISA and fluorometric assays, respectively. SOD and Cat activities were determined using standard enzymatic assays.*

Results: *Significant sex- and organ- specific differences in response to Sirt3 loss were detected. Sirt3 knockout affected kidneys more than brain tissue, with females showing lower levels PC and LPO. In kidneys, female KO showed higher MnSOD, but lower CuZnSOD and Cat activity compared to males. In brains, WT females show higher activities of these enzymes than males, suggesting a sex-specific protection mechanism, but female KO brains show a larger decrease in these parameters.*

Conclusion: *Our study provides comprehensive insights into the complex interplay of Sirt3, oxidative stress, and antioxidant defenses in murine kidney and brain. The observed differences between the two organs and the impact of sex highlight the need for studying Sirt3 function in diverse physiological contexts. The tissue-specific responses and sex-related variations underscore the importance of considering these factors in the development of therapeutic strategies targeting mitochondrial function and redox homeostasis.*

INTRODUCTION

Tissue- and organ-specific regulation of oxidative and antioxidant status is a topic of growing scientific interest, particularly in the context of sex-related differences. Emerging evidence suggests that changes in sex hormones, genetics and lifestyle factors contribute to differential responses to oxidative stress between males and females *(1–3)*. Recognizing the complex nature of this imbalance, recent research has focused attention on the role of sirtuin 3 (Sirt3), a major mitochondrial deacetylase, and its potential influence on the balance between oxidants and antioxidants in these organs. Sirt3, a member of the sirtuin family, has emerged as a central player in orchestrating mitochondrial function and redox balance *(4, 5)*. Its role in modulating

protein and lipid oxidation, as well as antioxidant enzyme activities, underscores its significance in maintaining cellular homeostasis.

Kidney and brain dysfunction are often linked to aging; both organs are rich in mitochondria and are susceptible to oxidative challenges that can affect overall health. Analysis of expression patterns has shown that high Sirt3 expression is related to enhanced oxidative capacity of the corresponding tissue (e.g., brain, heart, liver, brown adipose tissue and skeletal muscle), while reduced expression levels have been observed in tissues with lower metabolic demand, such as white adipose tissue, lung and small intestine *(6)*. It has been reported that Sirt3 deficiency in mice exacerbates mitochondrial damage and accelerates kidney disease during high-fat diet (HFD) feeding *(7)*. Sirt3 has been shown to be protective against acute kidney injury *(8)*. In addition, disruption of mitochondrial homeostasis is thought to play a pathogenic role in a number of neurological diseases and conditions, with Sirt3 playing a central role in these processes (reviewed in *(6)*). Given the high aerobic metabolic rate of the brain and its proportional consumption of significant amounts of ATP *(9)*, there has been a growing interest in elucidating the regulation of Sirt3 expression and functional activity in the nervous system. Our recent results have shown that Sirt3 exhibits sex-specific variations, contributing to the observed differences in oxidative stress responses between males and females and also their specific susceptibility towards various stressors *(1, 10)*.

In our current work, we hypothesize that Sirt3 affects sex-specific differences in oxidative and antioxidant parameters in both kidney and brain tissue in mice. In particular, we anticipate observing distinct patterns in the levels of oxidative stress markers and antioxidant defenses will differ between males and females. Furthermore, we expect that the absence of Sirt3 will modulate these differences, indicating the central role of Sirt3 in modulating the redox balance in these organs. Studying these sex-specific differences will improve our understanding of related physiological mechanisms, which in turn has implications for the development of sex-specific therapeutic interventions.

MATERIALS AND METHODS

Experimental design

Sirt3 WT (stock no. 000664, Jackson Laboratory) and KO mice (stock no. 027975, Jackson Laboratory) of both sexes at 8 weeks of age, were fed with a standard fat diet (SFD, 11.4% fat, 62,8% carbohydrates, 25,8% proteins; Mucedola) during 10 weeks. The mice were age-matched and housed in following conditions: 3 mice per cage, 22°C, 50–70% humidity, and a cycle of 12 h light and 12 h darkness. The mice were divided into 4 groups (4 mice per group): SFD-fed WT males, SFD-fed KO males, SFD-fed WT females and SFD-fed KO females. After 10 weeks, mice were anesthetized by intraperitoneal injection of ketamine/xylazine (Ketamidor 10%, Richter pharma Ag; Xylazine 2%, Alfasan International), kidney and brain were obtained and stored in liquid nitrogen until analysis. Animal experiments were done within the project funded by Croatian Science Foundation, project ID: IP-014-09-4533, approved on 01/09/2015. All procedures were approved by the Ministry of Agriculture of Croatia, (No: UP/I-322-01/15-01/25 525-10/0255-15-2 from 20 July 2015) and carried out in accordance with the associated guidelines EU Directive 2010/63/EU.

Protein Carbonylation

Protein carbonylation was performed with an ELISA-based assay. Kidney (10% homogenates) and brain (20% homogenates) were prepared in PBS with protease inhibitors (Roche). Organs were homogenized with Potter S Homogenizer (B. Braun Biotech International), sonicated 2x30 seconds, followed by centrifugation for 20 min at 16,000 g, 4 °C. A Pierce™ BCA Protein Assay Kit (Thermo Fischer Scientific) was used to determine the protein concentration, and 1 µg/100µL lysate was incubated overnight at 4°C using Maxisorb wells (Sigma Aldrich). 2,4-dinitrophenylhydrazine (DNPH; 12 µg/mL) was used for derivatization of adsorbed proteins. A rabbit anti-DNP primary (D9656, Sigma Aldrich) followed by goat anti-rabbit secondary antibody conjugated to HRP (1706515, Bio-Rad) were used to detect the derivatized dinitrophenol (DNP)-carbonyl. Enzyme substrate 3,3′,5,5′-tetramethylbenzidine (Sigma Aldrich) was added into samples and incubated until color developed, followed by stopping the reaction using 0.3 M H_2SO_4. The absorbance was measured at 450 nm on a microplate reader (Bio-Tek Instruments).

Lipid peroxidation (LPO)

The protocol involves the reaction of lipid peroxidation products, primarily malondialdehyde (MDA), with thiobarbituric acid (TBA), which leads to the formation of MDA-TBA2 adducts called TBARS. Standard curve was prepared with serial dilutions of 1,1,3,3-tetramethoxypropane (1,1,3,3 TMP-MDA standard) in ReH_2O ranging from 0,06 - 10 µM. In brief, kidney and brain tissue was homogenized (5%) using an ice-packed Potter S Homogenizer (B. Braun Biotech International), in 1.15% KCl. Homogenates were sonicated 2x30 seconds, and centrifuged on 16,000 g for 15 min, 4 °C. Afterwards, 200 µL of supernatants/MDA standards were treated with 200 µL of 2% SDS, vortexed, and incubated for 5 min at RT. After that, 500 µL of thiobarbituric acid (TBA) was added, and the samples were vortexed and incubated for 60 min at 95 °C. The samples and standards were cooled to RT and centrifuged for 10 min 4,000 g, RT. 200 µL of sample/MDA standards in duplicate were transferred to 96-well plate and the sample fluorescence was measured on Infinite M Nano+ (Tecan) at Ex/Em = 532/553 nm.

The results were expressed as nmol TBARS/mg of protein according to a standard curve which was prepared with serial dilutions of MDA standard.

Antioxidative enzyme activities

Antioxidative enzyme activities were analyzed in kidney and brain tissue lysates prepared in PBS supplemented with protease inhibitors (Roche) using an ice-jacketed Potter-S Homogenizer (B. Braun Biotech International), at 1,300 rpm. Superoxide dismutase (SOD) activities were determined measuring inhibition of the xanthine/xanthine oxidase-mediated reduction of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (I.N.T.) using a Ransod kit (Randox Laboratories) at 505 nm according to the manufacturer's recommendations. To obtain the MnSOD activity a 4 mM of KCN was used to selectively inhibit CuZnSOD. CuZnSOD activity was obtained by subtracting the MnSOD activity from the total SOD activity. The Catalase (Cat) activity was done as previously described *(11)*, by measuring the change in absorbance (at 240 nm) in the reaction mixture during the interval of 30 s following sample addition. The final concentrations of 10 mM H_2O_2 and 50 mM PBS (pH 7.0) were used. Antioxidative enzyme activities were analyzed using Tecan Infinite M Nano$^+$ (Tecan).

Protein isolation and Western blot analysis

Kidney and brain were homogenized in an ice-cold lysis buffer (RIPA buffer supplemented with protease inhibitors (Roche)) using an ice-jacketed Potter S Homogenizer (B. Braun Biotech International), at 1,300 rpm. Homogenates were sonicated 2x30 seconds and centrifuged at 16,000 g for 20 min at 4 °C. The resulting lysate was transferred to a new tube and the protein concentration was determined with Pierce™ BCA Protein Assay Kit (Thermo Fischer Scientific). Proteins (20 µg/20 µL) were resolved by SDS-PAGE and transferred onto a PVDF membrane (Roche). Membranes were blocked with an I-Block™ Protein-Based Blocking Reagent (Invitrogen) for 1 hour at room temperature and incubated with primary antibodies overnight at 4 °C (anti-Sirt3, Cell Signaling, D22A3; anti- PGC-1α, Novus, NBP1_04676; anti-CAT, Abcam, ab1877; anti-CuZnSOD, Abcam, ab16831; anti-MnSOD, Abcam, ab13533) An appropriate horseradish peroxidase (HRP)-conjugated secondary antibody (goat-anti rabbit IgG, Bio-Rad, 1706515) was used for chemiluminiscence detection. AmidoBlack (Sigma Aldrich) was used for total protein normalization. The Alliance Q9 mini Imaging System (UVITEC) was used for the detection of immunoblots using an enhanced chemiluminscence kit (Bio RAD). We performed western blot analyses with n=3 mice per each group.

Statistical analysis

For the statistical analysis of Western blot data, SPSS for Windows (v.17.0, IBM, Armonk, NY, USA) was used. A two-way ANOVA was performed to reveal the interaction effect of Sirt-3 and sex. If a significant interaction was observed, a Bonferroni adjustment was made to correct for multiple comparisons within each simple main effect separately. Significance was set at $p<0.05$.

RESULTS

Oxidative damage of proteins (carbonylation)

To elucidate the impact of Sirt3 deficiency and its potential sex-specific variations on redox homeostasis in kidney and brain, we examined sensitivity to oxidative stress with respect to Sirt3 or sex by measuring protein carbonylation (PC), a marker of protein oxidative damage. The absence of Sirt3 correlated with elevated carbonylation levels in kidneys of both male and female mice (Figure 1A). Conversely, in the brain, an inverse pattern of damage was observed, as Sirt3 KO groups demon-

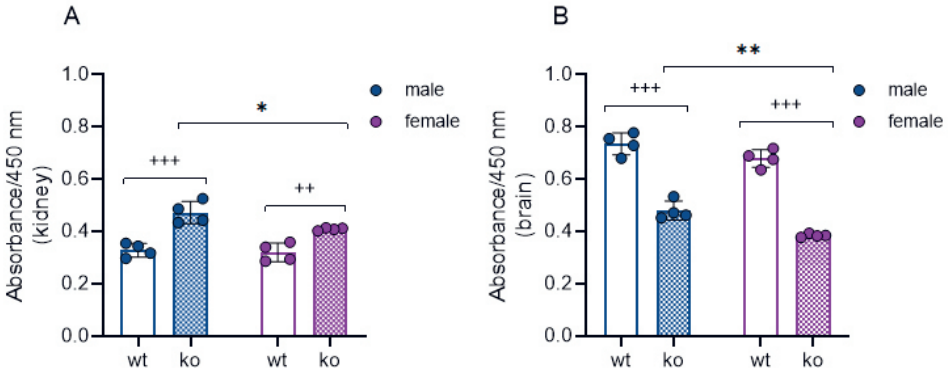

Figure 1. *The total amount of protein carbonyls measured with an ELISA-based assay at 450 nm in* ***(A)*** *kidney and* ***(B)*** *brain. A two-way ANOVA was conducted to determine the effects of Sirt3 and sex in each tissue. All simple pairwise comparisons were run with a Bonferroni adjustment applied. $^{+++}p<0.001$, $^{++}p<0.01$, WT vs. KO; $^{**}p<0.01$, $^{*}p<0.05$, male vs. female. Data are shown as mean ± SD. N=4 samples per group.*

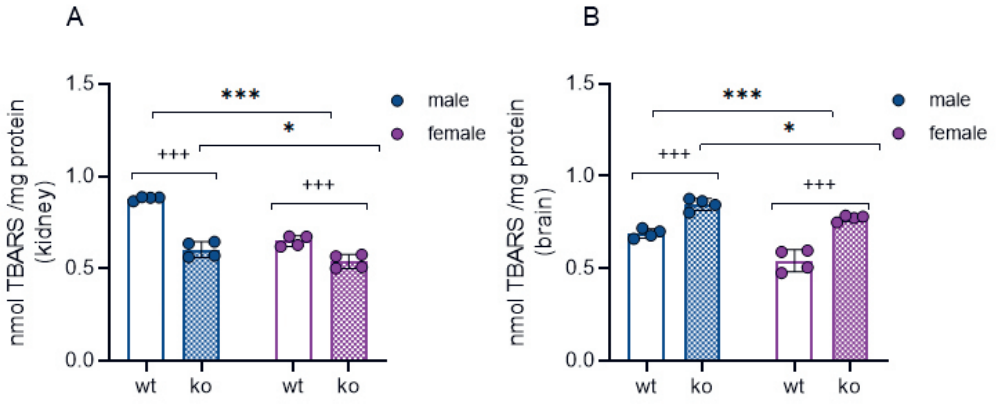

Figure 2. *The total amount of lipid peroxidation products (TBARS) measured fluorometrically at 532 nm in* ***(A)*** *kidney and* ***(B)*** *brain. A two-way ANOVA was conducted to determine the effects of Sirt3 and sex in each tissue. All simple pairwise comparisons were run with a Bonferroni adjustment applied. +++p<0.001, WT vs. KO; ***p<0.001, *p<0.05, male vs. female. Data are shown as mean ± SD. N=4 samples per group.*

strated lower protein carbonylation in comparison to Sirt3 WT groups, with Sirt3 KO females having slightly lower carbonylation than Sirt3 KO males (Figure 1B).

Oxidative damage of lipids (lipid peroxidation)

To further elucidate potential lipid-specific responses and their interaction with protein oxidation with respect to Sirt3 and sex, we performed lipid peroxidation analysis. Kidneys of Sirt3 KO mice exhibited lower LPO levels compared to their WT counterparts, indicating a potential protective effect of Sirt3 deficiency against lipid peroxidation in the kidney. Furthermore, females consistently displayed lower LPO levels than males irrespective of genotype (Figure 2A). Contrary to LPO in kidney, brain showed the inverse pattern of lipid oxidative damage, with higher LPO in Sirt3 KO brains. However, Sirt3 KO females exhibited reduced damage in comparison to Sirt3 KO males in both genotypes (Figure 2B).

Antioxidant enzyme activities

In addition to assessing lipid peroxidation (LPO) and protein carbonylation as indicators of oxidative damage, we investigated antioxidant activities, specifically copper-zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), and catalase (Cat), aiming to provide a comprehensive understanding of the redox balance in the kidney and brain, with respect to Sirt3 and sex. CuZnSOD functions as a cytoplasmic enzyme that catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, contributing to cellular antioxidant defense and redox regulation *(12)*. Males exhibited consistent CuZnSOD levels across genotypes, whereas Sirt3 KO females demonstrated decreased CuZnSOD activity compared to both their WT counterparts and male KO mice (Figure 3A). In the brain, the impact of Sirt3 deficiency exhibited a sex-dependent differences in CuZnSOD activity: males displayed higher activity, whereas females demonstrated decreased enzyme activity (Figure 3B).

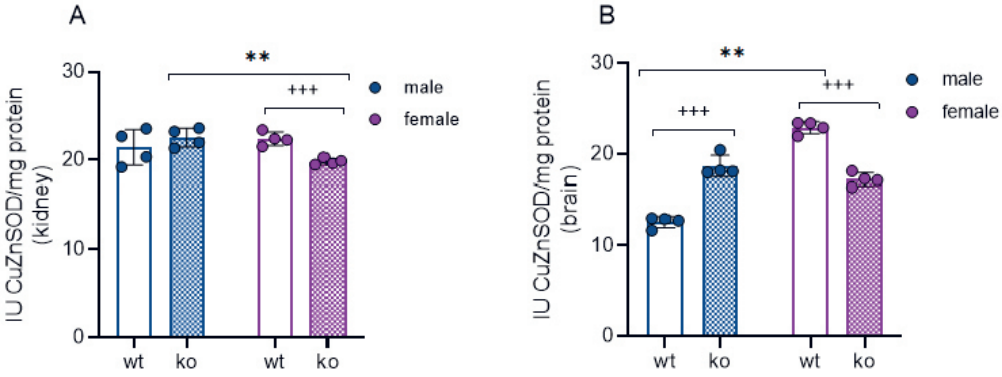

Figure 3. *CuZnSOD activity measured spectrophotometrically at 505 nm in* ***(A)*** *kidney and* ***(B)*** *brain. A two-way ANOVA was conducted to determine the effects of Sirt3 and sex in each tissue. All simple pairwise comparisons were run with a Bonferroni adjustment applied. +++p<0.001, , WT vs. KO; **p<0.01, , male vs. female. Data are shown as mean ± SD. N=4 samples per group.*

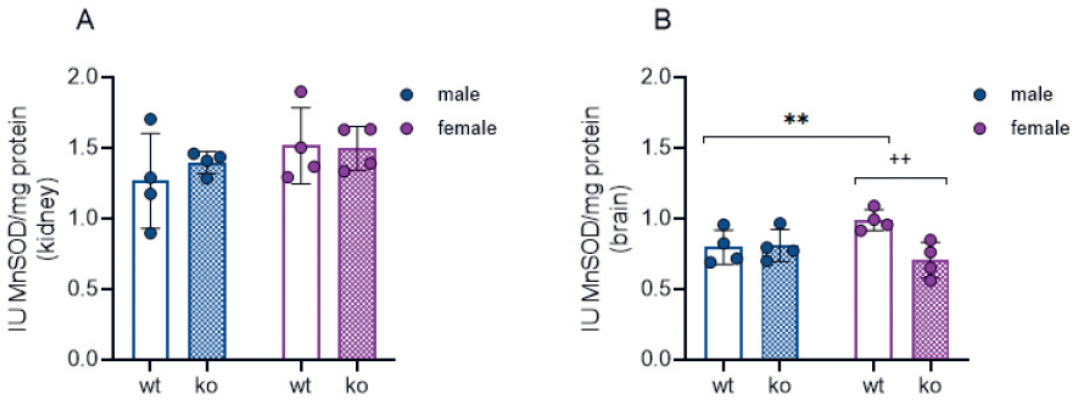

Figure 4. *MnSOD activity measured spectrophotometrically at 505 nm in* ***(A)*** *kidney and* ***(B)*** *brain. A two-way ANOVA was conducted to determine the effects of Sirt3 and sex in each tissue. All simple pairwise comparisons were run with a Bonferroni adjustment applied. ++p<0.01, WT vs. KO; **p<0.01, male vs. female. Data are shown as mean ± SD. N=4 samples per group.*

Manganese superoxide dismutase (MnSOD) is a mitochondrial enzyme that catalyzes the conversion of superoxide radicals to hydrogen peroxide, thereby playing a pivotal role in antioxidant defence and maintaining cellular redox homeostasis *(13)*. While renal MnSOD activity remained constant (Figure 4A), we identified a significant interaction effect between Sirt3 and sex on brain MnSOD activity. WT females exhibited elevated MnSOD compared to WT males, whereas the impact of Sirt3 loss was evident only in females, leading to a reduction in MnSOD levels (Figure 4B).

Catalase (Cat) functions as a pivotal antioxidant enzyme within the cellular defense system, complementing the activities of superoxide dismutases (CuZnSOD and MnSOD) by catalyzing the decomposition of hydrogen peroxide into water and oxygen, thus contributing to the comprehensive protection against oxidative stress. In the kidney, Sirt3 deficiency resulted in elevated Cat activity in males but reduced activity in females, with females consistently exhibiting lower Cat levels than males in both genotypes (Figure 5A). Conversely, in the brain, the impact of Sirt3 loss was specific to females, leading to a reduction in Cat activity compared to wild-type females (Figure 5B).

Protein expression in kidney and brain

To explore the status of Sirt3 and proteins involved in energy metabolism and antioxidant status in kidney and brain, we performed western blot analysis. Results showed that Sirt3 was abundant in kidney, while its expression was significantly lower in brain. Contrary to kidney, in brain females exhibited reduced protein level of Sirt3 in comparison to males (Figure 6A-C).

Because Sirt3 is under the control of PGC-1α, which is master regulator of mitochondrial biogenesis *(14)*, we also determined PGC-1α expression, aiming to elucidate the regulatory interplay between PGC-1α and Sirt3. In the kidney, females exhibited diminished PGC-1α protein

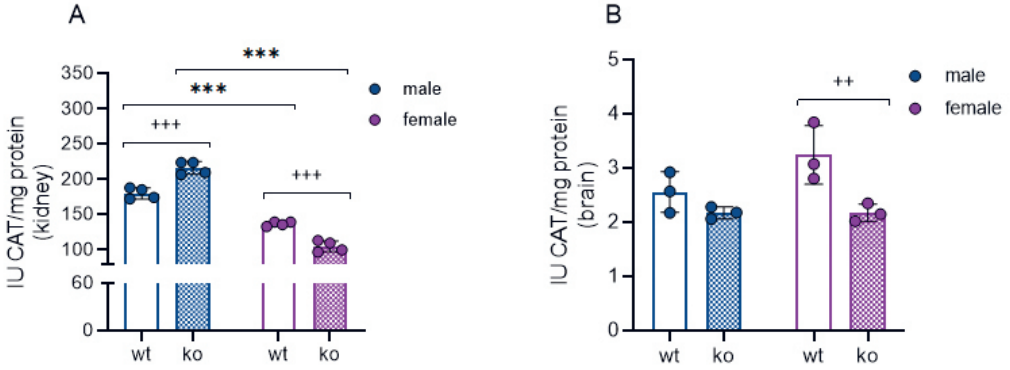

Figure 5. *Catalase (Cat) activity mesasured spectrophotometrically at 240 nm in* ***(A)*** *kidney and* ***(B)*** *brain. A two-way ANOVA was conducted to determine the effects of Sirt3 and sex in each tissue. All simple pairwise comparisons were run with a Bonferroni adjustment applied. ++p<0.01, +++p<0.001, WT vs. KO; ***p<0.001, male vs. female. Data are shown as mean ± SD. N=4 samples per group.*

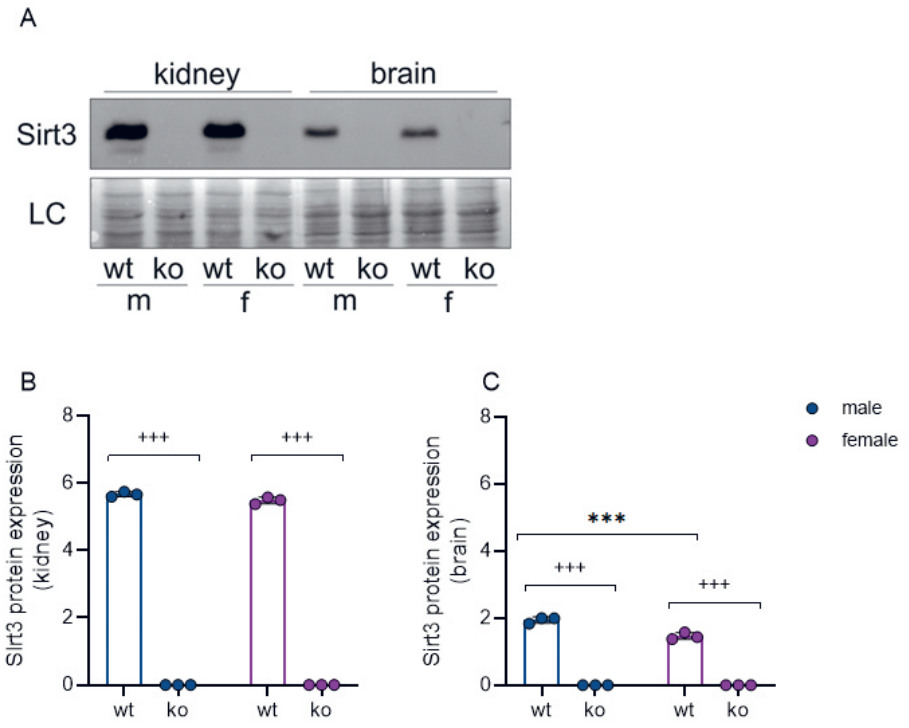

Figure 6. *Protein expression revealed by western blot analysis in kidney and brain of Sirt3 WT and KO mice of both sexes.* ***(A)*** *Representative immunoblot of Sirt3.* ***(B)*** *Graphical display of Sirt3 relative protein expression in kidney.* ***(C)*** *Graphical display of Sirt3 relative protein expression in brain. A two-way ANOVA was conducted to determine the effects of Sirt3 and sex in each tissue. All simple pairwise comparisons were run with a Bonferroni adjustment applied. $^{+++}p<0.001$, WT vs. KO; $^{***}p<0.001$, male vs. female. Data are shown as mean ± SD. N=3 samples per group.*

expression compared to males. Furthermore, in both sexes, the brain displayed elevated PGC-1α protein expression relative to the kidney, with females demonstrating higher expression than males (Figure 7A-C). To further explore the mitochondrial regulatory network, we investigated the expression of MnSOD, a main antioxidant enzyme in mitochondria and a known target regulated by Sirt3 *(15)*. In the kidney, our observations revealed a diminished expression of Sod2 in a sex-specific manner, with females having lower levels of MnSOD than males, similarly to PGC-1α. Conversely, in the brain, MnSOD expression was elevated in Sirt3 KO mice, with females displaying higher expression than males. It is noteworthy that the brain exhibited lower levels of MnSOD protein compared to the kidney (Figure 7A, D, E).

DISCUSSION

This work investigated the effects of Sirt3 deficiency and its potential sex-specific variations on oxidative/antioxidative homeostasis in the kidney and brain of mice and provided valuable insights into the regulatory mechanisms controlling oxidative stress and antioxidant defense. Recent studies have emphasized the central role of Sirt3 in the modulation of oxidative stress and highlighted its importance in the maintenance of redox homeostasis *(16, 17)*. Our previous findings have shown that there is a specific relationship between Sirt3 and sex, with its effect on energy homeostasis being sex-specific and dependent on the action of sex hormones. To date, no comprehensive study has elucidated the relationship between Sirt3 and sex concerning the comparative analysis of oxidative and antioxidative status in kidney and brain of mice.

Our data show differences in Sirt3 expression both between sexes and between different organs (i.e. kidney and brain). In both sexes, Sirt3 is abundant in the kidney and shows significantly lower expression in the brain, in accordance to previous study *(18)*. On the other hand, there is a clear sex-specific difference in Sirt3 levels, with females showing significantly lower Sirt3 expression in brain tissue. In kidney this difference is not significant.

Protein carbonylation (PC) is generally considered a proxy for conditions such as oxidative and metabolic stress, as well as aging *(19)*. PC can impair the functions of essential proteins, thus compromising cellular homeostasis *(20)*. We recently showed that nutritive stress induces a female-specific increase in the PC of WT mice *(1)*. The increased PC levels observed in both male and female Sirt3 KO mice, particularly in the kidney, emphasize the crucial role of Sirt3 in mitigating oxidative dam-

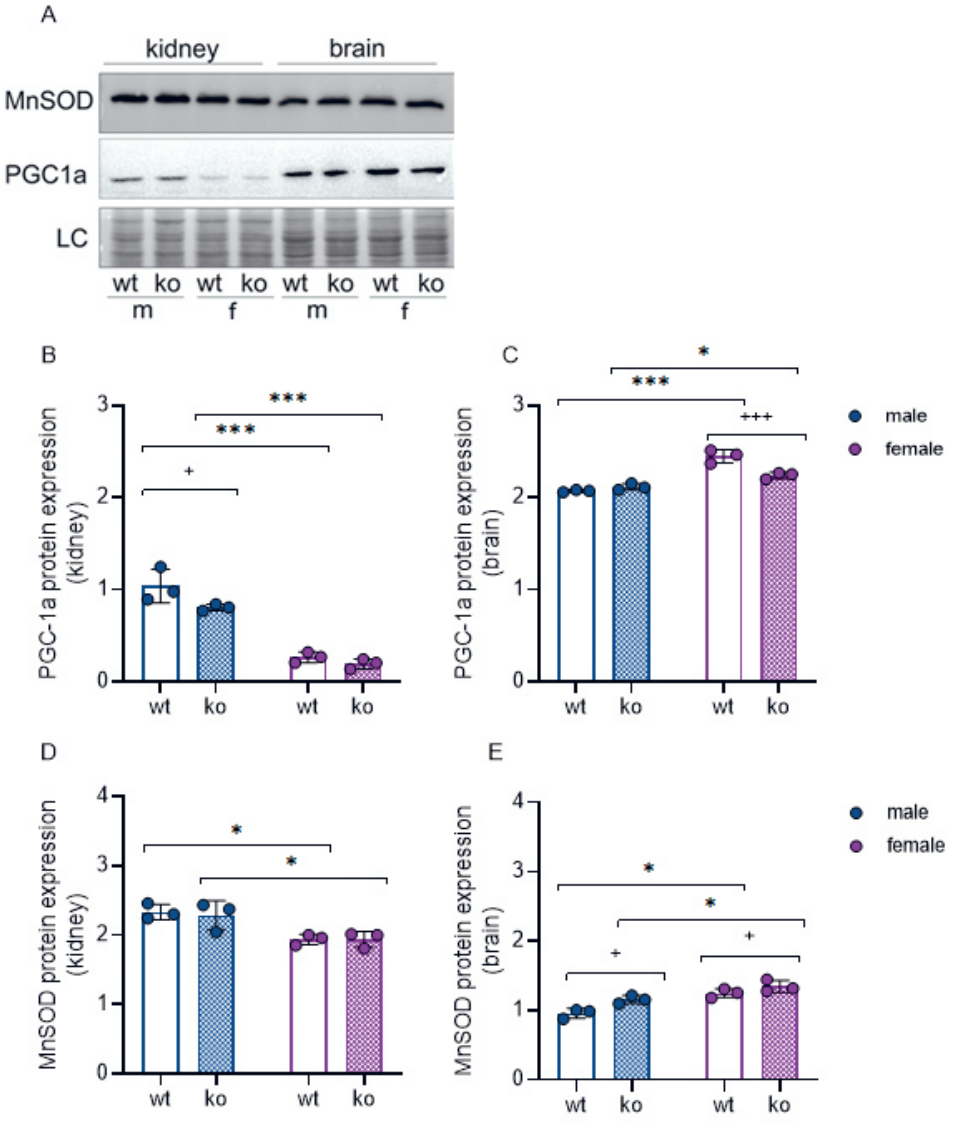

Figure 7. *Protein expression revealed by western blot analysis in kidney and brain of Sirt3 WT and KO mice of both sexes.* ***(A)*** *Representative immunoblots of PGC-1α and MnSOD.* ***(B)*** *Graphical display of PGC-1α relative protein expression in kidney.* ***(C)*** *Graphical display of PGC-1α relative protein expression in brain.* ***(D)*** *Graphical display of MnSOD relative protein expression in kidney.* ***(E)*** *Graphical display of MnSOD relative protein expression in brain. A two-way ANOVA was conducted to determine the effects of Sirt3 and sex in each tissue. All simple pairwise comparisons were run with a Bonferroni adjustment applied.* $^{+}p<0.05$, $^{+++}p<0.001$, *WT vs. KO;* $^{*}p<0.05$,$^{***}p<0.001$, *male vs. female. Data are shown as mean ± SD. N=3 samples per group.*

age of proteins. This aligns with the well-established Sirt3 function in preserving redox balance *(21)*: Higher Sirt3 levels in kidneys are suggestive of higher kidney-specific dependence on Sirt3 function, resulting in more pronounced protein damage resulting from Sirt3 knock-out. In the brain, PC levels follow an inverse pattern, with Sirt3 KO groups demonstrating lower protein carbonylation than their wild-type counterparts. Such contrasting response points to existence of an alternative mechanism of oxidative balance maintenance, apart from Sirt3, used by brain tissue after Sirt3 depletion. This tissue-specific effect will certainly be a subject of further explorations.

Similarly, organ-specific patterns of LPO as a response to Sirt3 deficiency levels were detected. In the kidney, Sirt3 KO mice exhibited lower LPO levels, indicating a potential protective effect of Sirt3 loss against lipid peroxidation. In contrast, the brain displayed an inverse pattern, with higher LPO levels in KOs. Sirt3 KO females showed reduced LPO levels compared to males. Such inverse pattern of oxidative damage suggests a tissue-specific regulatory mechanisms in response to Sirt3 deficiency. In terms of oxidative damage, females generally show lower kidney and brain oxidative damage parameters than males, and the loss of Sirt3 had the opposite effect with respect to

each organ. Lower damage in females is not in the direct correlation with antioxidative enzyme activities, which implicates that other mechanisms are involved in observed differences. In addition, females proved to be much more responsive to Sirt3 loss than males, given that they had modulated all tested antioxidant enzymes upon Sirt3 depletion, while males were significantly less affected.

The examination of PGC-1α, a master regulator of mitochondrial biogenesis known to control Sirt3 expression, further elucidated the regulatory interplay. In the kidney, females displayed diminished PGC-1α expression, while in the brain, elevated expression was observed, with females exhibiting higher levels than males. The interesting finding was that the abundant expression of Sirt3 in kidney was associated with low expression of PGC-1α, its regulator. However, in brain, Sirt3 was expressed three times lower than in kidney, which coincided with high expression of PGC-1α. This suggests a complex regulatory network wherein PGC-1α might influence Sirt3 expression in a tissue-specific and sex-dependent manner.

MnSOD expression, a key antioxidant enzyme regulated by Sirt3, unveiled distinct patterns in the kidney and brain. In the kidney and brain lower PGC-1α expression in KO was not associated with lower MnSOD protein expression. However, this association was evident with respect to sex, where females exhibited reduced levels of both PGC-1α and MnSOD when compared to males. PGC-1α is known as a positive regulator of MnSOD and other antioxidant enzymes *(22)*. Compared to kidney, brain tissue showed significantly higher PGC-1α expression and, surprisingly, lower MnSOD levels. The new finding is attributed to sex-specific renal expression of the PGC-1α-MnSOD axis, where we have shown that females generally have lower levels than males. These results suggest that a functional relationship between MnSOD and PGC-1α in the context of mitochondrial function and oxidative stress response is highly tissue-dependent and that previous findings cannot be universally applied.

In conclusion, our study provides comprehensive insights into the complex interplay of Sirt3, oxidative stress, and antioxidant defences in the kidney and brain. The observed differences between the two organs and the impact of sex highlight the need for understanding of Sirt3 function in diverse physiological contexts. The tissue-specific responses and sex-related variations underscore the importance of considering these factors in the development of therapeutic strategies targeting mitochondrial function and redox homeostasis in different murine organs.

REFERENCES

1. PINTERIĆ M, PODGORSKI II, HADŽIJA MP, BUJAK IT, DEKANIĆ A, BAGARIĆ R, FARKAŠ V, SOBOČANEC S, BALOG T 2020 Role of Sirt3 in differential sex-related responses to a high-fat diet in mice. Antioxidants 9:1–19 https://doi.org/10.3390/antiox9020174
2. TOWER J, POMATTO LCD, DAVIES KJA 2020 Sex differences in the response to oxidative and proteolytic stress. Redox Biol 31:101488 https://doi.org/10.1016/j.redox.2020.101488
3. BALABAN RS, NEMOTO S, FINKEL T 2005 Mitochondria, oxidants, and aging. cell 120:483–495 https://doi.org/10.1016/j.cell.2005.02.001
4. SCHWER B, NORTH BJ, FRYE RA, OTT M, VERDIN E 2002 The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide–dependent deacetylase. J Cell Biol 158:647–657 https://doi.org/10.1083/jcb.200205057
5. ANSARI A, RAHMAN MS, SAHA SK, SAIKOT FK, DEEP A, KIM K 2017 Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. Aging Cell 16:4–16 https://doi.org/10.1111/acel.12538
6. SIDOROVA-DARMOS E, SOMMER R, EUBANKS JH 2018 The role of SIRT3 in the brain under physiological and pathological conditions. Front Cell Neurosci 12:1–16 https://doi.org/10.3389/fncel.2018.00196
7. LOCATELLI M, MACCONI D, CORNA D, CERULLO D, ROTTOLI D, REMUZZI G, BENIGNI A, ZOJA C 2022 Sirtuin 3 deficiency aggravates kidney disease in response to high-fat diet through lipotoxicity-induced mitochondrial damage. Int J Mol Sci. https://doi.org/10.3390/ijms23158345
8. MORIGI M, PERICO L, ROTA C, LONGARETTI L, CONTI S, ROTTOLI D, NOVELLI R, REMUZZI G, BENIGNI A 2015 Sirtuin 3-dependent mitochondrial dynamic improvements protect against acute kidney injury. J Clin Invest 125:715–726 https://doi.org/10.1172/jci77632
9. FALKOWSKA A, GUTOWSKA I, GOSCHORSKA M, NOWACKI P, CHLUBEK D, BARANOWSKA-BOSIACKA I 2015 Energy Metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. Int J Mol Sci 16:25959–25981 https://doi.org/10.3390%2Fijms161125939
10. PINTERIĆ M, PODGORSKI II, POPOVIĆ HADŽIJA M, TARTARO BUJAK I, TADIJAN A, BALOG T, SOBOČANEC S 2021 Chronic high fat diet intake impairs hepatic metabolic parameters in ovariectomized Sirt3 KO mice. Int J Mol Sci. https://doi.org/10.3390/ijms22084277
11. AEBI H 1984 Catalase in vitro. In: methods in enzymology: Oxyg. Radicals Biol. Syst. Academic Press, pp 121–126 https://doi.org/10.1016/S0076-6879(84)05016-3
12. TRIST BG, HILTON JB, HARE DJ, CROUCH PJ, DOUBLE KL 2021 Superoxide Dismutase 1 in health and disease: how a frontline antioxidant becomes neurotoxic. Angew Chemie Int Ed 60:9215–9246 https://doi.org/10.1002/anie.202000451
13. BIRBEN E, MURAT U, MD S, SACKESEN C, ERZURUM S, KALAYCI O 2012 Oxidative stress and antioxidant defense. WAO J 5:9–19 https://doi.org/10.1097/WOX.0b013e3182439613
14. HALLING JF, PILEGAARD H 2020 PGC-1α-mediated regulation of mitochondrial function and physiological implications. Appl Physiol Nutr Metab 45:927–936 https://doi.org/10.1139/apnm-2020-0005
15. DIKALOVA AE, ITANI HA, NAZAREWICZ RR, MCMASTER WG, FLYNN CR, UZHACHENKO R, FESSEL JP, GAMBOA JL, HARRISON DG, DIKALOV SI 2017 Sirt3 Impairment and SOD2 hyperacetylation in vascular oxidative stress and hypertension. Circ Res 121:564–574 https://doi.org/10.1161/circresaha.117.310933
16. MARTINO E, BALESTRIERI A, ANASTASIO C, MAIONE M, MELE L, CAUTELA D, CAMPANILE G, BALESTRIERI ML, D'ONOFRIO N 2022 SIRT3 modulates endothelial mito-

chondrial redox state during insulin resistance. antioxidants. https://doi.org/10.3390/antiox11081611

17. CHEN X, LIU J 2023 The role of cellular lipid metabolism in aging. Cell Lipid Heal Dis. https://doi.org/10.1016/b978-0-323-95582-9.00013-9
18. JIN L, GALONEK H, ISRAELIAN K, CHOY W, MORRISON M, XIA Y, WANG X, XU Y, YANG Y, SMITH J J, HOFFMANN E, CARNEY D P, PERNI R B, JIROUSEK M R, BEMIS J E, MILNE J C, SINCLAIR D A, WESTPHAL C H 2009 Biochemical characterization, localization, and tissue distribution of the longer form of mouse SIRT3. Protein Sci 18:514–525 https://doi.org/10.1002/pro.50
19. GONOS ES, KAPETANOU M, SEREIKAITE J, BARTOSZ G, NAPARLO K, GRZESIK M, SADOWSKA-BARTOSZ I 2018 Origin and pathophysiology of protein carbonylation, nitration and chlorination in age-related brain diseases and aging. Aging (Albany NY) 10:868–901 https://doi.org/10.18632/aging.101450
20. ESTÉVEZ M, DÍAZ-VELASCO S, MARTÍNEZ R 2022 Protein carbonylation in food and nutrition: a concise update. Amino Acids 54:559–573 https://doi.org/10.1007/s00726-021-03085-6
21. GIRALT A, VILLARROYA F 2012 SIRT3, a pivotal actor in mitochondrial functions: metabolism, cell death and aging. Biochem J 10:1–10 https://doi.org/10.1042/bj20120030
22. RIUS-PÉREZ S, TORRES-CUEVAS I, MILLÁN I, ORTEGA ÁL, PÉREZ S 2020 PGC-1α, inflammation, and oxidative stress: an integrative view in metabolism. Oxid Med Cell Longev 2020:1452696 https://doi.org/10.1155/2020/1452696