The effect of zinc status on cognitive and vascular function in a mouse model of Alzheimer's disease

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Abstract

Exploring potential treatments for Alzheimer's disease (AD) is important as the aging population continues to increase (Cummings, 2004). A hallmark of AD is excessive oxidative stress (Cheignon et al., 2018). Previous research has identified zinc as an antioxidant and regulator of vasodilation, associated positively and negatively with cognitive function (Betrie et al., 2021; Cuajungco & Fagét, 2003). However, its relationship with vasodilation in AD has not been fully elucidated. We hypothesize that maintaining zinc status will improve cognitive and vascular function in a mouse model of AD. We treated male and female AD model mice (6 m) with zinc-normal (30 ppm), low (2-5 ppm) and high (300 ppm) diets for 2 m. We evaluated cognition through rotarod and nest building tests. We assessed large artery stiffness by pulse wave velocity and cerebrovascular function by pressure myography. There was an interaction effect of zinc status and sex (p=0.047) and a main effect of sex (p=0.02) on time spent on rotarod. There was no effect of zinc status or sex on nest building. We identified a main effect of sex on pulse wave velocity (p=0.01). We are still analyzing cerebrovascular function but preliminary results indicate sex-differences in response to zinc diets, where low zinc may be detrimental to males but not females (p<0.05). We find that zinc status may impact cognitive and cerebrovascular function, indicating the need for further research into zinc's effect on AD . This research is aimed at determining potential future nutritional interventions for reducing the risk of AD.

Background

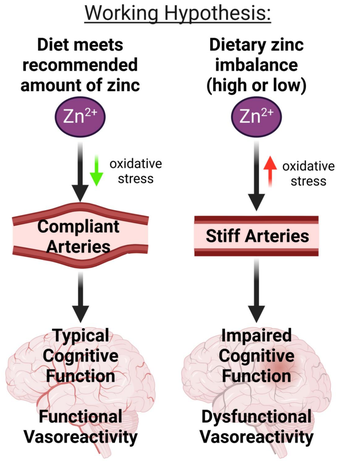
Alzheimer's Disease (AD):

- Neurovascular inflammation triggered by oxidative stress is an important precursor to the development of dementias, like AD, and contributes to harmful amyloid-β plaque formation (Cheignon et al., 2018).
- The risk of AD is highly linked to increasing cerebrovascular dysfunction and arterial stiffness, which leads to reduced blood flow and neuroinflammation (Reeve et al., 2024).

Zinc and Oxidative Stress:

- Zinc has antioxidant and anti-inflammatory properties, reducing harmful oxidative stress (Cuajungco & Fagét, 2003).
- The zinc pathway is crucial for neuronal development and is linked positively and negatively to cognitive dysfunction with age.
- Zinc is an integral part of arterial vasorelaxation mechanisms regulating blood flow to the brain (Betrie et al., 2021).

Working Hypothesis:



Research Question: Does dietary zinc impact cerebrovascular health in a mouse model of AD?

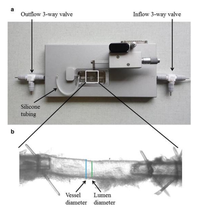
Methods

Animals:

- To model AD, APP^{NL-G-F} mice were studied, which develop amyloid-beta plaques by 6 m of age by knock-in of the human amyloid precursor protein mutations. Male and female mice were studied at 6 m of age.
- Mice were randomly assigned to zinc-normal (ZnN, 30 ppm), low (ZnL, 2-5 ppm) and high (ZnH, 300 ppm) diets at 4 m for 2 m.

Cerebrovascular Health:

- Endothelial function was assessed via isolation of posterior cerebral arteries ex vivo and placement in a myograph chamber under a normalized pressure of 68 mmHg in a physiological salt solution. After 20% pre-constriction to 1-6 µM phenylephrine, endothelium-dependent vasodilation was tested by measuring the change in lumina diameter under increasing concentrations of vasodilator, acetylcholine (Ach).



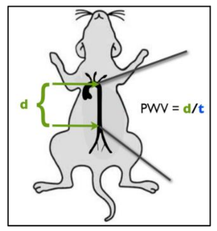
("Pressure Myography to Study the Function and Structure of Isolated Small Arteries" Musculoskeletal Key)

- Acetylcholine responses were repeated post 30-minute incubation under 0.25 µM Nω-nitro-L-arginine methyl ester hydrocholoride (L-NAME), an eNOS inhibitor. This informs us of dilation pathways independent of nitric oxide.
- Endothelium-independent dilation was measured in increasing concentrations of vasodilator sodium nitroprusside (SNP).

Methods

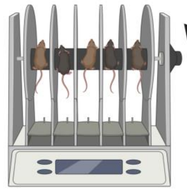
Cerebrovascular Health (cont.):

- Arterial stiffness was assessed by aortic pulse wave velocity. Isoflurane was used to anesthetize the mice, Doppler ultrasound probes were placed on the ascending aorta and abdominal aorta to collect wave forms and measure their velocity (distance/time). The collected data was analyzed using Doppler Signal Processing Station (Scintica Instruments, Webster, TX).



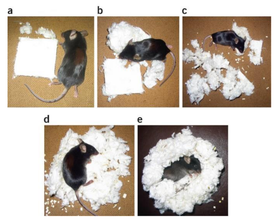
Cognitive Function:

- Motor coordination and balance was assessed via the rotarod test. Mice were habituated to the test 24 hours prior to test by staying on the rotating rod (4 rpm) for 90 seconds. On test day, mice were placed on an accelerating rod, from 4-40 rpm in 5 mins. Time spent running on rod prior to dismantling was measured. The trial ends after 6 minutes. The test is repeated for a total of 3 trials with 10-minute breaks in between each.



("Snapshot: What is the Rotarod Test?", Xhako, National Ataxia Foundation)

- Instinctual behavior was tested via a nest-building test. Rodents are separated into new cages with food, water, and a square cotton nestlet. After one night, rodent nests are ranked on a five-point scale from no nest (score of 0) to fully-shredded and well-constructed nest (score of 5). Poor nesting behavior is an indication of cognitive decline and poor overall comfortability of the animal.



("Assessing nest building in mice", Deacon, Nature Protocols 2006)

Results

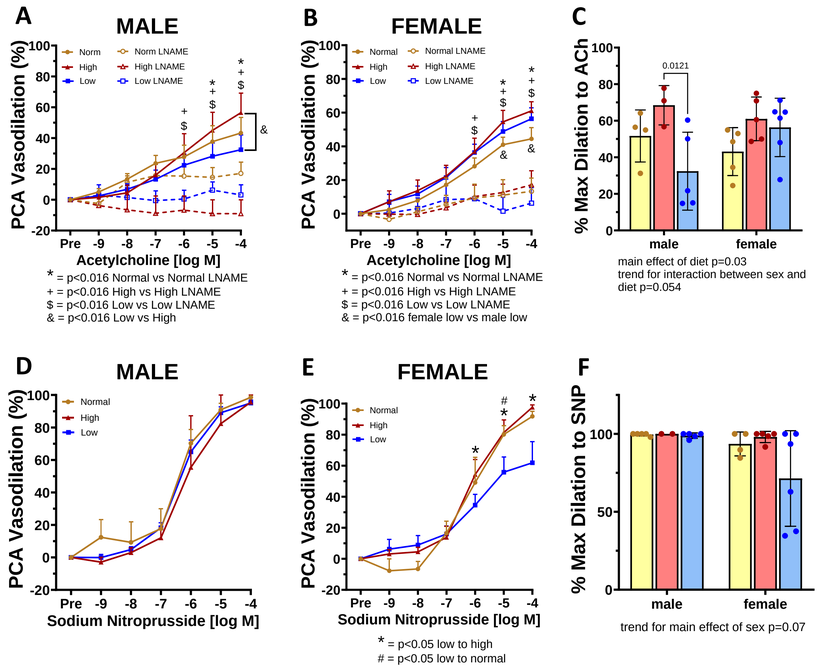


Figure 1: Zinc diet influences endothelium-dependent and independent dilation. Acetylcholine and LNAME were used to measure endothelium-dependent dilation in male (**A**) and female (**B**) mice on normal, low, and high zinc diets. Max dilation to Ach is represented in C. SNP was used to measure endothelium-independent dilation (**D** and **E**). Max dilation to SNP is represented in **F**. Data shown are mean ± SEM, p-value of 0.05 was considered to be significant.

Results cont.

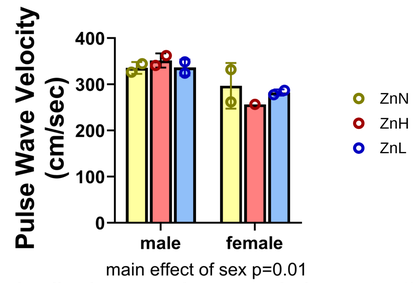


Figure 2: Sex, but not zinc diet, impacts pulse wave velocity. Aortic stiffness was measured as mean pulse wave velocity (cm/sec) across male and female mice on zinc low, normal, and high diets. Data shown are mean ± SD, p-value of 0.05 was considered to be significant.

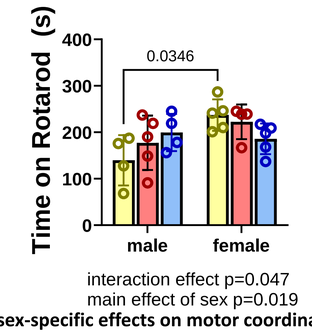


Figure 3: Zinc diet has sex-specific effects on motor coordination. Motor coordination measured as time on rotarod (s) across male and female mice on zinc low, normal, and high diets. Data shown are mean ± SD, p-value of 0.05 was considered to be significant.

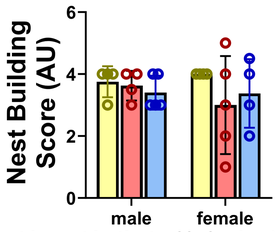


Figure 4: Zinc diet does not impact instinctual behavior. Nesting instincts measured as Nest Building Score (AU) across male and female mice on zinc low, normal, and high diets. Data shown are mean ± SD, p-value of 0.05 was considered to be significant.

Conclusions

We find a that zinc diet has significant and sex-dependent effects on endothelium-dependent and independent dilation. Furthermore, we find an interaction effect between zinc diet and sex on motor coordination. However, we did not find an effect of zinc diet on instinctual behavior or pulse wave velocity. Our pulse wave velocity results do support the current literature indicating lower pulse wave velocity in females compared to males. The sex differences in preliminary data indicate a higher detriment of zinc-deficiency in male mice over female mice. Additionally, endothelium-independent vasodilation pathways might mask the dysfunction of endothelium-dependent vasodilation. These findings indicate a need for further research of the effect of zinc on cerebrovascular function and AD.

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