The Effect of Zinc Status and Age on Liver Health in Mice

by
Kendra Nicole Braun

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Nutrition
(Honors Scholar)

Presented March 12, 2021
Commencement June 2021

AN ABSTRACT OF THE THESIS OF

Kendra Nicole Braun for the degree of Honors Baccalaureate of Science in Nutrition presented on March 12, 2021. Title: The Effect of Zinc Status and Age on Liver Health in Mice.

Abstract approved:_____

Emily Ho

Age-related diseases have been associated with chronic inflammation and impaired immune response, one of the hallmarks of aging. Aging individuals also have a higher susceptibility to zinc deficiency. The effects of zinc deficiency share many commonalities with the effects of aging on the immune system and zinc deficiency may be a contributing factor to aging of the immune system. Understanding the relationship between zinc status and age-related inflammation and immune decline may offer insight on how to mediate the negative age effects through nutritional intervention. We hypothesized that in the liver, aging increases inflammation and increased zinc status via dietary zinc supplementation will reduce age-related inflammation, while decreased zinc status due to marginal zinc deficiency will further increase age-related inflammation. In a mouse model, we observed significant effects of age and diet on serum zinc status. Serum inflammatory marker IL6 increased with marginal zinc deficiency and MCP1 decreased with zinc supplementation. Four inflammatory markers, s100a8, s100a9, IL1β, and MCP1, increased with age but were not affected by zinc supplementation or deficiency. T cell related markers IFNγ and IL22 in the liver changed with zinc intervention. These results indicate

the marked increase in inflammation that occurs with age and suggest zinc may play a role in proinflammatory immune response.

Key Words: zinc, aging, inflammation
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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Kendra N Braun, Author

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Chapter 1: Introduction of Thesis

Aging

Although the exact mechanisms of aging are largely unknown, it is well understood that age is an important risk factor for many diseases including dementia, heart disease, cancer, and type II diabetes mellitus. Aging is a complicated process that involves changes to the body's physiology over time affecting fertility, lung function, bone density, and cholesterol levels (Zierer et al., 2015). In particular the immune system is impaired, which includes reduced or dysregulated immune response. Collectively, these changes and impairments to the immune system associated with age are referred to as immunosenescence, as senescence is synonymous with aging (Fulop et al., 2018). Complications of a deficient immune system with age include reduced immune response and decreased vaccine efficiency, which can increase risk of developing infectious diseases including those that are typically prevented by vaccines (Fulop et al., 2018). Deficits of the immune system have been attributed in part to changes in T cell function. Naive T cells are those that have not yet encountered an antigen; once the naive T cell encounters an antigen, the cell can proliferate and differentiate into various types of T cells to respond to the antigen presenting cell (Pennock et al., 2013). Thymus is the immune organ where T cell development and maturation occurs. With age, the thymus experiences thymic involution leading to a decreased amount of new naive T cells that are generated and shuttled to the periphery pool, affecting the overall ability of the adaptive immune system to respond to an antigen (Nikolich-Zugich, 2014).

In addition, aging is associated with chronic inflammation, often referred to as "inflamm-aging". Inflammation is a normal and essential innate immune system response to injury in the body

including exposure to pathogens, toxins, or cell damage. Acute inflammatory responses keep injury or infection in check by activating leukocytes to produce proinflammatory cell-signaling proteins, known as cytokines, to induce inflammatory response and remove the harmful stimuli, initiating the repair process (Chen et al., 2017). Chronic inflammation is identified by prolonged and heightened levels of proinflammatory cytokines in the body and is one of the hallmarks of aging (Rea et al., 2018). When left uncontrolled, chronic inflammation can cause oxidative stress which can damage DNA and lead to diseases. Certain age-related diseases including cancer, arthritis, Alzheimer's, type II diabetes mellitus, cardiovascular disease, and autoimmune diseases have been associated with chronic inflammation. A better understanding of aging mechanisms and what contributes to immunosenescence and inflamm-aging may help identify factors that can be modified to improve the aging immune system.

Nutrition: Zinc

Zinc is an essential micronutrient of interest due to its important role in various cellular processes, including immune function, cell growth, and reproduction as well as enzyme function, protein folding, gene expression and cell signaling (Ho, 2004; *Zinc*, 2014). Zinc is found in a multitude of proteins in the body, particularly those involved with DNA binding and DNA damage repair as well as enzymes like copper/zinc superoxide dismutase (Ho & Ames, 2002). Reactive oxygen species are partially reduced forms of oxygen that are hazardous to the cell by causing oxidative damage; superoxide dismutase (SOD) plays an important role in converting reactive oxygen species into non-hazardous oxygen compounds (Berg et al., 2012). Zinc is also essential for DNA repair (Berg et al., 2012; Song et al., 2009). The role of zinc in DNA repair and protection against oxidative stress makes it essential for healthy fetal and child growth and neurological development (Wessells & Brown, 2012). In addition, zinc is essential for the

development and function of the immune system along with immune signaling and activation (Barnett et al., 2016). Since the human body cannot store significant amounts of excess zinc, it must be obtained through the diet via food or supplements (Skrajnowska & Bobrowska-Korczak, 2019). The Recommended Daily Allowance (RDA) for zinc is 8 mg per day for women and 11 mg per day for men, which can be met by eating zinc-rich foods like oysters and other shellfish, beef, turkey, and fortified cereals (Micronutrients, 2001; *Zinc*, 2014). While zinc can be obtained by eating plant-based foods like legumes and nuts, the bioavailability of zinc from these foods is lower because absorption is inhibited by components in those foods including phytate, oxalate, and other divalent minerals. (Luo & Xie, 2012). The aging population is particularly at risk for marginal zinc deficiency, which can be attributed to the fact that intake is low and serum zinc levels have been observed to decline with age (Barnett et al., 2016). Overall, 17.3% of the world population is estimated to be at risk for zinc deficiency, but this estimation ranges from 7.5% in high-resource regions, and up to 30% in low-resource regions (Wessells & Brown, 2012). This poses zinc deficiency as an important public health issue. It is estimated that 12% of the United States' population does not consume the RDA for zinc — within the elderly population, an estimated 40% of the US population does not consume enough zinc (Ma & Betts, 2000; Mares-Perlman et al., 1995; Prasad et al., 1993). Furthermore, even if dietary zinc intake is adequate, the elderly population has reduced absorption and utilization of zinc (August et al., 1989; Turnlund et al., 1986). Zinc deficiency is linked to increased oxidative stress, decreased DNA repair capabilities, and impaired cell signaling and apoptosis (Ho, 2004). Zinc deficiency poses the risk for impaired immune function and a higher susceptibility to infections because zinc is needed for the development and function of the immune system, including naive T cell activation. Zinc deficiency may also result in thymic involution and lymphopenia (Fischer

Walker & Black, 2004; Shankar & Prasad, 1998). Due to the aforementioned estimate of the US' elderly population not consuming enough zinc, studies have investigated the effects of supplementation to alleviate the negative effects of deficiency but the results can be unclear and therefore inconclusive (Barnett et al., 2016). Furthermore, there are multiple factors that potentially contribute to zinc deficiency including lack of zinc intake in the diet or dysfunction of zinc transporters in the body which affect the uptake, retention, and secretion of zinc (Wong et al., 2009). Largely, the effects of zinc deficiency share many commonalities with the effects of aging on the immune system and zinc deficiency may be a contributing factor to aging of the immune system (Fraker & King, 2004). Understanding the interrelatedness of zinc status and age-related inflammation and immune decline may offer insight on how to mediate the negative effects through nutritional intervention.

Chapter 2

The Effect of Zinc Status and Age on Liver Health in Mice

1 INTRODUCTION

Zinc deficiency is a potential factor contributing to immune system aging and the effects of zinc deficiency parallel the general effects of aging on the immune system. The overarching goal is to understand the how zinc status and age-related inflammation and immune decline relate to one another to offer insight on how nutritional intervention can help with these negative effects. Due to the increasing aging population, the prevalence of age-related diseases has also increased, affecting the mortality rates of the elderly along with their quality of life (Wong & Ho, 2012). Understanding chronic inflammation related to older age, and strategies to limit and control age-related inflammation is ultimately important in order to improve the health and wellbeing of the aging population. Zinc is an essential micronutrient of interest due to its important role in the function of the immune system, and its anti-inflammatory properties. Furthermore, the effects of zinc deficiency are similar to dysfunction of the immune system seen with that of aging. The elderly population is at high risk for zinc deficiency, and inadequate zinc consumption may be contributing to age-related inflammation and morbidities. In the United States, it is estimated that inadequate zinc intake is significantly higher in individuals over 50, with 40% of men and 45% of women consuming less than the estimated average requirement (Micronutrients, 2001). Improving zinc status in aged individuals can potentially mitigate age-related inflammation and may ultimately improve health outcomes in the elderly.

The goal of my honors thesis is to examine how zinc status and age affect liver health. Liver is an organ of interest due to the physiologic changes it goes through with increasing age. In

humans, it has been observed that total liver volume declines as much as 20-40% through the lifespan (Schmucker, 2005). While the liver is known for its metabolic and detoxification roles in the body, its role in immune function is also essential, participating in the body's innate immunity (Gao, 2016). After food is ingested and digested, nutrients are absorbed and the liver takes up nutrient-rich blood, filtering it before it is sent to the circulatory system. Taking a key role in the body's immunity, the liver filters pathogens that enter the body through the gastrointestinal tract (Gropper et al., 2018; Kubes & Jenne, 2018). With regards to age, liver tissue shows an increase in inflammation with age, and in mice models has been noted as a tissue with high levels of inflammatory cytokines and inflammatory genes when compared to younger mice (Singh et al., 2008). These high levels of inflammatory signals indicate that the liver is potentially responding to inflammation-related damage, which makes the liver an organ of interest. Aging is a potential risk factor increasing susceptibility of hepatic inflammation and liver fibrosis. Liver inflammation with regards to age is important since it is known that long-term liver fibrosis leads to cirrhosis, liver failure, and portal hypertension (Robinson et al., 2016). Because the liver is an important organ related to immune function, home to hepatocytes and immune cells known as macrophages, understanding the changes in the liver in relation to age is imperative. With age, function of hepatocytes declines, negatively impacting the liver's capability of regeneration causing the liver to become more susceptible to damage via alcohol, drugs, and other toxins (Stahl et al., 2018). Thus, reducing liver inflammation in the aging population is an important health intervention in order to mitigate the consequential health issues. Regarding zinc, the liver is the main organ where zinc metabolism occurs. While the majority of zinc in the body is stored in the bone, prostate, skin, and retina, some is stored in the liver and can be accessed when needed by the body. When the liver is damaged or diseased, zinc

levels are altered and deficiency can lead to further impaired liver function. Impaired function can manifest as liver fibrosis, mentioned prior as a complication of chronic inflammation associated with age (Grüngreiff et al., 2016; Tuerk & Fazel, 2009). This interrelatedness of the liver's zinc needs, effects of deficiency, and impairment due to inflammation, indicate that the liver is an important organ of interest. Yet to date, very little is known regarding the effects of altering zinc status on liver health.

Significance and Aim of the Thesis

Prior research in the Ho lab has shown that immune cells in the aged thymus and spleen have lower intracellular levels of zinc (Wong et al., 2013). Additionally, it has been shown that zinc supplementation improves thymopoiesis in aged mice by improving zinc status (Wong et al., 2009). The effects of improved thymopoiesis via zinc supplementation on T cell development and function were shown to improve and restore function that had been previously impaired by zinc deficiency (Wong et al., 2021).

The aim of this thesis was to determine the effects of zinc status and age on biomarkers associated with liver inflammation and immune response, studied using an aging mouse model. We hypothesized that in the liver, aging increases inflammation and increased zinc status via dietary zinc supplementation will reduce age-related inflammation, while decreased zinc status due to marginal zinc deficiency will further increase age-related inflammation.

2 METHODS

2.1 Animals, diets, and study design

Young and old mice were used to study the effects of adequate zinc intake, zinc supplementation, and marginal zinc deficiency. Marginal zinc deficiency is a level of deficiency that is considered low but more relevant to the body's physiology than a diet that is completely free of zinc. Young C57BI/6 male mice (2 months old) were purchased from Jackson Laboratories (Bar Harbor, ME). Old C57BI/6 male mice (24 months old) were obtained from the National Institute of Aging. For 1 month before the start of the study, all mice were given the control zinc adequate diet, 30 mg/kg zinc. After 1 month of the control diet, young and old mice were randomly assigned to different dietary treatments for 6 weeks. To control temperature and humidity, mice were housed individually in ventilated cages kept at 72°F, 50% humidity, with a 12-hour light cycle. At the beginning of the study, old mice were free from obvious signs of illness, tumors, or lesions.

To study the effects of marginal zinc deficiency, groups of young and old mice were fed a purified diet containing either 30 mg/kg zinc (zinc adequate group, further referred to as ZA) or 6 mg/kg zinc (marginal zinc deficient group, further referred to as MZD) for 6 weeks. To study the effects of zinc supplementation, groups of young and old mice were fed a purified diet containing either 30 mg/kg zinc (ZA) or 300 mg/kg zinc (zinc supplemented group, further referred to as ZS) for 6 weeks. Purified ZA, MZD, and ZS diets were custom formulated using a modified egg white-based AIN-93G diet and purchased from Research Diets (New Brunswick, NJ). Zinc in the diet was provided as zinc carbonate. In previous studies, a MZD diet had been shown to reduce zinc status in mice, and a ZS diet had been shown to increase zinc status in mice (Wong et al., 2019; Wong et al., 2009). Food and water were provided ad libitum. Food intakes

and body weights of all mice were monitored throughout the study; no difference in food intake and body weight due to the different dietary treatments was observed. At the end of the experiments, mice were euthanized by CO_2 asphyxiation and sera and tissues were collected. Liver tissue was collected from the mice and preserved in RNALater (Life Technologies, Grand Island, NY) for RNA isolation. The animal protocol was approved by the Oregon State University Institutional Laboratory Animal Care and Use Committee. Methods for determining serum zinc, IL6 and MCP1 have been previously published (Wong et al., 2021)

2.2 RNA isolation, spectrophotometric quantification of RNA, and purity of RNA

Total RNA from mouse liver was isolated using TRIzol Reagent (Life Technologies). Quantification of RNA and purity of the RNA was determined using a spectrophotometer to measure the absorbance of diluted RNA samples at a 260 nanometer wavelength. One microgram of total RNA was reverse transcribed into cDNA using SuperScript III First-Strand Synthesis SuperMix for quantitative real-time PCR (Life Technologies).

2.3 Gene expression

Expression of genes related to immune function and inflammation were analyzed using quantitative real-time PCR, further abbreviated as qRT-PCR. qRT-PCR was performed using Fast SYBR Green Mastermix (Life Technologies) on 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). qRT-PCR was performed to examine gene expression of the following: 18s ribosomal RNA (housekeeping gene), CD3, F4/80, IFNγ, IL4, IL17, IL22, IL1β, IL6, TNFα, MCP1, s100a8, s100a9. PCR primer sequences for PCR data included in this thesis are detailed in Table 1. Gene copy numbers were determined using a standard curve, generated from serial dilutions of DNA for each of the aforementioned genes. The dilution series

began with 10^8 copies and ended with 10^4 copies. Data represent the copy number of the gene of interest normalized to the number of 18s, the housekeeping gene.

Gene name	Forward primer sequence (5' – 3')	Reverse primer sequence (5' – 3')
IFNγ	ACTGGCAAAAGGATGGTGAC	TGAGCTCATTGAATGCTTGG
IL1β	AAGATGAAGGGCTGCTTCCAA	TGAAGGAAAAGAAGGTGCTCATG
IL6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
IL22	ATACATCGTCAACCGCACCTTT	AGCCGGACATCTGTGTTGTTAT
MCP1	TCCCAATGAGTAGGCTGGA	TCTGGACCCATTCCTTCTTG
s100a8	TGTCCTCAGTTTGTGCAGAATATAAA	TCACCATCGCAAGGAACTCC
s100a9	GGTGGAAGCACAGTTGGCA	GTGTCCAGGTCCTCCATGATG

Table 1: Primer sequences for real time RT-PCR

2.4 Statistical analyses

Statistical analyses were performed using GraphPad Prism (Graph-Pad, La Jolla, CA). Two-way ANOVA was used to determine the significance of the effects of zinc status and age and their interaction. Statistical significance was defined as $p \leq 0.05$.

3 RESULTS

3.1 Serum zinc levels in mice are influenced by age and dietary zinc intake

The effects of aging and dietary zinc intake (marginal zinc deficient diet or zinc supplemented diet) on serum zinc levels were evaluated in two mouse feeding studies that were completed in the Ho lab and have been recently published (Wong et al., 2021). In both studies, aged mice have decreased zinc status compared to young mice despite being fed a zinc adequate diet. In the first study, the zinc status in both young and old mice decreases when mice are fed a marginal zinc deficient diet. This decreased zinc status is more apparent in old mice and it further exaggerated

when fed a marginal zinc deficient diet (Figure 1). In the second study, it was found that the decreased zinc status in aged mice could be restored to levels similar to young mice by feeding the mice a zinc supplemented diet. Young mice also achieved a heightened zinc status when fed a zinc supplemented diet versus a zinc adequate diet (Figure 2). Serum zinc levels are correlated with age and are responsive to dietary intervention, including supplementation or marginal deficiency.

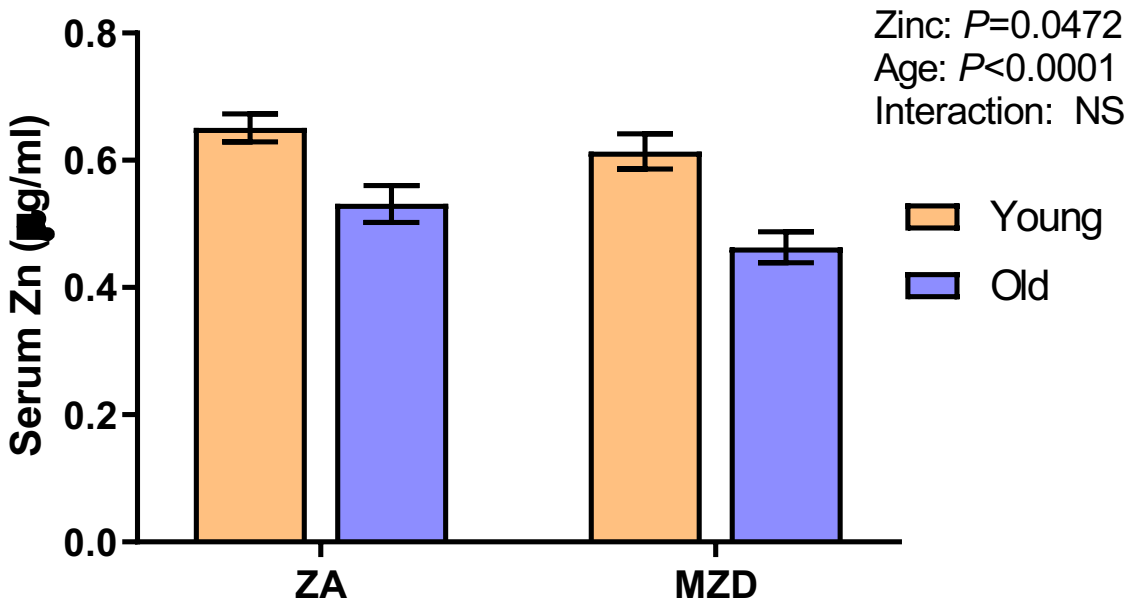

Figure 1. **Aging is associated with decreases in zinc status and was further decreased in old mice fed a marginal zinc deficient diet**. Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a zinc adequate (ZA) diet or marginal zinc deficient (MZD) diet for 6 wks. Serum zinc levels were measured by ICP-OES at the end of the study. Data represent mean ± SEM (n = 7-10 per treatment group). Two-way ANOVA to test for main effects of zinc status and age and their interaction. NS = not significant.

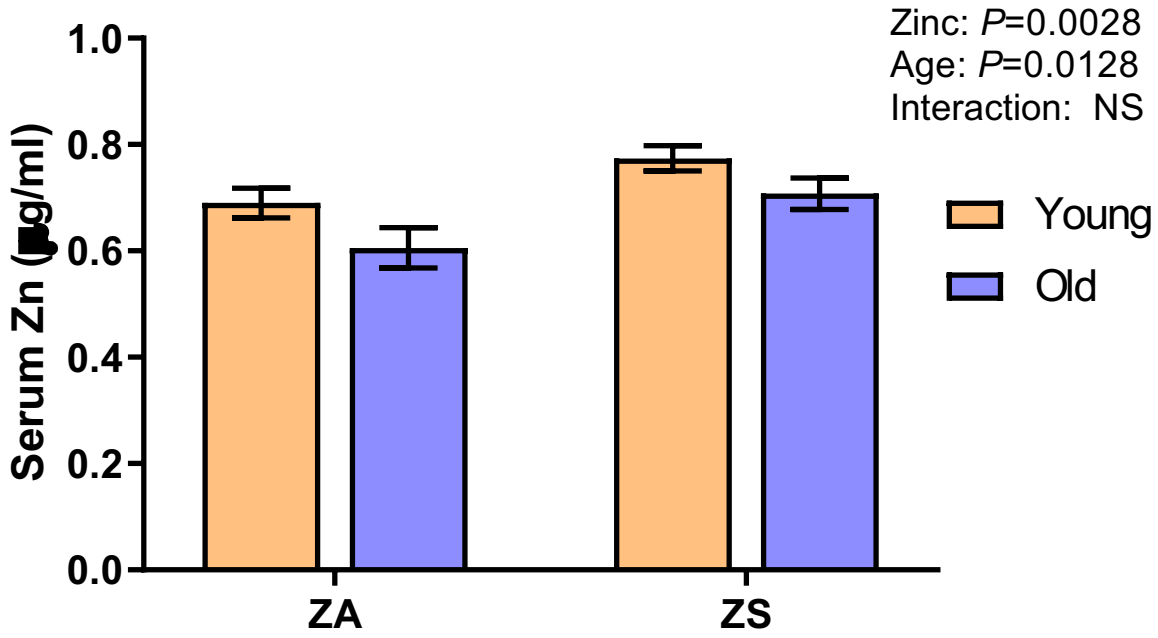

Figure 2. Age-related decline in serum zinc was reversed with dietary zinc supplementation. Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a zinc adequate (ZA) diet or zinc supplemented (ZS) diet for 6 wks. Serum zinc levels were measured by ICP-OES at the end of the study. Data represent mean ± SEM (n = 7-10 per treatment group). Two-way ANOVA to test for main effects of zinc status and age and their interaction. NS = not significant.

3.2 Serum inflammatory markers: Marginal zinc deficiency increases IL6 response and zinc supplementation decreases MCP1 response in serum

The effects of aging and dietary zinc intake on serum inflammatory markers were examined and recently reported (Wong et al., 2021). Old mice fed a zinc adequate diet have increased serum levels of MCP1 and IL6 (inflammatory cytokines) in comparison to young mice. Manipulation of diet via marginal zinc deficiency further exaggerates the increase in serum IL6 levels (Figure 3). In contrast, old mice fed a zinc supplemented diet had decreased MCP1 levels compared to old mice fed zinc adequate diet (Figure 4). Manipulation of diet via zinc supplementation or

marginal zinc deficiency alters abundance or serum inflammatory markers, specifically MCP1 and IL6.

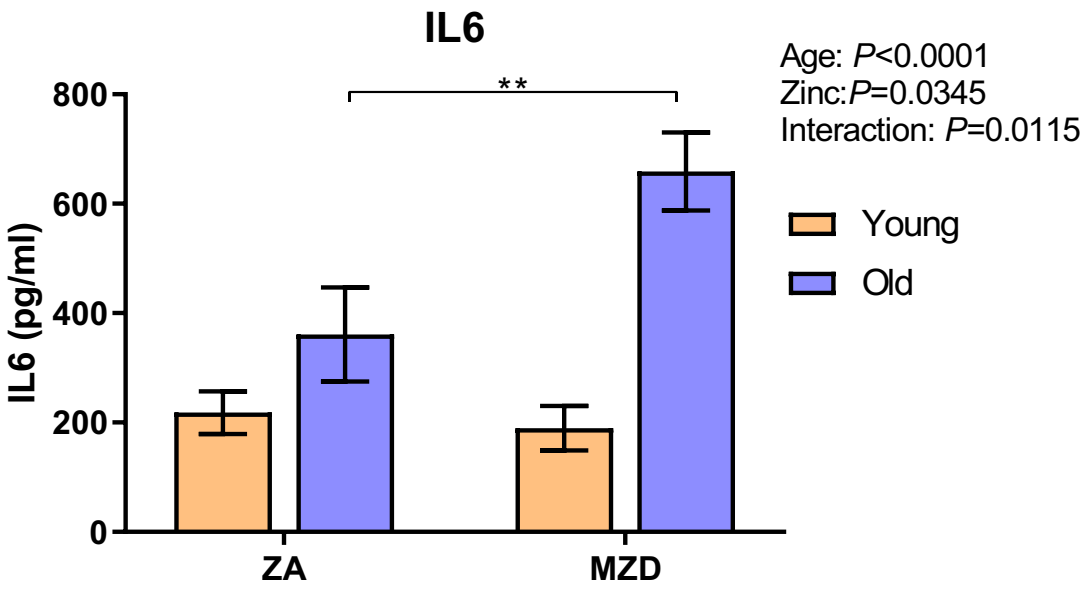

Figure 3. Age-related increase in whole blood IL6 response was further enhanced by marginal zinc deficiency. Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a zinc adequate (ZA) diet or marginal zinc deficient (MZD) diet for 6 wks. Whole blood were collected at the end of study to determine whole blood IL6 response after 24h ex vivo lipopolysaccharide (LPS) stimulation (10 ng/ml). Data represent mean ± SEM (n = 7-10 per treatment group). Two-way ANOVA to test for main effects of zinc status and age and their interaction, followed by Tukey's multiple comparisons test where appropriate. ** $p < 0.01$.

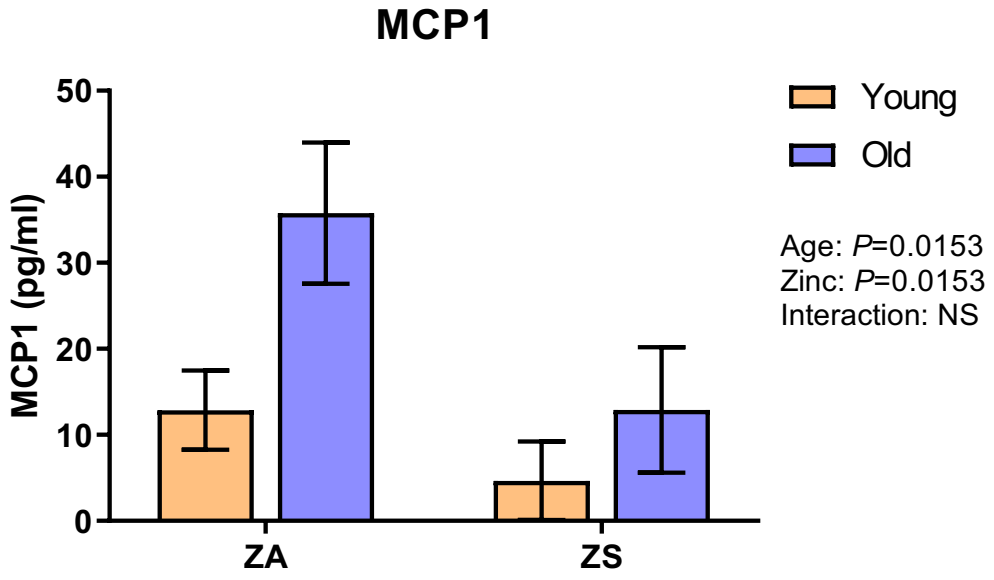

Figure 4. Zinc supplementation reduced MCP1 in whole blood. Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a zinc adequate (ZA) diet or zinc supplemented (ZS) diet for 6 wks. Whole blood were collected at the end of study to determine MCP1 response in unstimulated whole blood. Data represent mean ± SEM (n = 7-10 per treatment group). Two-way ANOVA to test for main effects of zinc status and age and their interaction. NS = not significant.

3.3 Inflammatory markers s100a8, s100a9, IL1β, and MCP1 in the liver change with age

The liver plays an essential role in the body's innate immune response, housing a variety of lymphocytes and screening incoming pathogens from the gastrointestinal tract. Additionally, mice models have exemplified the liver as a tissue with heightened levels of inflammatory cytokines and inflammatory genes. High levels of inflammation suggest the body is potentially responding to inflammation, making the liver an organ of interest. The effects of changing zinc status on the liver's immune response is relatively unknown, and due to the observed effects of dietary zinc on serum inflammatory markers in young and old mice, we decided to investigate the effects of zinc supplementation and deficiency on the expression of gene associated with

inflammation in the liver. In particular, we examined the inflammation-related genes IL1β, MCP1, s100a8, and s100a9.

We found that in liver tissue, there is an age effect resulting in the increased expression of multiple inflammatory genes, including s100a8, s100a9, IL1β, and MCP1. In the study where mice were fed either a zinc adequate or zinc supplemented diet, old mice consistently displayed statistically significant higher levels of s100a8, s100a9, and MCP1 (Figure 5). While the levels of IL1β were not statistically significant, we observed a trend (near significance, $p < 0.08$) that is in agreement with the significant age effect observed in the aforementioned genes. Zinc supplementation did not alter the expression of proinflammatory genes we tested. We observed the same effect in the study where mice were fed either a marginal zinc deficient diet or zinc adequate diet; old mice consistently displayed statistically significant higher levels of s100a8, s100a9, and MCP1 and IL1β followed the trend (Figure 6). Marginal zinc deficiency did not result in further increased in proinflammatory genes expression.

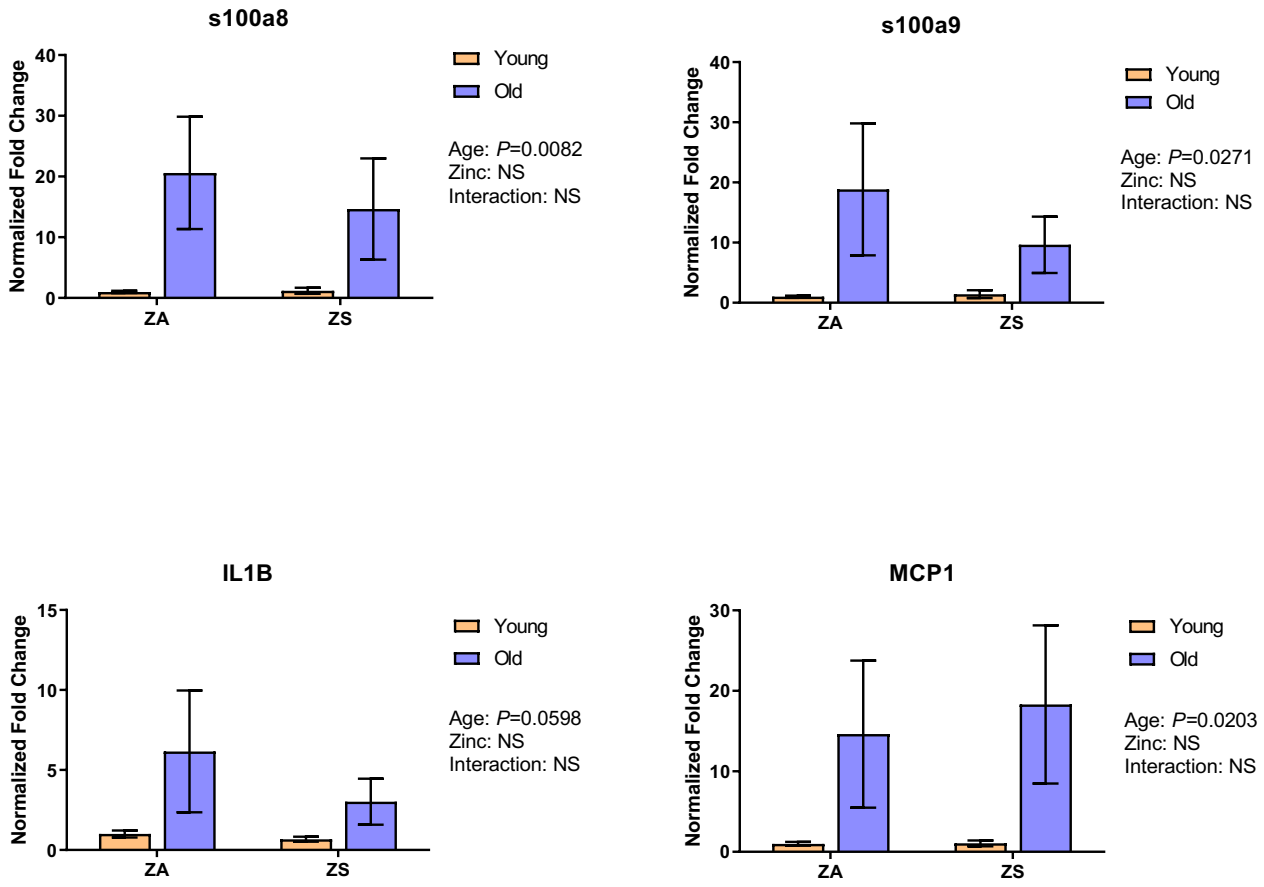

Figure 5. Inflammatory markers in the liver increase with age and were not affected by zinc supplementation. Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a zinc adequate (ZA) diet or zinc supplemented (ZS) diet for 6 wks. Liver tissue was collected from mice after 6 weeks and RNA was isolated from liver to determine inflammation status. Two-way ANOVA to test for main effects of zinc status and age and their interaction. Significance is determined by p value < 0.05.

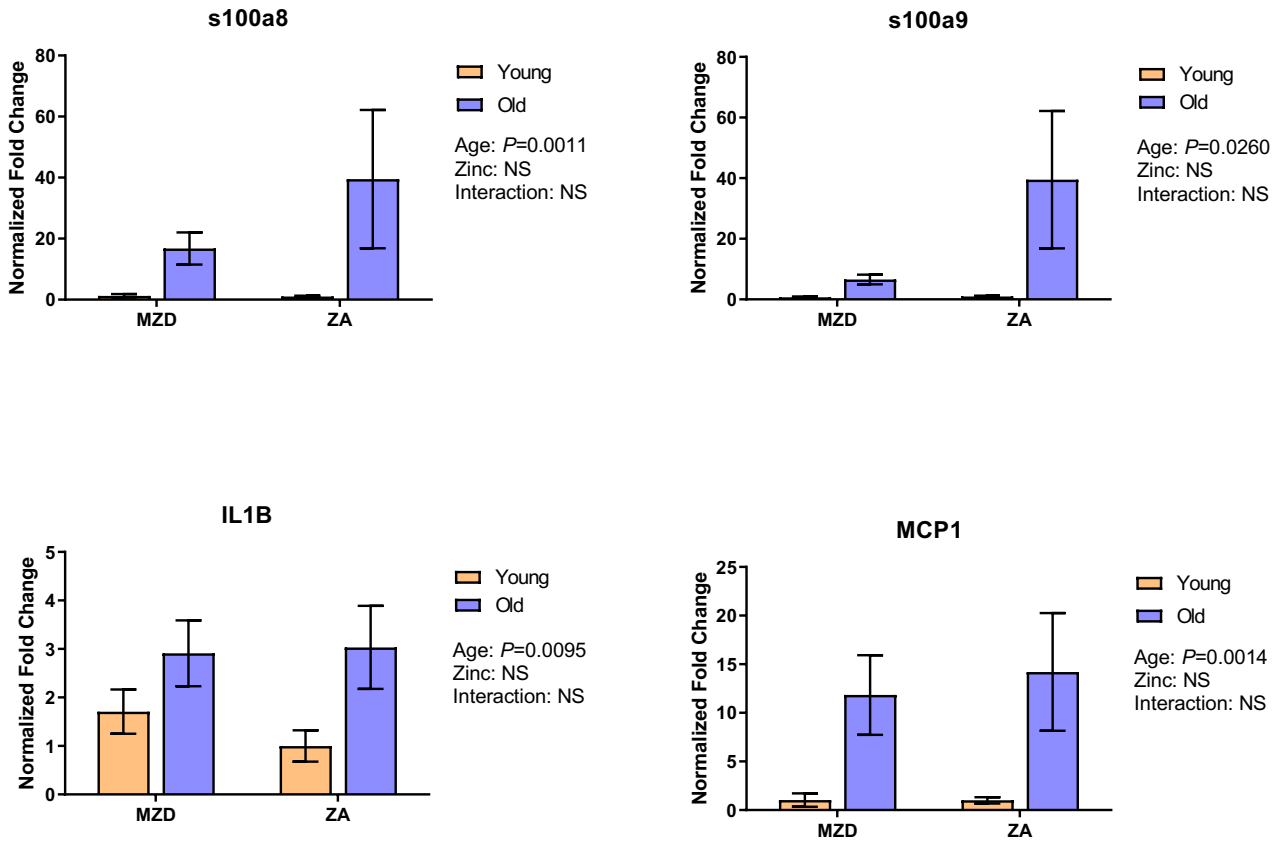

Figure 6. Inflammatory markers in the liver increase with age and were not affected by marginal zinc deficiency. Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a moderate zinc deficient (MZD) diet or zinc adequate (ZA) diet for 6 wks. Liver tissue was collected from mice after 6 weeks and RNA was isolated from liver to determine inflammation status. Two-way ANOVA to test for main effects of zinc status and age and their interaction. Significance is determined by p value < 0.05.

3.4 T cell related markers IFNγ and IL22 in the liver change with zinc intervention

It has been previously been reported that cytokines (soluble mediators of immune response) associated with inflammation are increased with age. We next examined the gene expression of two cytokines that are associated with T cell proinflammatory response (IFNγ and IL22). IFNγ and IL22 are increased in old mice that are fed a zinc adequate diet (Figure 7). Our data shows a promising association that suggests that zinc supplementation may have an effect on T cell response in both young and old mice. Although the data was not statistically significant, we observed that young and old mice express decreased levels of IFNγ and IL22 when fed a zinc supplemented diet compared to those fed a zinc adequate diet (Figure 7). Additionally, we observed that young and old mice express increased levels of IFNγ and IL22 when fed a marginally zinc deficient diet compared to those fed a zinc adequate diet (Figure 8).

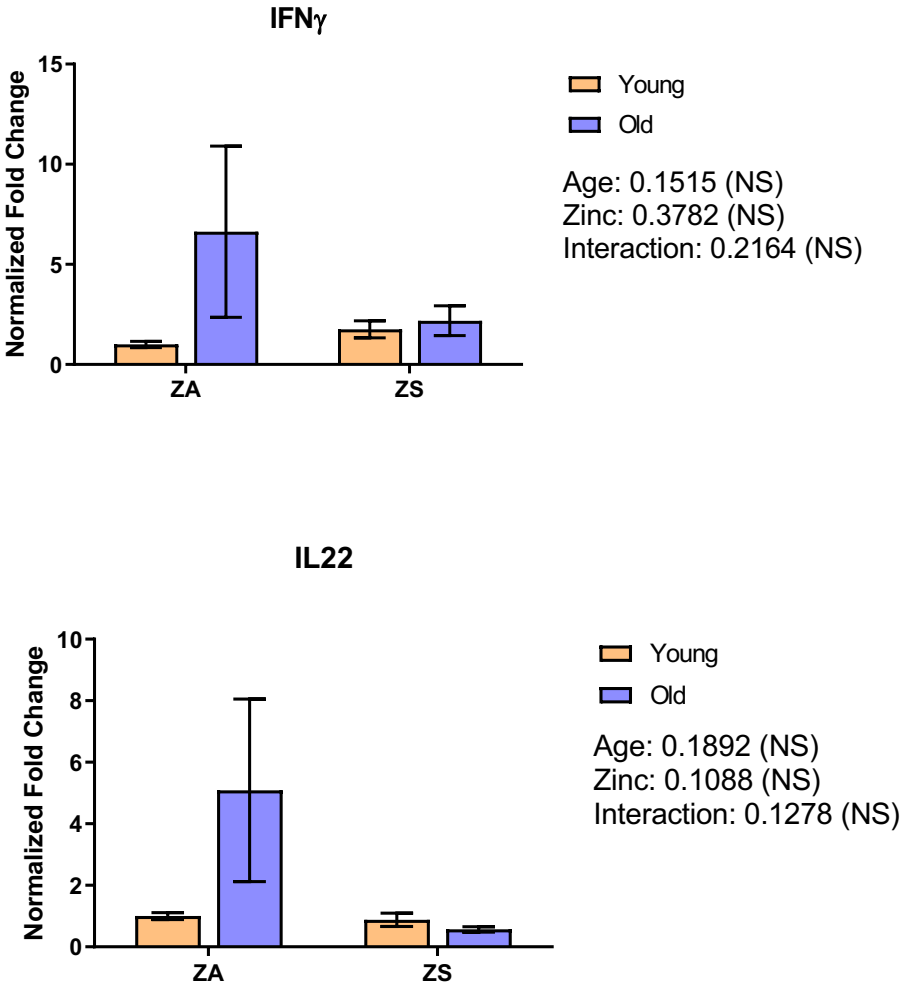

Figure 7. Zinc supplementation is associated with changes in IFNγ and IL22.
Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a zinc adequate (ZA) diet or zinc supplemented (ZS) diet for 6 wks. Liver tissue was collected from mice after 6 weeks and RNA was isolated from liver to determine changes in IFNγ and IL22 gene expression. Two-way ANOVA to test for main effects of zinc status and age and their interaction. Significance is determined by p value < 0.05.

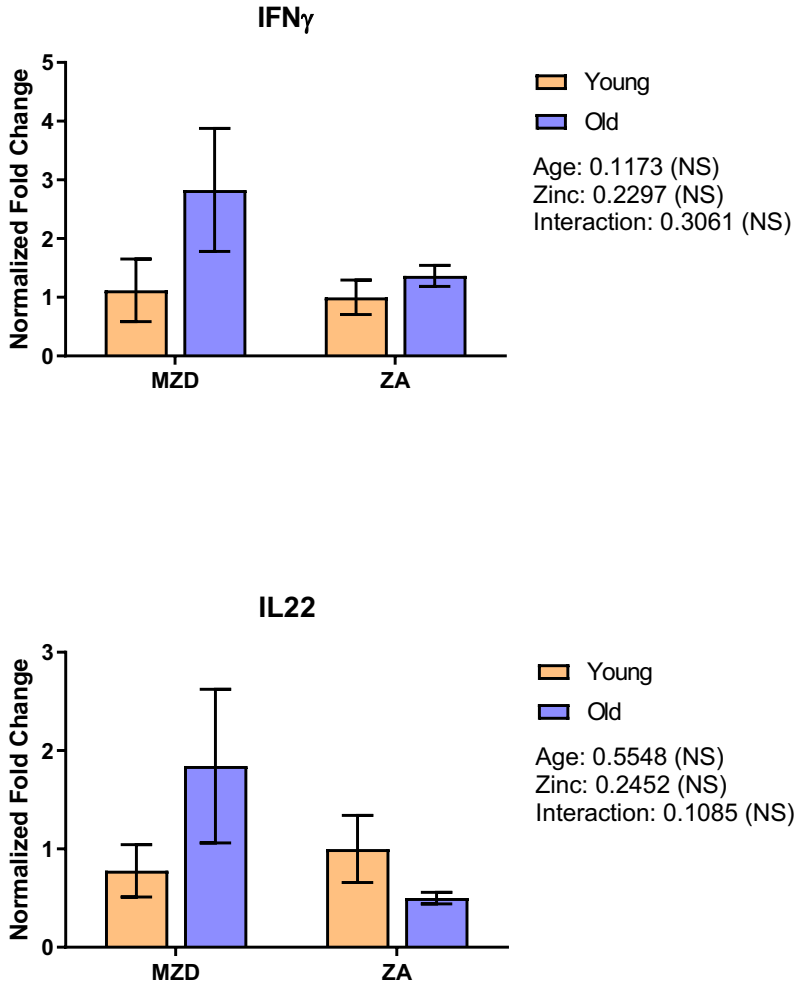

Figure 8. Marginal zinc deficiency is associated with changes in IFNγ and IL22. Young (2 mo) and old (24 mo) C57Bl/6 mice were fed a zinc adequate (ZA) diet or marginally zinc deficient (MZD) diet for 6 wks. Liver tissue was collected from mice after 6 weeks and RNA was isolated from liver to determine changes in IFNγ and IL22 gene expression. Two-way ANOVA to test for main effects of zinc status and age and their interaction. Significance is determined by p value < 0.05.

4 DISCUSSION

In this study, we used quantitative real-time PCR to examine expression of genes related to liver inflammation and immune response in relation to zinc status and age in mice. Based on the

notion that the effects of aging share many commonalities with the effects of zinc deficiency, we investigated the potential impact of dietary zinc supplementation to reduce age-related inflammation. At the same time, we examined whether marginal zinc deficiency further exacerbates age-related inflammation.

Summary of Findings

We were able to show that multiple inflammatory markers in the liver increase with age. We illustrate the fact that regardless of diet, old mice consistently exhibit higher levels of genes related to inflammation including s100a8, s100a9, IL1β, and MCP1. Old mice fed zinc supplemented, zinc adequate, and marginally zinc deficient diets exhibit higher levels of these inflammation related genes compared to their younger counterparts.

While our results did not show a significant interaction between zinc status and age on T cell response, we note an interesting effect of zinc status on two cytokines related to T cell proinflammatory response. Both IFNγ and IL22 changed in relation to zinc supplementation or deficiency. When fed a zinc supplemented diet, old mice expressed decreased levels of IFNγ and IL22 indicating decreased T cell proinflammatory response. Similarly, when fed a marginally zinc deficient diet, old mice expressed increased levels of IFNγ and IL22 compared to those fed a zinc adequate diet indicating that marginal zinc deficiency is related to a heightened T cell proinflammatory response. Although these results were not statistically significant, they provide potentially interesting starting points for follow up studies to further investigate the significance of dietary zinc on immune response and T cell markers.

Future Directions + Final Conclusions

Immunosenescence and chronic inflammation both contribute to an increased risk for diseases related to aging (Fulop et al., 2018). The elderly population largely does not consume enough zinc, on top of the idea that zinc may not be optimally absorbed as we age (Ma & Betts, 2000; Mares-Perlman et al., 1995; Prasad et al., 1993). Because zinc plays an important role in the immune response, adequate zinc nutrition in the aging population is thought to be beneficial and improve the negative effects of immunosenescence. Although this study showed no significant impact with zinc supplementation and marginal zinc deficiency on changes in inflammatory or T cell markers in the liver, it is still evident that with increasing age important inflammatory signals are increased and promising changes in proinflammatory T cell response with zinc supplementation further exemplify the potential effects of improved zinc status on immune response. More work will be needed to allow us to better understand how improving zinc status achieved through nutritional supplementation is beneficial for the aging population.

Chapter 3: References

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