THE EFFECTS OF DIETARY ZINC IMBALANCE ON COGNTIVE AND VASCULAR FUNCTION IN A YOUNG MOUSE MODEL OF ALZHEIMER'S DISEASE

by

LAZAR ISAKHAROV

A THESIS

Presented to the Department of Human Physiology
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

February 2026

An Abstract of the Thesis of

Lazar Isakharov for the degree of Bachelor of Science
in the Department of Human Physiology to be taken June 2026

Title: The effects of dietary zinc imbalance on cognitive and vascular function in a young mouse model of Alzheimer's disease

Approved: *Ashley Walker, Ph.D.*
Primary Thesis Advisor

The prevalence of Alzheimer's disease (AD) continues to rise concurrently with life expectancy and the older population. Although the exact mechanisms of AD are unclear, one proposed hypothesis is that chronic oxidative stress with age, along with cerebrovascular stiffness and dysfunction, contributes to the formation of harmful amyloid-beta (Aβ) plaques in the brain, triggering disease pathology. Zinc deficiency is a leading nutritional deficiency worldwide and is naturally reduced in the diet of older adults. Zinc is a prominent antioxidant and trace metal. Limited research has connected zinc imbalance to AD; however, the exact effects and mechanisms are still unknown. The purpose of this study was to examine the association between dietary zinc imbalance and AD to elucidate a potential long-term preventive treatment for the disease. I hypothesized that a low zinc diet would impair both cognitive and vascular function, through deregulation of oxidative-stress-relief by superoxide dismutase, in comparison to a zinc-normal and -high diet in a mouse model of AD. Cognitive dysfunction was not observed between the groups, but sex-dependent cerebrovascular dysfunction was noted. Male mice on a low zinc diet showed signs of endothelial cell dysfunction, not present in males on a high or normal zinc diet. Conversely, female mice on a low zinc diet showed vascular smooth muscle cell dysfunction, not present in females on a high or normal zinc diet. There were

no differences in NADPH oxidase or superoxide dismutase gene or protein expression between the groups. This study provides evidence for a sex-dependent effect of dietary zinc-imbalance, independent of superoxide dismutase. These results contribute to the current gap in literature connecting dietary zinc to AD.

Acknowledgements

I am deeply grateful to the many people whose support made this thesis possible. I would like to thank my principal investigator and primary thesis advisor, Dr. Ashley Walker, for trusting me with the opportunity to contribute to the Aging and Vascular Physiology (AVP) Lab as an Undergraduate Research Assistant. I would like to thank my post-doctoral scholar mentor, Dr. Abby Cullen, for her close and attentive mentorship, and for allowing me to join her project and explore my research interests. Dr. Cullen's commitment to my growth as a researcher and scientific professional has been fundamental to the completion of this research project. I would like to extend my gratitude to the entire AVP lab team for their support, and particularly to my peer, Kiara Gardley, who helped with data collection for this project. My time in the AVP lab has been a defining factor of my undergraduate experience.

I would like to thank the Clark Honors College and specifically my CHC Thesis Advisor, Dr. Barbara Mossberg, for her guidance and support throughout the thesis writing process.

I feel incredibly lucky to have received multiple funding awards for this project, including the Excellence in Human Physiology Undergraduate Research Stipend, the Clark Honors College Mentored Research Program, and the Oregon Vice President for Research and Innovation Fellowship, for which I am very grateful.

I would like to thank the Center for Undergraduate Research and Engagement for the opportunities to present this research at the Undergraduate Research Symposium.

Lastly, I would like to thank my family and friends, who kept me motivated and excited throughout my undergraduate research and academic journey.

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Introduction

As the aging population continues to increase, so too does the prevalence of Alzheimer's disease (Winder et al., 2021). In fact, global life expectancy has risen by close to 40 years in the last century alone, expanding the threat of chronic diseases of aging (Twiss et al., 2025). As such, it is more important than ever to understand these diseases and how to prevent them. In the backdrop of decades of research, there is still currently no known treatment for Alzheimer's disease (AD) prevention or reversal (Wang et al., 2025). However, disease pathology has been associated with markers of oxidative stress, leading to neurodegeneration and cognitive decline. Zinc is a vital antioxidant and is a leading nutritional deficiency globally (Mocchegiani et al., 2012). Connecting dietary zinc imbalance to AD could provide a long-term nutritional intervention as a preventative treatment for the disease.

AD is the most common form of dementia. In fact, three fourths of all dementia cases are AD (Kamatham et al., 2024). Additionally, the prevalence of AD is rapidly increasing. Currently, 50 million people worldwide are in some stage of AD, which is expected to triple by 2050. The physical impairments of AD are only worsened by the global stigma and fear of an AD diagnosis. Studies find that being diagnosed with AD lowers self-esteem and mental health, and reduces help-seeking behaviors (Rosin et al., 2020). The lack of a cure makes the illness seem like a death sentence, and the portrayal of AD in the media only makes it worse. Moreover, AD is more prevalent in women and marginalized groups and not only affects those directly afflicted with the disease, but also caretakers and families, who endure stress and worry in watching disease progression. The current most commonly accepted form of AD therapy is directed toward symptom alleviation, rather than early-diagnosis and prevention.

As one form of dementia, AD is characterized by neurodegeneration and cognitive decline. Although the exact disease pathology of AD is still unknown, a prominent explanation is the amyloid-beta (Aβ) hypothesis (Kamatham et al., 2024). The Aβ hypothesis states that with age, Aβ protein in the brain aggregates into plaques which increases cell death and cerebrovascular damage, leading to neurodegeneration and dementia. The Aβ peptide is cleaved from amyloid precursor protein, an enhancer of brain function. However, although not conclusively determined, aggregation of Aβ has been found to increase with chronic neuroinflammation and oxidative stress (Arimon et al., 2015). Oxidative stress is the accumulation of reactive oxygen species (ROS), highly reactive molecules that damage cell function. Consequently, Aβ plaques activate more oxidative agents, stimulating a dangerous and exponential positive feedback loop. While genetics and environment both play a role in AD pathogenesis, aging is the biggest risk factor (Modrick et al., 2009). Oxidative stress increases with age, as redox reactions become less efficient. Exposure to chronic oxidative stress in the brain damages endothelial cells and impairs cerebral arterial vasodilation, decreasing blood flow to the brain and breaking down the blood-brain barrier. For this reason, oxidative species and harmful inflammatory markers can enter the brain and trigger Aβ aggregation.

Zinc is a prominent antioxidant. In the AD pathway, zinc can help reduce oxidate stress by inhibiting NADPH oxidase (NOX), an enzyme that produces ROS, such as $\cdot OH$ and $\cdot O_2^-$ (Sun et al., 2022). Zinc is also a cofactor, or activator, along with copper, of superoxide dismutase (SOD), an enzyme that converts the unstable ROS, $\cdot O_2^-$, into the safer H_2O_2. Sadly, AD pathology exacerbates zinc imbalance in the brain, with Aβ plaques absorbing extracellular zinc (Shen et al., 2022). Contrary to its seemingly protective effects, zinc has also been shown to induce neurotoxicity and worsen vascular function by promoting cell death (Tanaka &

Kawahara, 2017). Current literature still questions the role of zinc as a double-edged sword, with some studies finding it protective as mentioned above, while others find it harmful to cognitive function (Sun et al., 2022).

Zinc imbalance is one of the most common forms of micronutrient malnutrition globally. In fact, 17% of the global population has inadequate zinc intake, rising to 24% in Asia and Africa (Lowe et al., 2024). Additionally, studies have correlated elevated atmospheric CO_2 with a decrease in zinc levels in cereal grains. On a global scale, with rising levels of CO_2 in the atmosphere, dietary zinc availability is projected to decrease by 14.6% by 2050. Furthermore, zinc naturally decreases in the diet and absorption of older adults (Cabrera, 2015). With age, adult diets trend toward eating less meat and tougher high-zinc foods, opting for more fiber-rich foods, which restrict intestinal absorption of zinc. These dietary changes can be exacerbated by pharmacological interactions with zinc internally (Mocchegiani et al., 2012). For example, some drugs can trigger oxidative stress pathways in the gut, activating metallothionein, a protein that balances zinc in cells, to capture zinc ions before they can be absorbed. With a higher chance of taking permanent daily medications, elderly people are more at risk for this zinc interaction. On a molecular level, age impairs the function of metallothionein and ZIP proteins, which transport zinc into the cell (Wong et al., 2013). Cumulatively, these factors reduce overall zinc intake and sequestration, leading to higher rates of zinc imbalance in the aging population.

Although zinc and AD have been studied together, we lack a comprehensive understanding of the mechanisms in which zinc and Aβ influence the cerebrovascular and cognitive components of AD. Additionally, the imbalance in zinc and the activation of SOD in the context of AD has not been succinctly determined. This thesis aims to address this gap in literature. The purpose of this research was to determine the impact of dietary zinc imbalance on

oxidative-stress-related cognitive decline and cerebrovascular function in the context of SOD in a mouse model of AD. I hypothesized that a zinc-deficient diet would increase oxidative stress and cognitive decline through decreased activation of SOD, and impair blood-brain barrier integrity and cerebrovascular function in comparison to a zinc-normal and -high diet in a mouse model of AD. The results of this study will enhance the understanding of how zinc works to reduce oxidative stress and the impact of this on the cerebrovascular and cognitive components of AD.

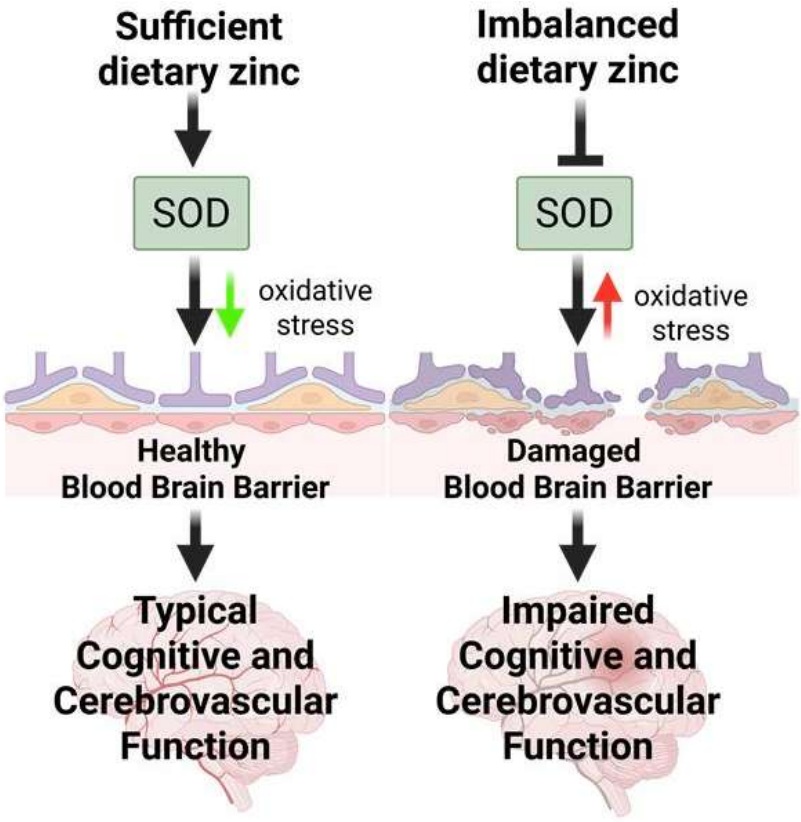

Figure 1: Initial hypothesis.

Methods

Animal Model

I used a transgenic rodent model of AD for this study. The APP-KI^{NL-G-F} mice model were used to simulate AD by knock-in of human amyloid precursor protein mutations, which contributes to a significant increase in Aβ plaques by 6 months of age. This model is ideal for determining the relationship between zinc, SOD, and AD because it eliminates confounding factors of aging, such as chronic exposure to oxidative stress. A total of 80 male and female mice were placed on either a zinc-deficient diet (2.5 mg/kg), zinc-normal diet (30 mg/kg), or zinc-high diet (300 mg/kg) at 4 months until 6 months of age.

Cognitive Function

To assess spatial learning and memory, I used the Morris Water Maze test (MWM, Figure 2). The MWM takes place over a four day period. The first three days are training days, in which the mice have two swimming sessions, one in the morning and one in the afternoon. During each training session, the mice swim three trials with 15 minutes between each trial. Time required to reach the platform was recorded. In each trial, mice have 60 seconds to find a hidden platform after being dropped into different drop zones. On the fourth day, mice swim the probe and beacon test. The probe test is conducted in the morning, where the platform is lowered and mice swim for the full 60 seconds. Time spent in area of the platform was recorded. In the afternoon of day 4, the beacon test was performed, in which the platform was raised and flagged with a visual cue. The beacon test measures differences in swimming speeds between mice that

could confound our training day learning results. The video recordings of each trial were analyzed via the EthoVision software.

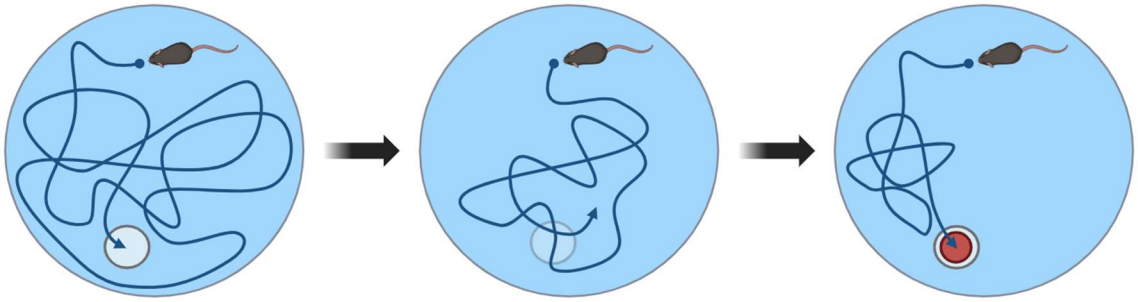

Figure 1: Morris Water Maze test.

Top view of Training Trial (left), Probe Trial (middle), and Beacon Trial (right) (biorender.com).

The Novel Object Recognition test was used to asses short-term recognition memory (Figure 3). Mice started with an open field trial in an arena to test anxiety levels prior to assessment. Mice with higher anxiety levels were expected to spend more time closer to the walls of the arena. The next day, mice were exposed to two identical objects in the same arena for 10 minutes. Two hours later, the trial was repeated with one of those objects replaced by a new, novel object. Time exploring each object was recorded using the EthoVision software.

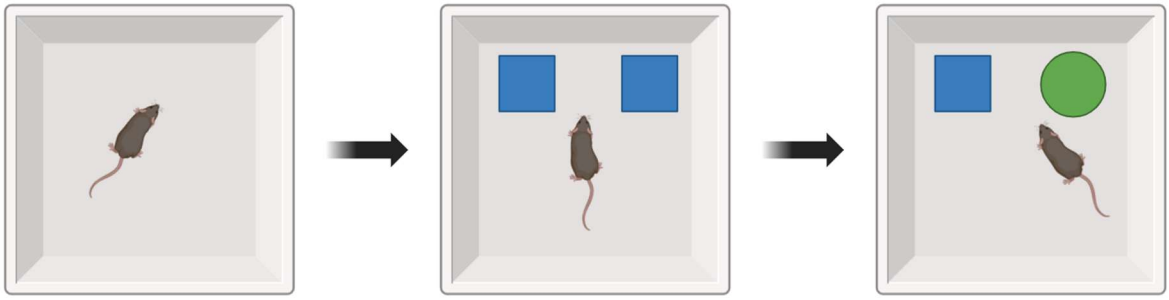

Figure 3: Novel Object Recognition test.

Top view of open field test (left), identical object test (middle), and novel object test (right) (biorender.com).

Instinctual behavior was tested via a nest-building test (Figure 4). Rodents are separated into new cages with food, water, and a square cotton nestlet. After one-night, rodent nests were ranked on a five-point scale from no nest (score of 1) to fully shredded and well-constructed nest (score of 5).

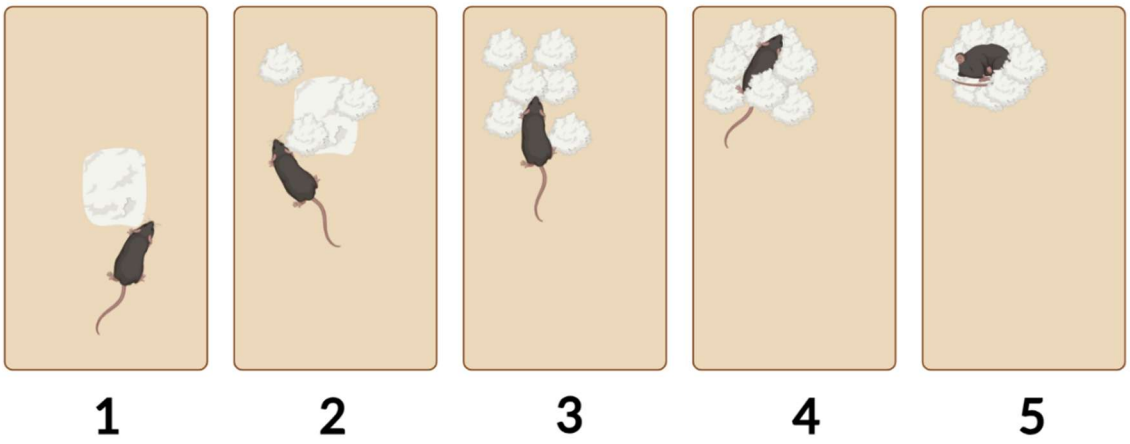

Figure 4: Nest building test.

Top view of nest scores 1 (left) to 5 (right) (biorender.com).

Motor coordination and balance were assessed via the rotarod test (Figure 5). Mice were habituated to the test 24 hours prior by staying on a rotating rod (4 rpm) for 90 seconds. On test day, mice were placed on an accelerating rod, from 4-40 rpm in 300 seconds. Time spent running on rod prior to falling off was recorded. If the mice remained on the rod for the full time, they were removed and given a time of 300s. The test is repeated for a total of 3 trials with 10-minute breaks in between each trial.

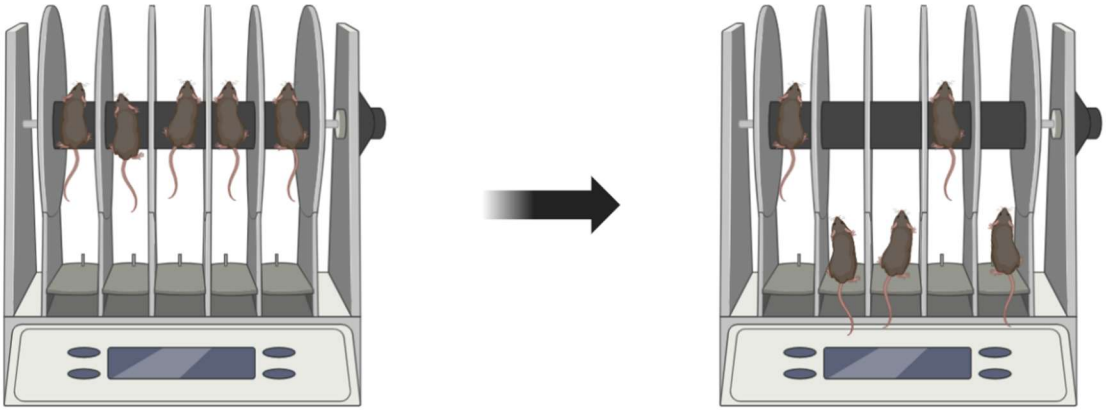

Figure 5: Rotorod test.

Front view of mice on rotating rod at the start (left) and end (right) of the test (biorender.com).

Vascular Function

Endothelial function was measured *ex vivo* by pressure myography of posterior cerebral arteries (PCAs) in a physiological salt solution bath (Figure 6). PCAs were cannulated onto glass micropipette tips within the salt solution. The bath was warmed to 35°C and PCAs were pressurized by gravity to 50 mmHg. Endothelium-dependent dilation was measured via dilation to increasing doses of vasodilators, acetylcholine (ACh, 10^{-9} to 10^{-4}M) and insulin (10^{-9} to 10^{-4}M), after 20% preconstriction to phenylephrine (PE, 2µm). To confirm dilation through the nitric oxide (NO) pathway, doses of Ach and insulin were repeated after a 30-minute incubation with N^{ω}-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor. To determine if vascular function was altered by endothelial cells independent of the smooth muscle lining, endothelium-independent dilation was measured via dilation to increasing doses of sodium nitroprusside (10^{-10} to 10^{-4}M, SNP) and constriction to increasing doses of endothelin-1 (10^{-9} to 10^{-5}, ET1).

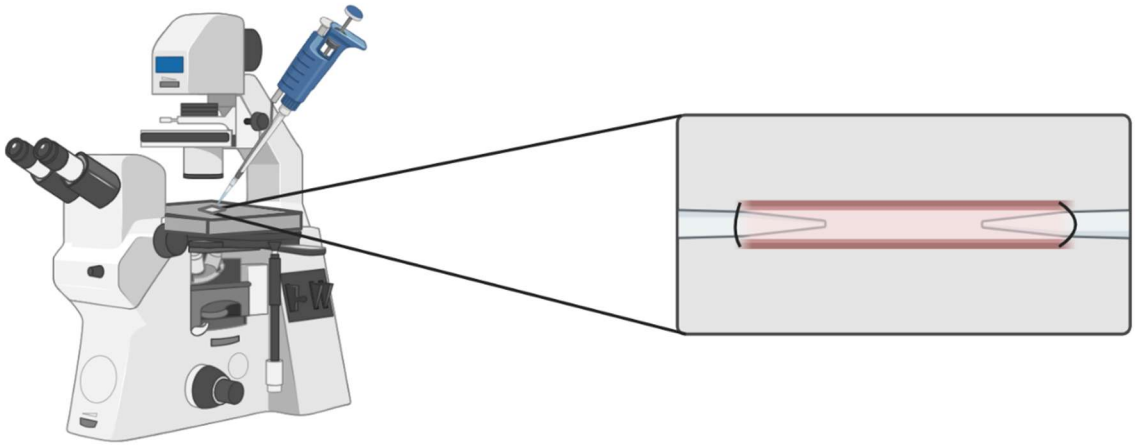

Figure 6: Pressure myography test.

(biorender.com)

Protein expression

Western blotting was used for protein quantification of NOX2 and SOD (Figure 7). Briefly, we isolated the hippocampus from the right hemisphere of the brain. Tissues were lysed using cell lysis buffer (Buffer A (150 mM NaCl, 10mM HEPES, 1mM EGTA, 0.1mM $MgCl_2$, pH 7.4), NaF (10mM), VO_4 (2mM), Protease inhibitor cocktail (10µL/mL)) followed by three times sonication (70% power, 10s, Qsonica) and a BCA assay (Thermo Scientific, Waltham, MA) was used to calculate total protein. 50µg of protein was added to 4-20% Tris-Glycine stain-free gels (Mini-PROTEAN TGX Stain-Free, Biorad) and gel electrophoresis was run at 125V for 40 minutes. Protein was transferred onto a PVDF membrane (0.45 µm, Fisher Scientific) using a semi-dry transfer system (HEP Semidry, Thermo Fisher). Total protein was imaged on stain-free gels and on the PVDF membrane post-transfer. To probe for proteins of interest, including NOX2 (AB129068, Abcam) and SOD1 (AB16831, Abcam), we incubated with primary antibodies overnight. The following day primary antibodies were washed off using TBS-t, and

secondary antibody (Goat anti-rabbit igG HRP-linked antibody, Cell Signaling) was incubated in 1.5% milk (Blotting-Grade Blocker, BioRad). Images were collected using the BioRad ChemiDoc imaging system with chemiluminescence and analyzed using ImageLab software by controlling against total protein.

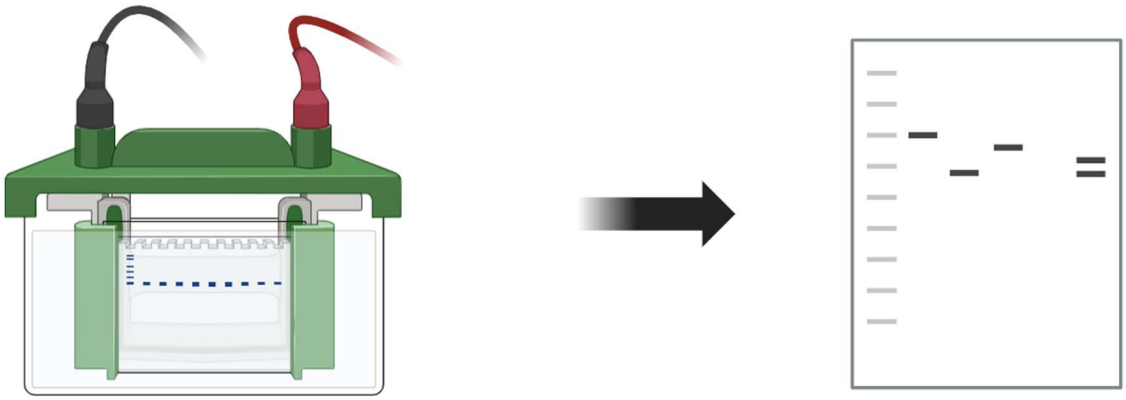

Figure 7: Western blot test.

(biorender.com)

RNA expression was performed using hippocampal and cortex tissue of NOX2 and SOD to elucidate path-specific barriers to final protein expression. Dissection and lysing of external cerebral arteries from the brain was performed. The Qiagen QIAzol and RNeasy microkits (Qiagen, Hilden, Germany) were used to prepare the arteries. Resulting RNA was quantified using the Nanodrop 2000 (Thermo Scientific, Waltham, MA). The Qiagen QuantiTect Reverse Transcription Kit was used to generate cDNA samples, which were then amplified using real-time qPCR by Thermo Fisher PowerUp SYBR Green, primers (IDT), and QuantStudion 5 Real-Time PCR System (Thermo Scientific, Waltham, MA). The $2^{-\Delta\Delta CT}$ method was used to calculated mRNA expression, using 18s for the housekeeping control gene and normalized values to males on a normal-zinc diet.

Results

Dietary zinc has a sex-dependent effect on endothelial vasodilation.

Male mice on a low-zinc diet had significantly lower vasodilation to ACh than their normal and high-zinc counterparts ($p<0.05$, Fig. 8A). Females on a high-zinc diet had significantly greater vasodilation than females on a normal-zinc diet ($p<0.05$, Fig. 8B). All groups had significantly lower vasodilation to ACh at doses 10^{-7} M through 10^{-4} M when incubated with LNAME (Fig. 8 A,B), confirming dilation to ACh was due to endothelium-mediated vasodilation pathways. Male mice on a low-zinc diet had significantly lower maximum dilation to ACh than male mice on a high-zinc diet ($p<0.05$) and female mice on a low-zinc diet ($p<0.05$, Fig. 8C). Female mice on a normal-zinc diet had significantly lower % maximum dilation to ACh than female mice on a high-zinc diet ($p<0.05$, Fig. 8C). There was a main effect of diet ($p=0.03$) on % maximum dilation to ACh (Fig. 8C).

Male mice on a high-zinc diet had significantly higher vasodilation to insulin than their low and normal-zinc counterparts ($p<0.05$, Fig. 8D). There were no significant differences in vasodilation to insulin across female groups ($p>0.05$). Female mice on a high-zinc diet experienced significantly less vasodilation to insulin than male mice on a high-zinc diet ($p<0.05$, Fig. 8D&E). At doses 10^{-8} M through 10^{-5} M, male mice on a high and low-zinc diet, and at doses 10^{-6} M through 10^{-4} M, female mice on a normal-zinc diet had significantly less vasodilation to insulin with LNAME than without ($p<0.05$); however, this significance was not seen in other groups ($p>0.05$, Fig. 8D&E). Male mice on a high-zinc diet had significantly higher % maximum dilation to insulin than male mice on a normal and low-zinc diet and female mice on a high-zinc diet ($p<0.05$, Fig. 8F). There were no significant differences between female groups in % maximum dilation to insulin ($p>0.05$, Fig. 8F). There was an interaction effect

(p=0.03), a main effect of sex (p=0.01), and a trend for main effect of diet (p=0.06) on % maximum dilation to insulin (Fig. 8F).

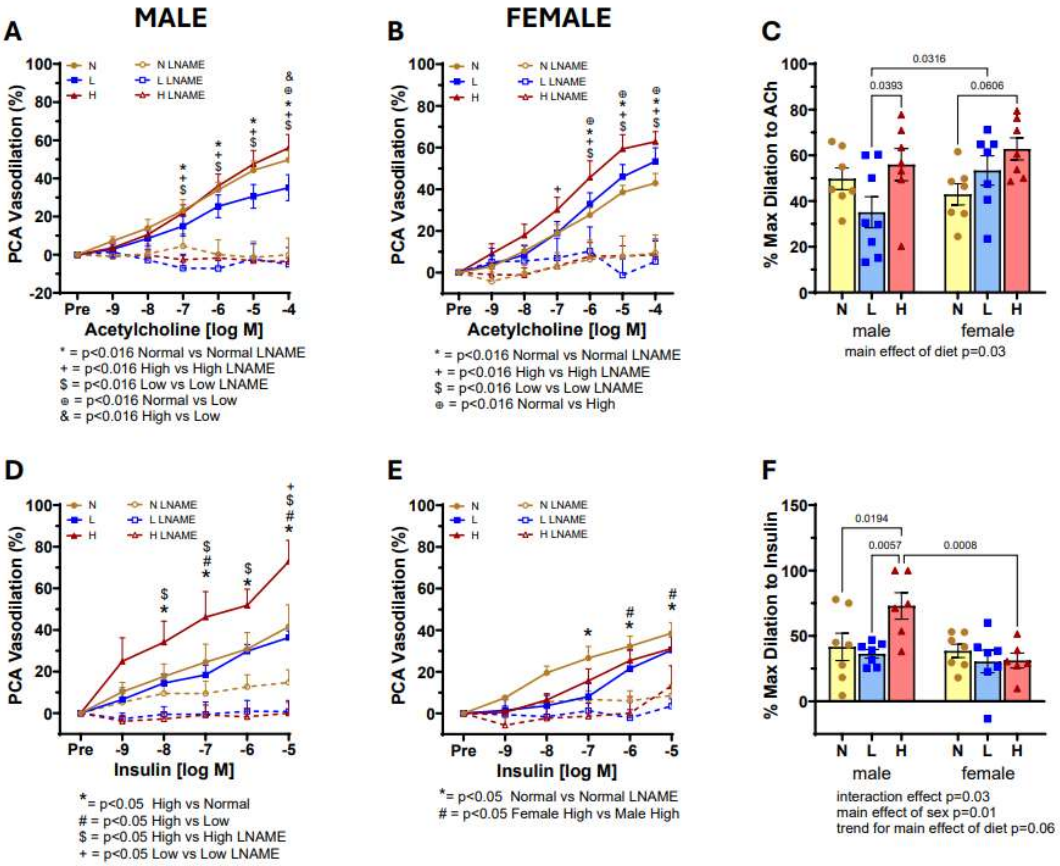

Figure 8: Endothelium-dependent vasodilation.

PCA vasodilation (%) to Acetylcholine (log M) in male (A) and female (B) mice with and without LNAME incubation. Percent maximum dilation to acetylcholine across male and female groups (C). PCA vasodilation (%) to insulin (log M) in male (D) and female (E) groups with and without LNAME incubation. Percent maximum dilation to insulin across male and female groups (F). $p<0.05$ was considered significant.

Dietary zinc has a sex-dependent effect on endothelium-independent vasodilation and vasoconstriction.

Male mice on a high-zinc diet had significantly lower vasodilation to SNP than male mice on a low and normal-zinc diet at doses 10-7 M to 10-5 M ($p<0.05$); however, these

differences were lost at the final dose ($p>0.05$, Fig. 9A). Female mice on a low-zinc diet had significantly lower vasodilation to SNP than female mice on a high and normal-zinc diet at dose 10-5 M ($p<0.05$), which continued in the last dose with female mice on a high zinc diet ($p<0.05$, Fig. 9B). There were no significant differences across diet and sex in % maximum dilation to SNP ($p>0.05$, Fig. 9C). There was a trend for main effect of sex ($p=0.08$) on % maximum dilation to SNP (Fig. 9C).

Male mice on a low-zinc diet had significantly lower vasoconstriction at the highest dose of ET1 than their normal and high-zinc counterparts ($p<0.05$, Fig 9D). There were no significant differences in vasoconstriction to ET1 across female groups ($p>0.05$, Fig. 9E). Female mice on a norma-zinc diet had significantly less % max constriction to ET1 than males on a normal-zinc diet ($p<0.05$), while there were no differences among dietary groups in males or females respectively ($p>0.05$, Fig. 9F). There was a main effect of sex ($p=0.04$) on % max constriction to ET1 (Fig. 9F).

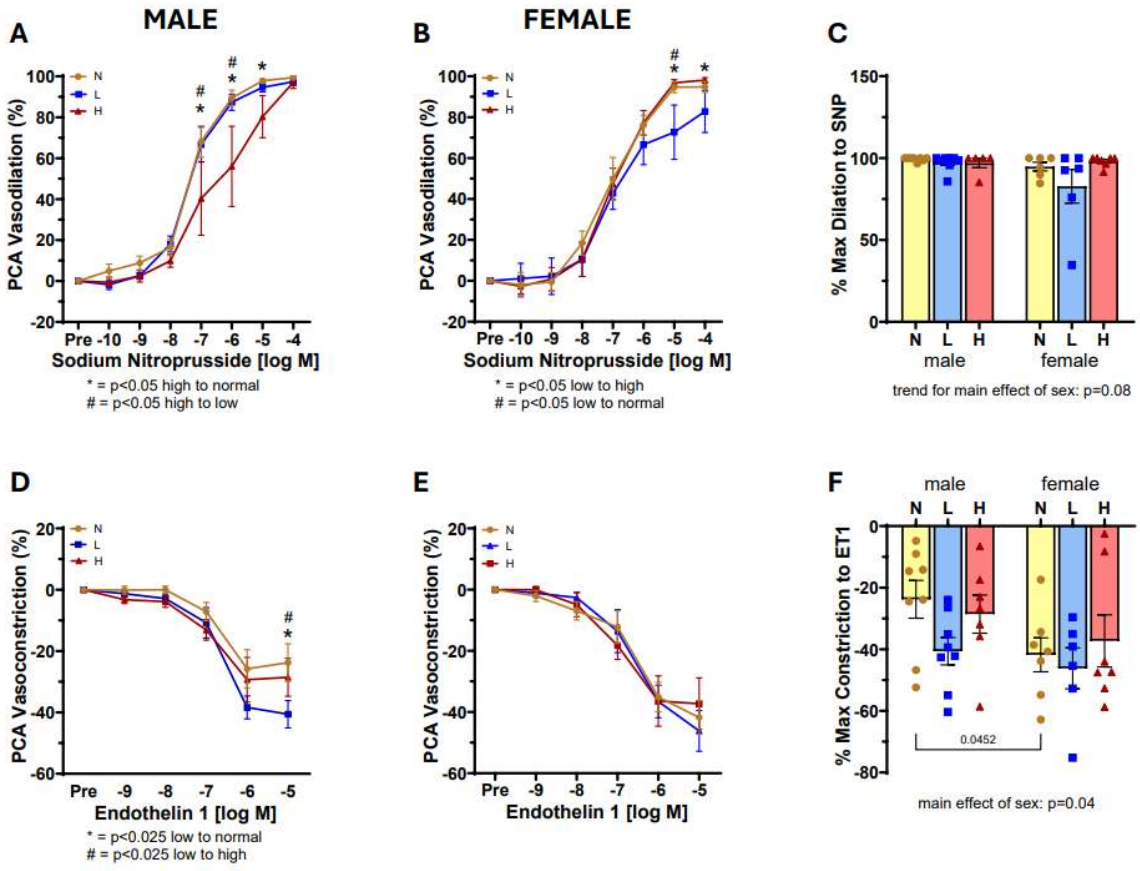

Figure 9: Endothelium-independent vasodilation and vasoconstriction.

PCA vasodilation (%) to sodium nitroprusside (log M) in male (A) and female (B) groups with and without LNAME incubation. Percent maximum dilation to sodium nitroprusside across male and female groups (C). PCA vasoconstriction (%) to ET1 (log M) in male (D) and female (E) groups with and without LNAME incubation. Percent maximum constriction to ET1 across male and female groups (F). $p<0.05$ was considered significant.

Dietary zinc, and sex, had no effect on cognitive function at young age.

Zinc diet had no effect on nest building scores (AU) across male and female groups ($p>0.05$, Fig. 3A). Zinc diet had no effect on time spent on the Rotorod (s) across male and female groups ($p>0.05$, Fig. 10B).

Zinc diet had no effect on time to platform (s) in the MWM training trials across sessions in male and female groups ($p>0.05$, Fig. 10C). Zinc diet had no effect on time spent in target quadrant in MWM probe trials across male and female groups ($p>0.05$, Fig. 10D). Female mice on a high-zinc diet had significantly higher velocity (cm/s) in MWM trials than male mice on a high-zinc diet ($p<0.05$), and there was a main effect of sex on MWM velocity ($p=0.003$, Fig. 10E). Zinc diet had no effect on time to platform region (s) in the MWM beacon trials across male and female groups ($p>0.05$).

Zinc diet had no effect on time in center (s) in NOR open field trials across male and female groups ($p>0.05$, Fig. 10G). Zinc diet had no effect on discrimination index in NOR trials across male and female groups ($p>0.05$, Fig. 10H). Zinc diet had no effect on time with novel versus familiar object (s) in NOR trials across male and female groups ($p>0.05$, Fig. 10I).

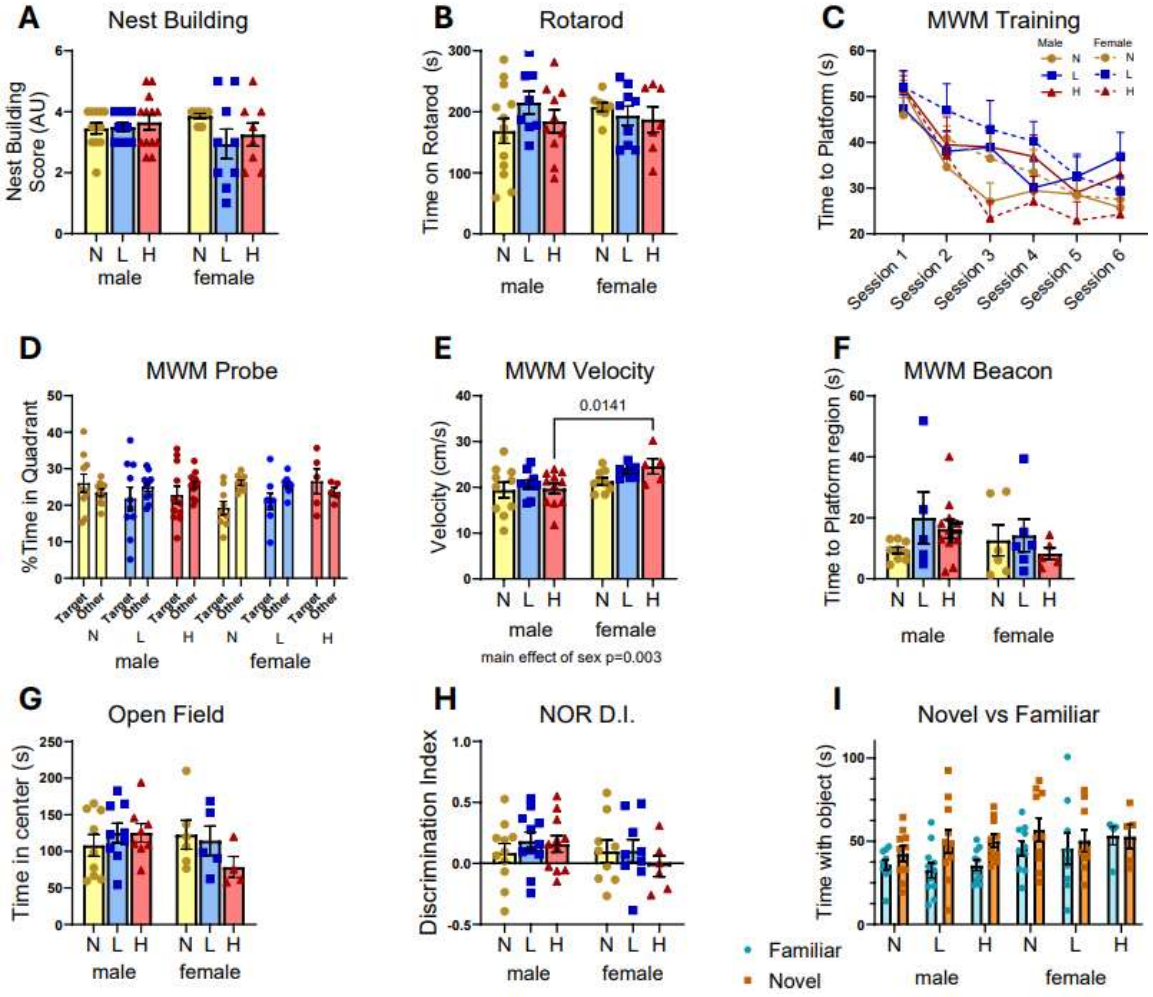

Figure 10: Cognitive testing.

Nest building score (AU) across male and female groups (A). Time on Rotorod (s) across male and female groups (B). Time to platform (s) in MWM training trials across sessions in male and female groups (C). Percent time in quadrant (%) in MWM probe trials across male and female groups (D). Velocity (cm/s) in MWM trials across male and female groups (E). Time to platform region (s) in MWM beacon trials across male and female groups (F). Time spent in center (s) in NOR open field trials across male and female groups (G). Discrimination index in NOR trials across male and female groups (H). Time spent with novel versus familiar object (s) in NOR trials across male and female groups (I).

Dietary zinc had no impact on NOX2 or SOD1 at young age.

Male mice on a high-zinc diet had significantly higher NOX2 gene expression in cerebral arteries than female mice on a high-zinc diet ($p<0.05$) and there was a main effect of sex ($p=0.03$), where females expressed significantly less NOX2 than males (Fig. 11A). There were no significant differences in NOX2/total protein (au) across male and female groups (Fig. 11B). There were no significant differences in SOD1 gene expression across male and female groups (Fig. 11D). There were no significant differences in SOD1/total protein (au) across male and female groups (Fig. 11E).

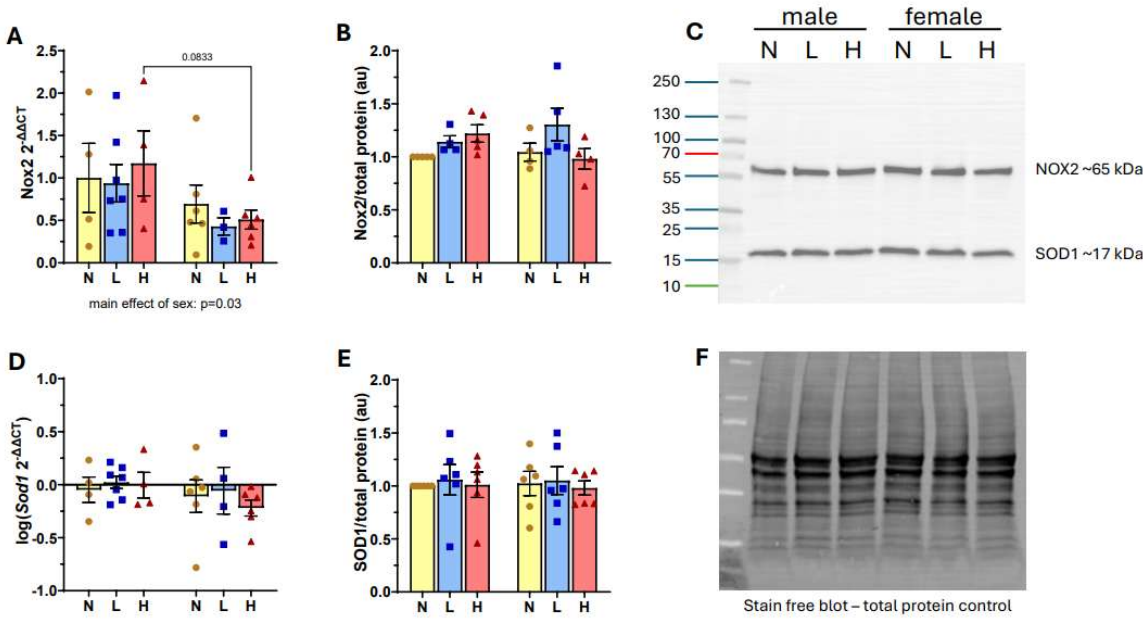

Figure 11: Protein and gene expression.

NOX2 gene expression (2-ddCT) across male and female groups (A). NOX2/total protein (au) across male and female groups (B). SOD1 gene expression (2-ddCT) across male and female groups (D). SOD1/total protein (au) across male and female groups (E). Representative image for A and D (C). Representative image for B and E (F).

Discussion

The purpose of this study was to elucidate the effects of dietary zinc imbalance on the cognitive and vascular components of AD model mice. I hypothesized that an imbalanced zinc diet would impair cognitive and vascular function by dysregulating SOD function. I found that dietary zinc imbalance in young AD model mice had no effect on cognitive function but had significant and sex-dependent effects on vascular endothelial/vascular smooth muscle cell function. Interestingly, there was no effect on the genetic expression or abundance of SOD. Therefore, the initial hypothesis was partially rejected, supported only by differences in cerebrovascular function.

Zinc diet affected cerebrovascular endothelium dependent dilation of PCAs (Figure 8). Under normal conditions, ACh binds to NO-receptors, releasing nitric oxide from endothelial cells which triggers vascular smooth muscle cells to relax, dilating the vessel's diameter (Walker et al., 2015). Low zinc diet resulted in impaired dilation to ACh in a sex-dependent manner, while a high zinc diet was partially restorative (Figure 8). The reduced zinc bioavailability could have increased ROS, which can react with NO to produce peroxynitrate, and reduce the amount of NO used for dilation. Since incubation with LNAME, an inhibitor of nitric oxide, significantly diminished these vasodilatory effects, dilation through the nitric oxide pathway of the endothelial cell, rather than through dilation of vascular smooth muscle cells, is presumed (Sventek et al., 1997). Zinc deficiency had a stronger effect on ACh-dependent dilation in males than in females, while a high-zinc diet enhanced ACh-dependent dilation in females, indicating a sex-specific effect. This positive effect in female mice could be due to estrogen accumulation. Although we did not track the estrous cycle, it is possible that excess estrogen in the blood stream during testing acted as a vasodilator and antioxidant, diminishing the effects of low zinc availability

(White, 2002). These results indicate a need for the relationship between zinc imbalance, sex, and AD to be studied further.

Conversely, a high-zinc diet enhanced insulin-dependent dilation in males, but not females (Figure 8). Insulin also operates through the nitric oxide pathway, making this difference very interesting (Hill et al., 2021). However, males on a normal zinc diet and females on a high and low zinc diet had no significant differences with LNAME incubation. This indicates impairment in NO-mediated dilation, which could explain the reduced vasodilation in males on a low zinc diet, and pattern differences in females (Sventek et al., 1997). The interaction effect and main effect of sex indicate a sex-dependent effect on insulin signaling, once again supporting the need for further research into these differences.

Interestingly, females on a low zinc diet had impaired maximum vascular smooth muscle function in dilation to SNP, while males had no difference in maximal dilation to SNP, highlighting a sex-specific sensitivity to endothelium-independent dilation (Figure 9). SNP relaxes the smooth muscle lining of the arterial wall by bypassing endothelial cells and releasing NO, causing vasodilation (Gagov et al., 2022). Therefore, zinc imbalance likely affects endothelium-dependent pathways in male mice and endothelium-independent pathways in females. Zinc deficiency increased vasoconstriction to ET1 in males, but not females, with a main effect of sex on maximum constriction, illuminating yet another sex-dependent effect (Figure 9). ET1 can bind to endothelial cells, producing a vasodilatory effect, or smooth muscle cells, causing vasoconstriction (Feng et al., 2022). ET1 production increases in response to endothelial stress and inflammation, indicating a likely increase in ROS in the arterial wall of males on a low zinc diet. Overall, the results of our pressure myography studies indicate the

dietary zinc levels have both a sex-dependent and mechanism-specific effect on cerebrovascular dilation and constriction pathways.

Zinc diet had no effect on cognitive function (Figure 10). However, given the indications of cerebrovascular impairment, the young age of the mice at only 6 months and short timeframe of the study could have had little effect on baseline cognitive function, as was seen in another study of the same young mouse model (Sakakibara et al., 2018). Further studies should be performed to see if these effects persist in older age and with longer zinc imbalance. The results could provide strong evidence that vascular dysfunction precedes cognitive decline. Although high-zinc males had significantly higher NOX2 expression than high zinc females, these differences did not persist in NOX2 protein quantification or SOD data, suggesting post-transcriptional regulation or limited functional impact of SOD at a young age (Figure 11).

Limitations and Future Directions

One limitation of this work is that only young mice were studied. Additionally, mice do not naturally develop AD. Our mouse model only induced Aβ accumulation, whereas AD pathology commonly includes tau protein tangles as well. Therefore, AD as a disease of aging in humans was only limitedly modeled, testing only a hypothetical pathology, the Aβ hypothesis. Moreover, we did not track the estrous cycle in female mice, which has been shown to affect arterial stiffness in mice and could skew the results of this study (Kehmeier et al., 2022).

Further investigation might identify the effect of zinc imbalance on its co-activator for SOD, copper, in which adequate copper intake might supplement zinc loss and could explain a null relationship between zinc deficiency and SOD activation. To illuminate the biomolecular pathways of zinc imbalance, future research should test expression and activity of zinc transporter proteins and markers of blood brain barrier integrity. Additionally, our control variable for this study was male APP-KI$^{NL\text{-}G\text{-}F}$ mice on a normal zinc diet. A wild-type control could elucidate further interactions between Aβ accumulation and zinc. Moreover, future replication of this study should determine differences in an older mouse model, which could elucidate cognitive differences. Since our mouse model may not accurately represent AD mice, more appropriate animal models and, ideally, clinical trials with dietary zinc supplementation could be the next step to confirm any significance in data.

Conclusion

This study found that zinc imbalance affects cerebrovascular, but not cognitive, function in a young mouse model of AD, independent of SOD or NOX regulation. While zinc supplementation trials in AD patients have been conducted before, the results lack consistency yielding confusion about the relationship between zinc and AD. This study refines the knowledge gap in the field and elucidates potential biomarkers of disease progression and proactive treatments to prevent early and late-onset AD. Further, health equity between sexes was placed at the forefront of this study to combat the medical disparity in biomedical research (Waters et al., 2021). I plan to carry the vital skills I learned from this project into my future career as a physician scientist and a leader of interdisciplinary biomedical innovation.

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