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Nutrition

Imbalance in zinc homeostasis enhances lung Tissue Loss following cigarette smoke exposure

Daren L. Knoell[a,*], Deandra Smith[a], Shengying Bao[b], Muna Sapkota[a], Todd A. Wyatt[c,d], Jay L. Zweier[b], Jennifer Flury[e], Michael T. Borchers[e], Mitch Knutson[f]

[a] The University of Nebraska Medical Center College of Pharmacy, Omaha, NE, 68198, United States
[b] The Ohio State University College of Medicine, Columbus, OH, 43210, United States
[c] The University of Nebraska Medical Center College of Public Health, Omaha, NE, 68198, United States
[d] VA Nebraska-Western Iowa Health Care System, Omaha, NE, 68105, United States
[e] The University of Cincinnati Department of Internal Medicine, United States
[f] The University of Florida Food Science and Nutrition Institute, United States

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ABSTRACT

Cigarette smoke exposure is a major cause of chronic obstructive pulmonary disease. Cadmium is a leading toxic component of cigarette smoke. Cadmium and zinc are highly related metals. Whereas, zinc is an essential metal required for normal health, cadmium is highly toxic. Zrt- and Irt-like protein 8 (ZIP8) is an avid transporter of both zinc and cadmium into cells and is abundantly expressed in the lung of smokers compared to nonsmokers. Our objective was to determine whether disturbed zinc homeostasis through diet or the zinc transporter ZIP8 increase susceptibility to lung damage following prolonged cigarette smoke exposure.

Methods: Cigarette smoke exposure was evaluated in the lungs of mice subject to insufficient and sufficient zinc intakes, in transgenic ZIP8 overexpressing mice, and a novel myeloid-specific ZIP8 knockout strain.

Results: Moderate depletion of zinc intakes in adult mice resulted in a significant increase in lung cadmium burden and permanent lung tissue loss following prolonged smoke exposure. Overexpression of ZIP8 resulted in increased lung cadmium burden and more extensive lung damage, whereas cigarette smoke exposure in ZIP8 knockout mice resulted in increased lung tissue loss without a change in lung cadmium content, but a decrease in zinc.

Conclusions: Overall, findings were consistent with past human studies. Imbalance in Zn homeostasis increases susceptibility to permanent lung injury following prolonged cigarette smoke exposure. Based on animal studies, both increased and decreased ZIP8 expression enhanced irreversible tissue damage in response to prolonged tobacco smoke exposure. We believe these findings represent an important advancement in our understanding of how imbalance in zinc homeostasis and cadmium exposure via tobacco smoke may increase susceptibility to smoking-induced lung disease.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide [1–5]. COPD is characterized by progressive, irreversible airflow obstruction in response to noxious gases and particles. The major causative factor of COPD in the United States is cigarette smoking (CS) [2,3,5,6]. Cadmium (Cd) is highly concentrated in tobacco and a leading hazardous substance [7,8]. Zinc (Zn) and Cd closely resemble each other. Consequently, both compete for cellular uptake through shared pathways, particularly the Zrt- and Irt-like protein 8 (ZIP8), as well as occupancy on protein binding sites. Whereas Cd is highly toxic to mammals, Zn is essential for normal

Abbreviations: BAL, Broncho-alveolar lavage; BMI, body mass index; Cd, cadmium; COPD, chronic obstructive pulmonary disease; CDC, Centers for Disease Control and Prevention; CS, cigarette smoke; NHANES, National Health and Nutrition Examination Survey; Zn, zinc; ROS, reactive oxygen species

* Corresponding author at: College of Pharmacy, Room 2039 The University of Nebraska Medical Center 986120 Nebraska Medical Center, Omaha, NE, 68198-6120, United States.

E-mail addresses: daren.knoell@unmc.edu (D.L. Knoell), deandra.smith@unmc.edu (D. Smith), Shengying.Bao@osumc.edu (S. Bao), muna.sapkota@unmc.edu (M. Sapkota), twyatt@unmc.edu (T.A. Wyatt), Jay.Zweier@osumc.edu (J.L. Zweier), Borchemt@ucmail.uc.edu (M.T. Borchers), mknutson@ufl.edu (M. Knutson).

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function. Zn also possesses antioxidant-like properties [9,10] whereas Cd catalyzes formation of reactive oxygen species (ROS) [11].

Smokers with low dietary Zn intakes have a higher Cd burden and increased incidence of COPD [12]. How deficient Zn intake causes increased pulmonary dysfunction remains unknown. Importantly, deficient Zn intake is common in COPD patients [13–15]. Further, randomized prospective clinical trials have revealed that oral nutrient supplementation has a beneficial role in COPD subjects [16]. We postulated that prolonged insufficient dietary Zn intake or alteration of zinc transport would accelerate CS-induced lung injury because of the relative loss of benefit from Zn and favoritism toward a Cd-rich environment.

In this investigation, we demonstrate that insufficient Zn intake enhances permanent lung tissue loss in response to chronic CS exposure. Further, ZIP8 overexpression as well as loss of ZIP8 expression resulted in more extensive tissue damage despite a normal Zn diet. These findings support further consideration of interventions with micronutrient-based strategies that overcome Zn deficits and identify a key Zn transporter that is instrumental in maintaining balance within the lung microenvironment through protection against cigarette smoke-induced lung disease.

2. Methods

2.1. Study design

2.1.1. Dietary zinc regulation in mice

All mouse experiments were conducted in accordance with the National Institutes of Health standards and the University of Cincinnati Medical Center and University of Nebraska Medical Center Institutional Animal Care and Use Committees. Mice were exposed to CS and compared against controls that were only exposed to room air. In particular, eight-week old, male, C57BL/6 mice (~25 g)(Jackson Labs, Bar Harbor, ME) were acclimatized to CS exposure and then randomly assigned in groups of a minimum of 6 and placed on one of two diets: a moderate zinc-deficient diet (MZ)(TD85421; 5 ppm) or a matched-control normal zinc diet (NZ)(TD85420; 50 ppm) and maintained on respective diets throughout the entirety of the exposure period (up to 28 weeks) for room air or CS exposures. Modification of Zn intakes was carefully considered before utilizing a moderate deficient Zn chow. Moderate deficiency, akin to the deficiency generated using the MZ diet, is prevalent within the COPD population. Mice were not pair-fed when maintained on the MZ diet since COPD patients are typically malnourished. A Zn-free environment was maintained using deionized water in Zn-free containers and stainless steel cages, along with routine cage changes. Animal weights, appearance, and activity levels were recorded weekly throughout all studies.

2.1.2. Cigarette smoke exposure models

Mice were exposed to either room air or CS, generated from 3R4 F Kentucky Reference Cigarettes (University of Kentucky) using a TE-10z smoking machine (Teague Enterprises, Woodland, CA) as described previously by our group [17]. Whole body exposure occurred at a concentration of 150 ± 15 mg/m^3 total suspended particulates for 4 h a day, 5 days a week. Control mice were exposed to HEPA-filtered room air. The transgenic ZIP8 overexpressing mouse model, herein referred to as Zip8tg, was also exposed to either room air or CS for a duration of up to seven months. The Zip8tg line possesses three additional copies of the 129/SvJ *Slc39a8* gene inserted into the C57BL/6 J genome as previously reported [18]. The extra Zip8 copies retain transcriptional control under the native promoter region and are abundantly expressed within the lung [19]. These animals have an otherwise normal phenotype. Zip8tg mice were a gift of Dr. Daniel Nebert. Conditional *Zip8* knockout mice; herein referred to as *Zip8KO*, were generated by first utilizing conditional-ready $Zip8^{flox/flox}$ mice from $Zip8^{flox-neo/+}$ mice (C57BL/6NTac-Slc39a8tm1a(EUCOMM)Wtsi/Cnrm) obtained from the European Mouse Mutant Archive. Briefly, $Zip8^{flox-neo/+}$ mice harbor two loxP sites that flank exon 3 of the *Zip8* (*Slc39a8*) gene. However, there is an additional loxP site upstream of exon 3 flanked by two FRT sites that encompass a neomycin gene. The FRT sites allow for specific removal of the neomycin gene (used as a selectable marker). Heterozygous $Zip8^{flox-neo/+}$ mice were bred to ROSA26:FLPe knock-in mice with ubiquitous expression of FLP1 recombinase (129S4/SvJaeSor- Gt (ROSA)26Sortm1(FLP1) Dym/J;The Jackson Laboratory) to delete the Neo cassette adjacent to the upstream loxP site. The resulting $Zip8^{flox/+}$ were mated to produce $Zip8^{flox/flox}$ mice. PCR and DNA sequencing confirmed removal of the FRT-flanked sequence and verified the loxP sites flanking exon 3. $ZIP8^{flox/flox}$ mice were crossed to myeloid cell specific LysMcre (The Jackson Laboratory) [20] to generate the conditional *ZIP8KO*.

2.1.3. Bronchoalveolar Lavage, leukocyte enumeration, and cytokine analysis

Lungs were lavaged three times with 1 ml of 1× Hank's balanced salt solution. Total cell counts were determined with a hemocytometer and differential counts were determined on Hemacolor-stained (EM Science, Gibbstown, NJ) slides. Cytokine levels were measured by ELISA according to manufacturer's instructions (eBioscience, San Diego, CA).

2.1.4. Mean linear intercept

Mean linear intercept (MLI), also referred to as chord length, is a measure of alveolar diameter and was determined on formalin-fixed, paraffin-embedded, H&E-stained mouse lung tissue as our group previously reported [17]. Sections (3 per lung) were blinded to two reviewers and measurements were averaged.

2.1.5. Cadmium and zinc measures

Cd and Zn were measured at baseline and then at the conclusion of each exposure period in lung and kidney tissue by either Atomic Adsorption or ICP-MS as previously published by our group [21].

2.1.6. Measurement of radical species

Lung samples were mixed with the cell-permeable cyclic hydroxylamine spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine to measure the formation of free radical oxygen species (CMH, Enzo Life Sciences Inc.) as previously reported [22]. ROS released by lung tissue reacts with CMH to form a stable nitroxide radical that can be measured using electron spin resonance (ESR) and expressed as nanomole per milligram of protein sample.

2.1.7. RNA extraction and quantitative RT-PCR

Total RNA was isolated from tissue using TRIZOL® reagent (Invitrogen). The cDNA synthesis was performed using ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis (Invitrogen). Real-time PCR was performed with the 7900 H T Real-Time PCR system (Applied Biosystems) using SYBR® Green reagents. All analysis was normalized against the cycle threshold number of GAPDH or cyclophilin genes, then calculated using the following equation: $RCN = 2^{-\Delta Ct} \times 100$, where ΔCt is the $Ct_{(target)} - Ct_{(reference)}$. The sequences of all the probes are available upon request.

2.2. Statistical methods

All animal data are presented as SD. For comparison between multiple groups, a one-way with post-hoc test, such as Tukey's or Bonferroni test, was used. A Student's *t*-test was used for comparison between two groups. Statistical significance was defined at a p-value of less than 0.05 ($p < 0.05$) or greater as designated in figure legends.

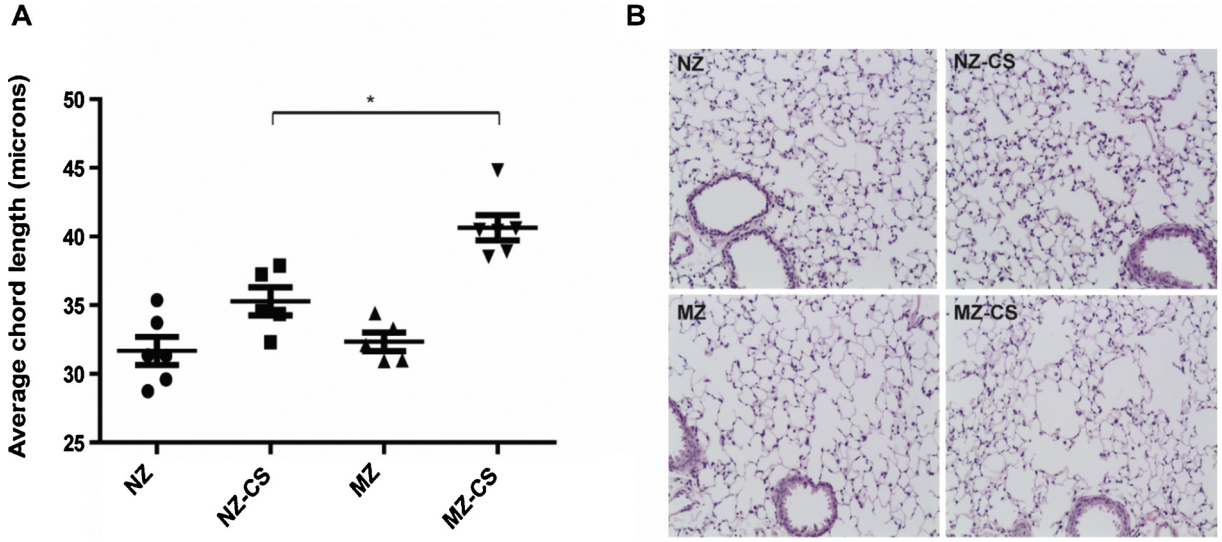

Fig. 1. (A) The average chord length measured across alveolar tissue obtained from adult mice that were maintained on a normal zinc containing (NZ) or moderately restricted zinc containing (MZ) diet was compared. Groups of mice on these diets were either exposed to room air or cigarette smoke (CS) for a period of four months. Data are representative of two independent experiments. **(B)** Representative photomicrographs of H&E stained lung sections obtained from each treatment group. Suboptimal zinc intake over a period of 4 months increased alveolar lung tissue loss in mice exposed to cigarette smoke. (Data shown are representative from two separate experiments with a minimum of six animals per treatment group. Bar graphs depict SD with error bars. Significance values for indicated comparisons by one-way ANOVA and Tukey's post test; * designates $p < 0.05$).

3. Results

3.1. Smoke exposure increases lung tissue loss in zinc deficient mice

C57BL/6 mice were maintained on either a normal Zn (NZ) or moderately deficient Zn diet (MZ) for up to four months. During this time, subgroups (a minimum of six mice in each group) were established within the dietary cohorts that were exposed daily to room air or cigarette smoke (CS). Mice, regardless of treatment condition, did not exhibit significant differences in appearance, activity, or weight gain. Most striking, mice with insufficient Zn intakes had a substantial increase in alveolar tissue loss following prolonged CS exposure compared to NZ-smoke-exposed mice (Fig. 1A,B). There was no evidence of lung tissue loss in either the MZ or NZ dietary cohorts exposed to room air.

As expected, mice exposed to CS had a significant increase in lung Cd content compared to mice exposed to room air (Fig. 2A). Further, mice that were maintained on the MZ diet had a significant increase in lung Cd content compared to CS-exposed mice maintained on a normal Zn diet. Corresponding measurement of Zn lung content revealed no major differences (Fig. 2B). Corresponding assessment of kidney metal content revealed as expected [23] significant accumulation of Cd in the kidney in all treatment groups but without significant differences, although CS exposed animals did have a moderate further elevation (Fig.2C). Zn content was significantly decreased in both MZ exposure groups consistent with a achieving a systemic Zn deficient state (Fig. 2D).

3.2. Evaluation of inflammation profiles in the lung

Having observed a significant increase in alveolar lung tissue loss in Zn restricted, CS-exposed mice, we examined cytokine, chemokine, cell content and ROS formation in bronchoalveolar lavage (BAL) or lung tissue. There were no major differences observed across all treatment groups in BAL IL-1β, CXCL1 or protein levels regardless of diet or exposure condition (Fig. 3A,B,C). Histologic enumeration of BAL cells revealed a significant increase in total cell number in both CS exposure groups (Fig. 3D) with the highest number of inflammatory cells, primarily macrophages and monocytes, in the MZ−CS treatment group (Fig. 3E). Knowing that Zn has antioxidant-like properties, we also measured the abundance of ROS in lung tissue. Insufficient Zn intake combined with CS exposure increased free radical formation in lung tissue that was higher than levels observed in CS-exposed mice maintained on Zn sufficient diets (Fig. 3F).

3.3. Evaluation of cigarette smoke exposure in ZIP8 overexpressing and ZIP8 knockout mice

The SLC39A8 gene codes for the zinc transporter protein ZIP8, a cellular importer of Zn and Cd [24]. We exposed Zip8tg mice that possess three extra copies of Zip8 [25] to either room air or CS out to seven months while being maintained on a normal Zn diet. Most notably, CS exposed Zip8tg mice exhibited a significant, dose responsive increase in lung Cd content out to seven months (Fig. 4A) and the extent of Cd accumulation was significantly greater in Zip8tg mice compared to WT CS-exposed mice (Fig. 4B). There were no significant differences in lung Zn content between treatment groups although Zip8tg did tend to have higher Zn levels (Fig. 4C). Consistent with previous observations, a significant increase in alveolar tissue loss was observed in CS-exposed Zip8tg mice compared to WT, CS-exposed mice (Fig. 4D). Further, Zip8tg mice did exhibit increased ROS lung content following CS exposure (Fig. 4E), but the extent of ROS formation was no different from WT, CS-exposed counterparts (Fig. 4E). CS exposure also resulted in Cd accumulation in the kidney although the amount of Cd was no different between WT and Zip8tg mice; however, we did observe an increase in Cd levels in Zip8tg mice exposed to room air compared to room air exposed WT mice (Fig. 4F). Knowing that other Cd transporters may contribute to lung Cd accumulation, we inspected lung tissue mRNA levels for other established transporters following prolonged CS exposure. Only ZIP8 mRNA levels were significantly higher in the lung of Zip8tg mice compared to wild type controls, whereas the expression of Nramp2/1 (DMT) and Zip14 were no different between treatment groups (data not shown).

Prior attempts to generate a Zip8 knockout mouse model failed due to embryo-lethality. Knowing this along with the instrumental role that macrophages play in COPD, we developed a myeloid-specific, Zip8 knockout mouse model. Importantly, this line maintains a normal phenotype during development and adulthood similar to WT

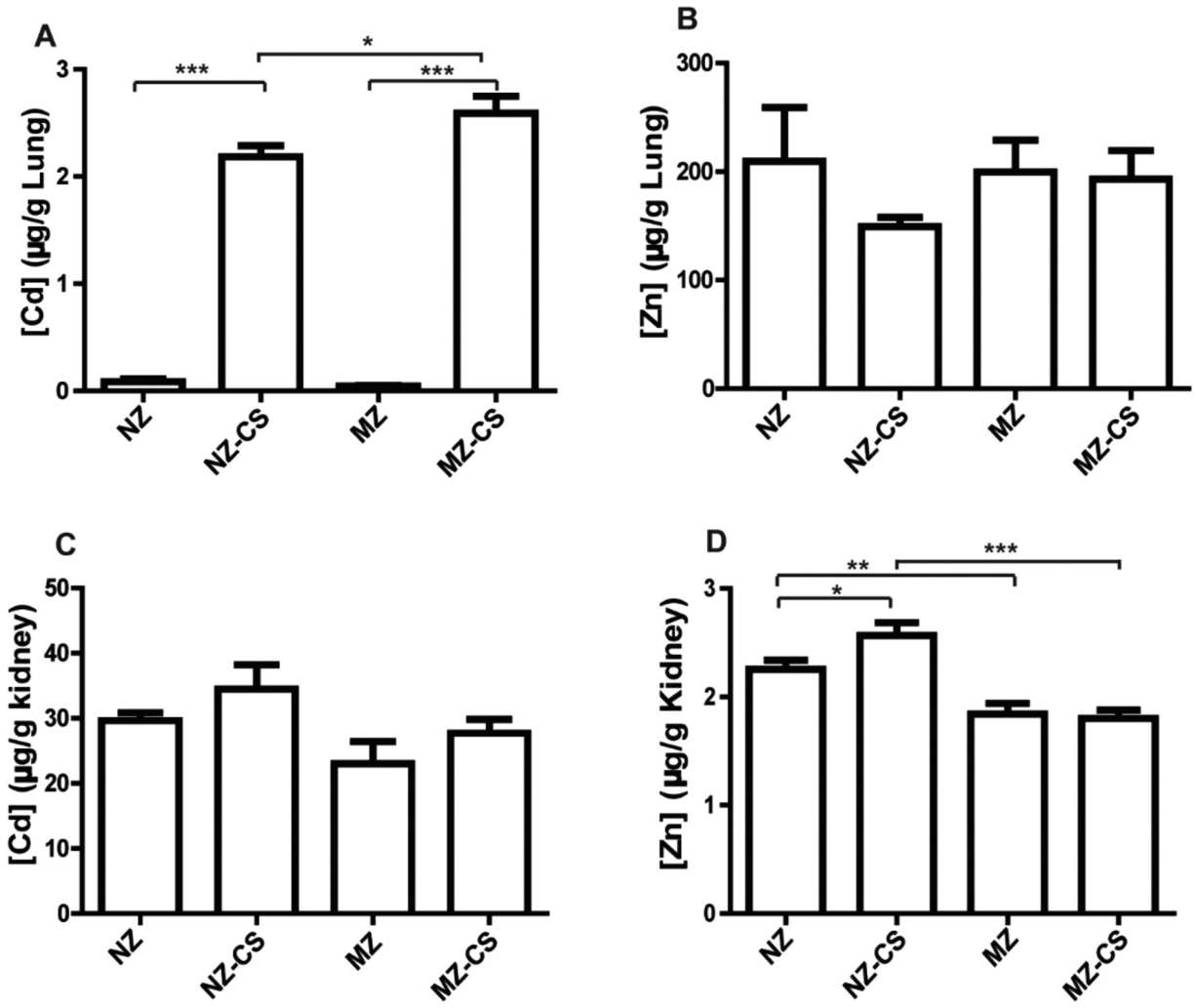

Fig. 2. (A) Cadmium (Cd) and **(B)** zinc (Zn) content were measured in the lungs obtained from adult mice that were maintained on a normal zinc containing (NZ) or moderately restricted zinc containing (MZ) diet. Groups of mice on these diets were either exposed to room air or cigarette smoke (CS) for a period of four months. CS exposure resulted in increased Cd content in the lung which was further significantly increased in mice maintained on a moderately restricted zinc diet **(A)**. Lung zinc content was not significantly different between groups **(B)**; however, zinc content in the kidney was significantly reduced in the MZ dietary group compared to mice maintained on a normal zinc (NZ) containing diet **(D)**. (Data are a combination of two independent experiments, each with a minimum of six mice per treatment group. Bar graphs depict SD with error bars. Significance values for indicated comparisons by one-way ANOVA and Tukey's post test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

counterparts. Using this novel model, we again exposed control WT and Zip8 knockout mice to either room air or CS for a four-month period while maintaining normal, Zn sufficient intake. Consistent with previous findings, loss of ZIP8 expression, in this case within myeloid-lineage cells, resulted in significantly more lung tissue destruction when compared to WT, CS-exposed counterparts (Fig. 5A,B). Further examination of lung tissue revealed that myeloid-specific Zip8KO mice had a significant decrease in lung Zn content compared to WT counterparts regardless of room air or CS exposure with a similar but insignificant trend observed in the kidney (Fig. 6A,C). Lung and kidney Cd content did not differ significantly between any of the treatment groups (Fig. 6B,D). Zip8 KO mice that were exposed to CS exhibited a significant increase in the total number of BAL cell counts and neutrophils (Fig. 7A,B) and with a corresponding significant increase in the neutrophil chemoattractant IL-23, when compared to WT counterparts (Fig. 7C).

4. Discussion and conclusion

Higher Zn intake reduces Cd burden in smokers [26]. Analysis of the NHANES III database revealed that adult smokers with low dietary Zn intakes have a higher Cd burden and increased risk of developing COPD [12]. Cross-sectional analysis of human data prohibits the establishment of a cause-and-effect relationship between Zn and Cd. Accordingly, for the first time, we evaluated three distinct animal models whereby Zn intake was restricted or Zn transport was modified during chronic CS exposure. Consistent with human studies, mice maintained on insufficient Zn intakes exhibited a substantial increase in alveolar tissue loss. Further, a lack of Zn intake was associated with higher Cd and ROS lung burden despite the capacity of mice to maintain normal serum Zn levels (not shown), also consistent with human findings.

Our findings generated in animals maintained on suboptimal Zn intakes raises the question whether Zn supplementation would be of benefit to prevent irreversible lung damage. Pulmonary rehabilitation, a cornerstone of COPD care, includes nutrition counseling [27]. Malnourishment is a common problem in individuals with COPD and accelerates pulmonary dysfunction [28]. A meta-analysis of randomized controlled trials demonstrated a positive role for oral nutritional supplementation in malnourished COPD subjects but also recognized multiple study limitations [16]. Further, Zn deficiency often occurs in tandem with other nutrient deficiencies making it difficult to determine whether one or a combination of nutrients would be required to counter

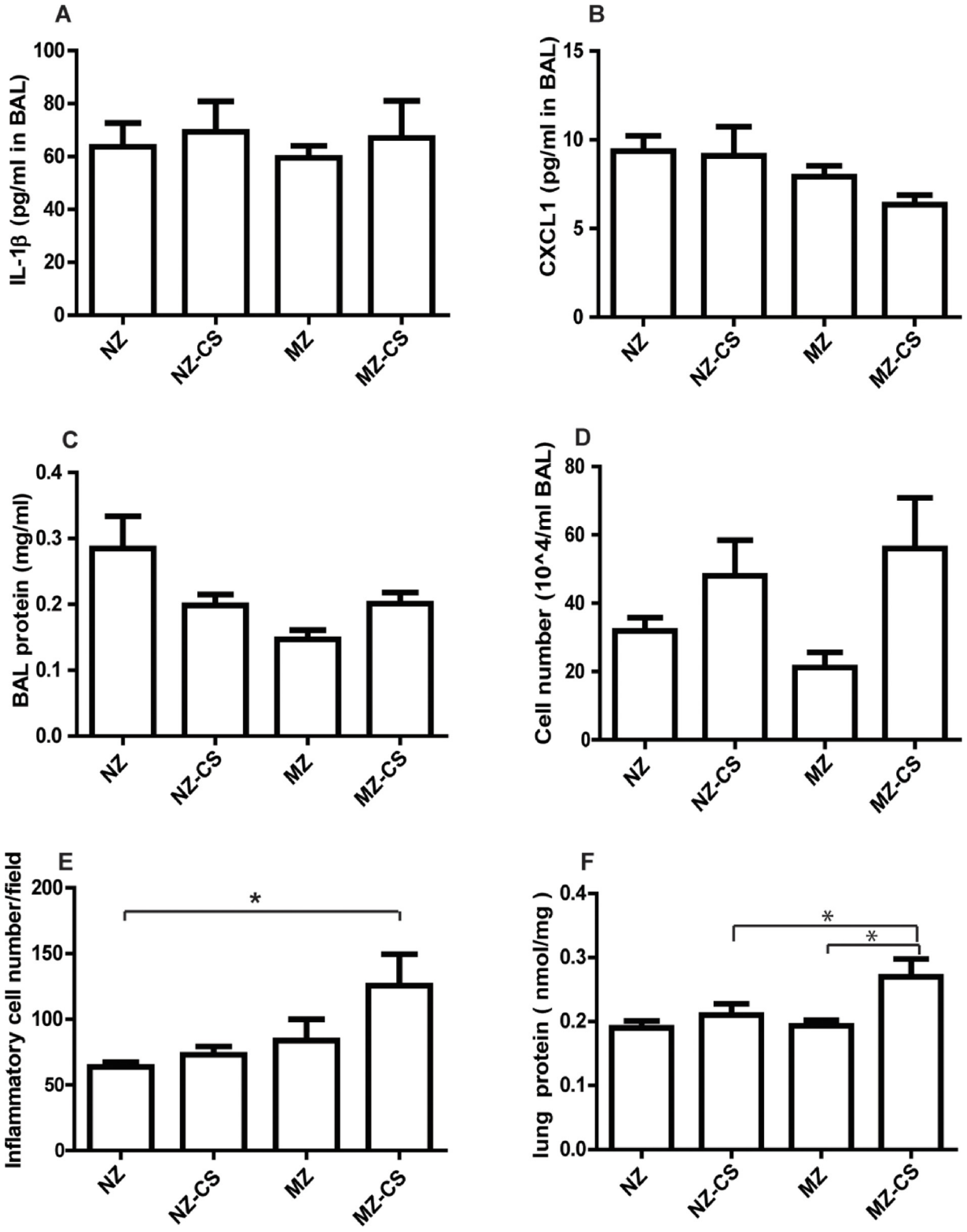

Fig. 3. Inspection of BAL samples revealed no difference in **(A)** cytokine (IL-1β) **(B)** chemokine (CXCL1), or **(C)** protein content. In contrast, smoke exposure (CS) resulted in increased number of cells present in BAL fluid **(D)** and when inflammatory cell types were enumerated, revealed the largest increase in MZ mice exposed to CS **(E)** although this did not achieve statistical significant between the NZ and MZ CS-exposed treatment groups. CS exposure also increased the amount of free radicals in lung tissue compared to room air exposed mice and prolonged insufficient Zn intake resulted in significantly more free radical formation **(F)** (Data shown are representative from two separate experiments with a minimum of six animals per treatment group. Bar graphs depict SD with error bars. Significance values for indicated comparisons by one-way ANOVA and Tukey's post test; * designates $p < 0.05$).

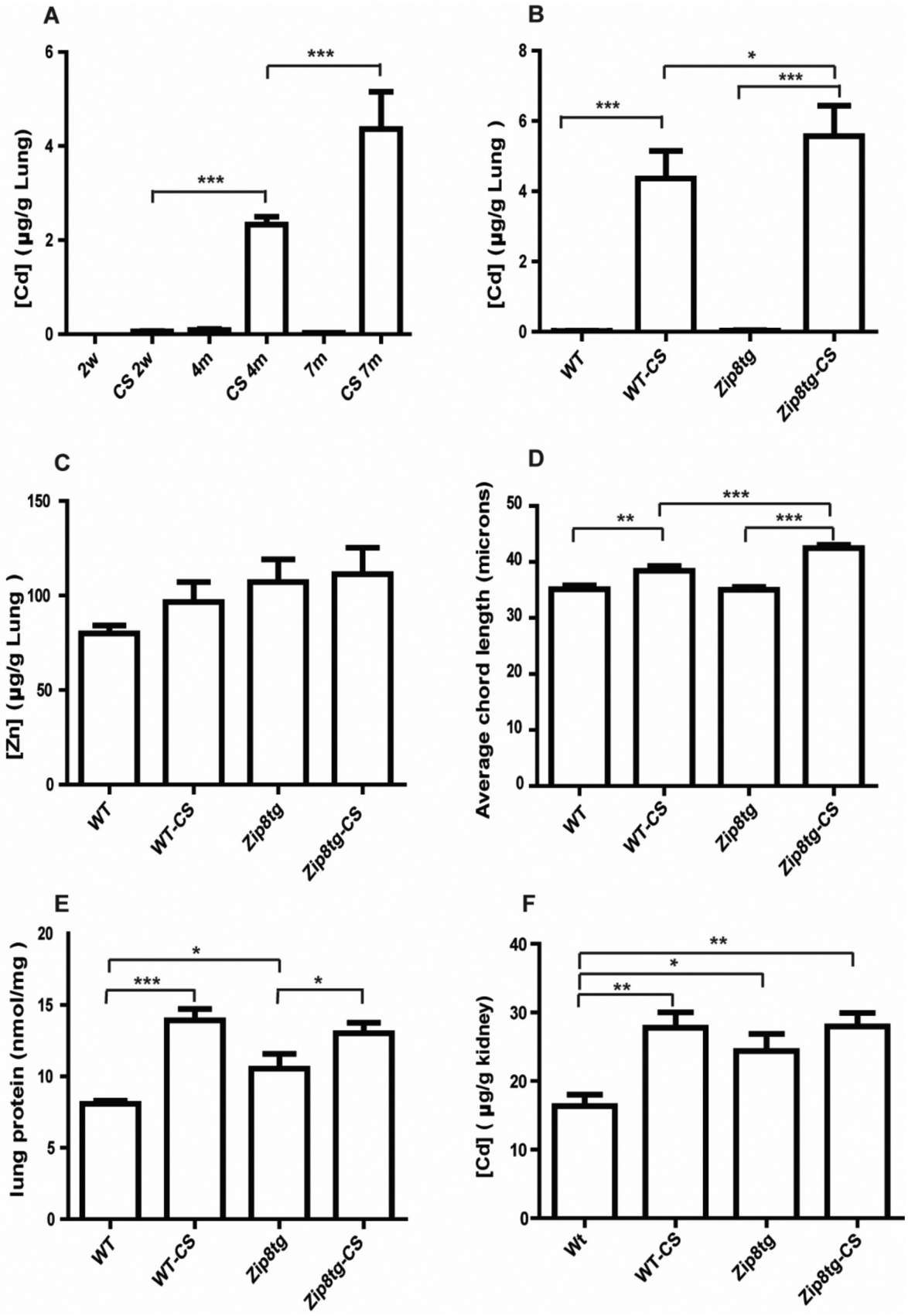

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Fig. 4. Transgenic ZIP8 overexpressing (Zip8tg) mice were exposed to CS daily for a period of 7 months. A time dependent increase in lung Cd content occurred over 7 months **(A).** Lung Cd deposition was significantly increased in Zip8tg compared to WT mice at 7 months **(B),** with no difference in lung Zn between the two groups **(C).** Alveolar loss at 7 months was increased in Zip8tg mice compared to WT, CS-exposed counterparts **(D)**. **(B)** Both WT and Zip8tg animals exhibited increased lung ROS content and increased kidney Cd content **(F)**. (Data shown are representative from two separate experiments with a minimum of six animals per treatment group. Bar graphs depict SD with error bars. Significance values for indicated comparisons by one-way ANOVA and Tukey's post test; * designates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

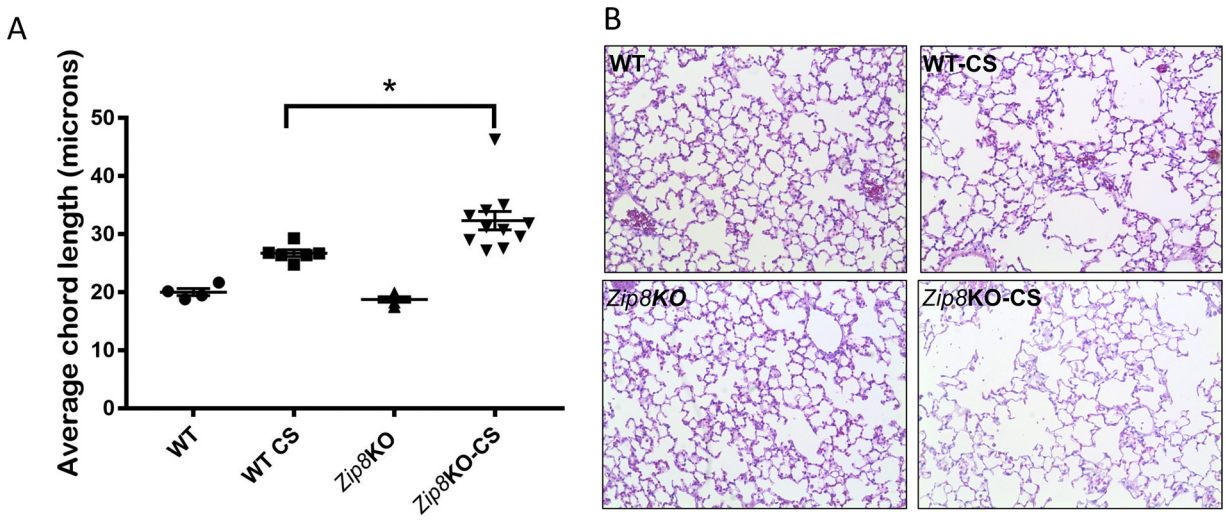

Fig. 5. (A) Comparison of average chord length measured across alveolar tissue obtained from Zip8KO mice or corresponding WT littermate controls. Groups of mice were either exposed to control room air or cigarette smoke (CS) for a period of four months. **(B)** Representative photomicrographs of H&E stained lung sections obtained from each treatment group. Ablation of ZIP8 expression resulted in increased alveolar tissue loss following 4 months of CS exposure over 4 months when compared to WT, CS-exposed mice. (Data shown are a combination from two separate experiments with a minimum of twelve animals per treatment group. Bar graphs depict SD with error bars. Significance values for indicated comparisons by one-way ANOVA and Tukey's post test; * designates $p < 0.05$).

the detrimental impact of prolonged tobacco smoke exposure. Additional investigation will be required in experimental models to first determine whether preventive supplementation is beneficial in the setting of moderately deprived Zn intake.

Cd is a "top ten" environmental toxicant. Each cigarette contains approximately 1–2 micrograms of Cd that is transferred to the lung following CS inhalation [29]. Smokers carry Cd burdens that are up to three times higher than nonsmokers. Relative to the greater than 7000 components found in cigarette smoke, Cd is distinct with a biologic [29] half-life in humans of 15–20 years [7,8]. Occupational exposure to high Cd concentrations can accelerate emphysema [30] and epidemiologic evidence has shown a significant association between urinary cadmium levels and air-flow obstruction among current and former smokers [31]. Cadmium causes direct damage to lung cells affecting DNA repair and cellular membrane integrity [32], increases oxidant stress, alters inflammatory pathways [33,34], and damages parenchymal cells [24]. We predict that Cd uptake and COPD susceptibility are inextricably linked through Zn-related trafficking pathways that contribute to the development of lung disease. Accordingly, a more precise understanding of toxicity relative to Cd exposure and alteration of Zn intake and transport may help guide risk assessment and prevention strategies particularly in higher risk, undernourished populations and genetically predisposed individuals. In particular, we observed that overexpression of ZIP8 resulted in a net increase in lung Cd content and more tissue loss whereas loss of ZIP8 expression caused a Zn deficit in the lung that was also associated with more alveolar destruction. Taken together, we believe these findings suggest that perturbations in Zn as well as Cd transport into cells within the lung as a consequence of genetic polymorphic variation may also warrant further investigation as a potential risk factor for the development of COPD in chronic smokers in addition to dietary Zn intake. We also recognize that a limitation of our approach is that it does not allow for valid comparison of tissue Cd and Zn content since the three models are all distinct from one another.

Oxidative stress induced by the formation of ROS following CS exposure plays a central role in the pathophysiology of COPD [35,36]. Given the known antioxidant properties of Zn, we examined lung tissue of Zn deficient mice for ROS formation. We observed that CS-exposed mice maintained on normal diets had increased ROS species compared to room air-exposed mice. Most striking, ROS formation in the lung increased as a result of prolonged insufficient Zn intake. Increased ROS formation occurred in tandem with a moderate increase in Cd accumulation, a known oxidant-inducing agent. Somewhat surprising, we did not observe a further increase in ROS formation in the lung of mice that overexpressed ZIP8 despite a significant increase in Cd accumulation with increased lung tissue loss. Taken together, we believe that ROS formation contributed to permanent lung damage in both models; however, the relative impact of ROS formation may have been more dominant as a consequence of restricted Zn intake when compared to ZIP8 overexpression.

ZIP8 is one of if not the only known transporters with competitive and high affinity for both Zn and Cd uptake. Our group was the first to demonstrate that ZIP8 is abundantly expressed in the lung of smokers and specifically localizes to the apical membrane of polarized lung epithelia in an optimal location for Cd uptake following smoke exposure [24]. Most striking, Zip8tg mice exposed to CS had more Cd accumulation in the lung with a corresponding increase in alveolar tissue loss. Further, Cd accumulation was most prevalent in CS-exposed lungs of Zip8tg mice. As anticipated, Cd accumulated to a higher extent in the kidney of all treatment groups including air exposed groups but at higher levels in CS exposed groups. Importantly, changes occurred despite maintaining mice on a normal zinc diet, although a longer duration of CS exposure (7 months) was required to reveal alveolar tissue loss. Somewhat surprising, we observed that abolition of ZIP8 in myeloid cells, including lung tissue macrophages, resulted in loss of alveolar tissue, but also increased the extent of neutrophil accumulation in the airway. Collectively, these findings demonstrate that Cd and Zn are likely at dynamic interplay in the lung of smokers and compete for uptake in lung tissue, in part through cellular uptake via ZIP8. An

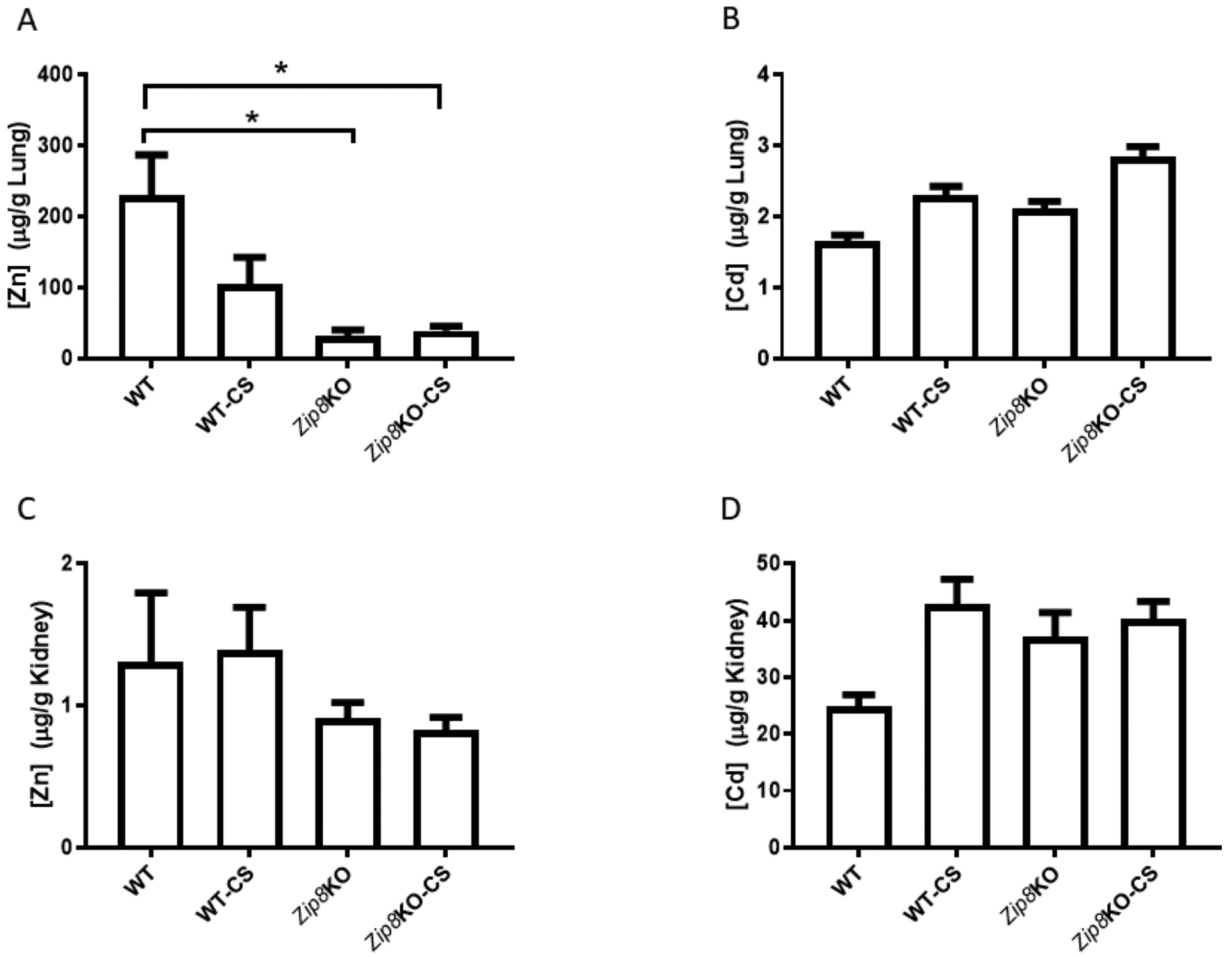

Fig. 6. Zinc (Zn) and cadmium (Cd) content in the lung and kidney of WT and Zip8KO adult mice exposed to cigarette smoke (CS) for four months. Lung Zn content was decreased in Zip8KO mice in all treatment conditions and significantly lower than WT mice **(A)**. A similar trend was observed in kidney tissue **(C),** but did not achieve statistical significance. No significant differences in lung or kidney Cd content were observed between groups **(B,D)**. (Data shown are a combination from two separate experiments with a minimum of twelve animals per treatment group. Bar graphs depict SD with error bars. Significance values for indicated comparisons by one-way ANOVA and Tukey's post test; * designates $p < 0.05$; ** $p < 0.01$).

important difference is that Zn has designated transporters that export Zn whereas Cd-specific exporters, if any exist, remain to be identified [11]. The incapacity of mammals to remove intracellular Cd undoubtedly accounts for its atypically long half-life. This may also explain in part why former smokers continue to exhibit lung inflammation and respiratory dysfunction years after smoking cessation [37]. What remains unresolved is how changes in ZIP8 expression in the lung as a result of overexpression or selective knockout cause alveolar tissue loss in response to chronic CS exposure. Future studies will be required to decipher what will likely be multiple mechanisms by which imbalance in Zn homeostasis in the setting of Cd exposure induce cellular changes that alter the host response in tissue function, ROS formation, chemokine formation and inflammation.

Given the high abundance of Cd in CS, we propose that both dietary Zn intake and genetic variation in proteins that regulate Zn homeostasis are important factors in COPD pathogenesis. While the majority of inter-individual variation in susceptibility to CS-induced lung damage cannot yet be attributed to known factors, we believe that findings from this investigation provide novel information that help us understand why certain smokers are more vulnerable to COPD. This is important not only for understanding disease pathogenesis, but may also guide more intensive micronutrient-based interventions that maximize risk-reduction strategies in addition to smoking cessation.

Ethics approval and consent to participate

Animal studies were approved by the Ohio State University, University of Cincinnati, and University of Nebraska Medical Center IACUCs.

Consent for publication

All authors provide consent to publish this work.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors contributions

Conceived and Designed Research – Knoell, Borchers.

Experimental Work – Smith, Sapkota, Bao, Borchers, Knutson, Wyatt.

Data Analysis and Figure Preparation – Smith, Sapkota, Bao, Knoell.

Statistics – Smith, Bao.

Writing – Knoell.

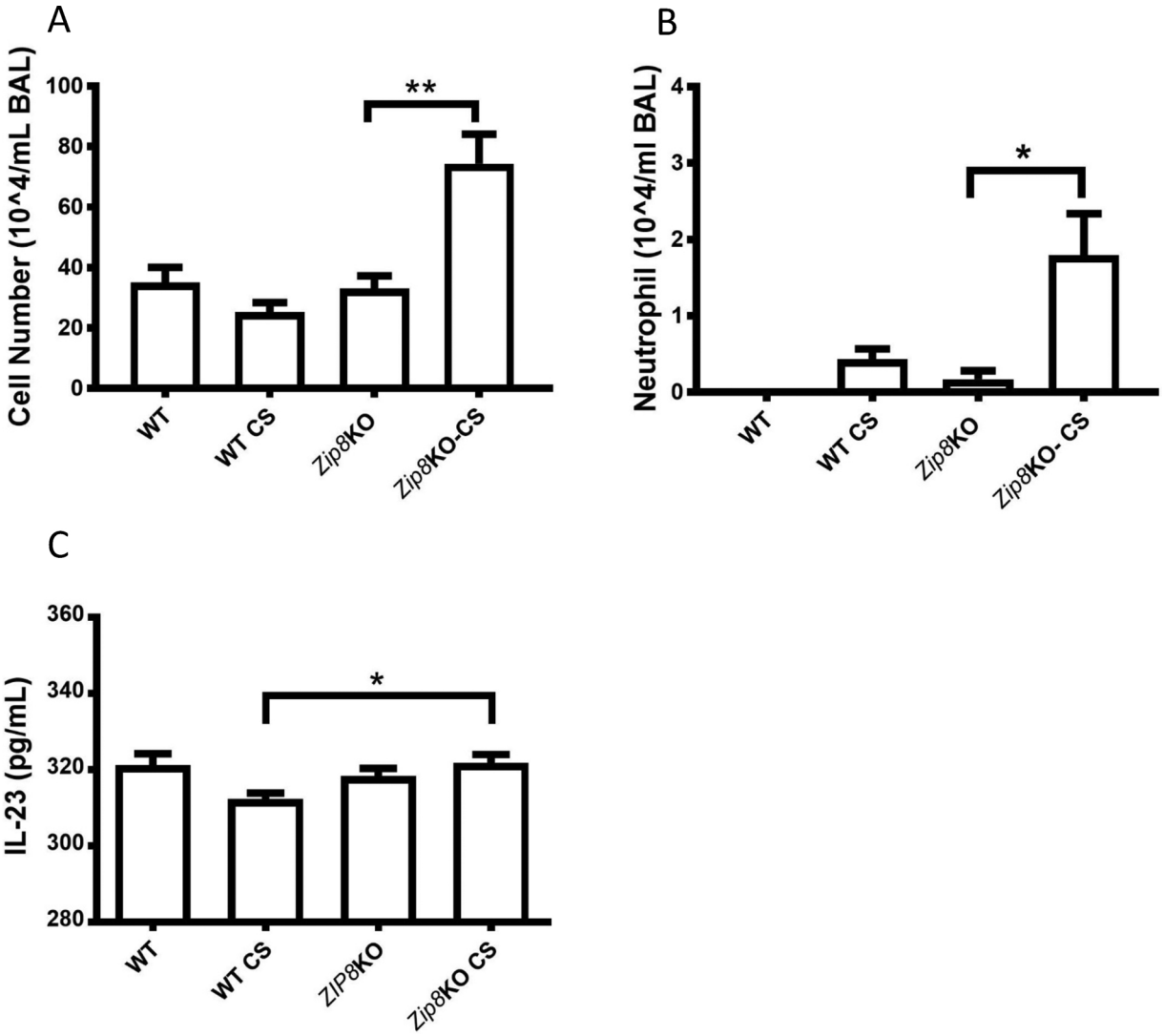

Fig. 7. Inspection of BAL samples revealed a significant increase in total BAL cell counts CS-exposed Zip8KO mice compared to CS-exposed WT mice **(A),** corresponding with an significant increase in neutrophils **(B)**. Analysis of BAL fluid also revealed a significant in increase in IL-23 in CS-exposed Zip8KO mice compared to CS-exposed WT mice. (Data shown are a combination from two separate experiments with a minimum of twelve animals per treatment group. Bar graphs depict SD with error bars. Significance values for indicated comparisons by one-way ANOVA and Tukey's post test; * designates $p < 0.05$).

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Declaration of Competing Interest

The authors declare that they have no conflict of interest. The authors claim no competing financial or nonfinancial interests.

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References

[1] C.J. Murray, A.D. Lopez, Alternative projections of mortality and disability by cause 1990-2020: global Burden of Disease Study, Lancet 349 (1997) 1498–1504.

[2] M.E. Wewers, W.C. Bailey, K.H. Carlsen, M.D. Eisner, P. Folan, J. Heath, M.D. Klinnert, T. Kovesi, G.W. Pien, V.C. Reichart, A. Talwar, K. Thompson, American Thoracic Society Subcommittee on Tobacco Control I. An official American Thoracic Society workshop report: tobacco control initiatives within the American Thoracic Society, Proc. Am. Thorac. Soc. 7 (2010) 1–7.

[3] M.D. Eisner, N. Anthonisen, D. Coultas, N. Kuenzli, R. Perez-Padilla, D. Postma, I. Romieu, E.K. Silverman, J.R. Balmes, Committee on Nonsmoking Copd E, Occupational Health A. An official American Thoracic Society public policy statement: novel risk factors and the global burden of chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 182 (2010) 693–718.

[4] R.A. Pauwels, C.G. Lofdahl, L.A. Laitinen, J.P. Schouten, D.S. Postma, N.B. Pride, S.V. Ohlsson, Long-term treatment with inhaled budesonide in persons with mild chronic obstructive pulmonary disease who continue smoking. European Respiratory Society Study on Chronic Obstructive Pulmonary Disease, N. Engl. J. Med. 340 (1999) 1948–1953.

[5] C.J. Murray, A.D. Lopez, Mortality by cause for eight regions of the world: global Burden of Disease Study, Lancet 349 (1997) 1269–1276.

[6] R.A. Pauwels, A.S. Buist, P.M. Calverley, C.R. Jenkins, S.S. Hurd, G.S. Committee, Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary, Am. J. Respir. Crit. Care Med. 163 (2001) 1256–1276.

[7] S. Satarug, J.R. Baker, S. Urbenjapol, M. Haswell-Elkins, P.E. Reilly, D.J. Williams, M.R. Moore, A global perspective on cadmium pollution and toxicity in non-occupationally exposed population, Toxicol. Lett. 137 (2003) 65–83.

[8] M.P. Waalkes, Cadmium carcinogenesis, Mutat. Res. 533 (2003) 107–120.

[9] W. Maret, H.H. Sandstead, Zinc requirements and the risks and benefits of zinc supplementation, J. Trace Elem. Med. Biol. 20 (2006) 3–18.

[10] A. Krezel, W. Maret, Zinc-buffering capacity of a eukaryotic cell at physiological

pZn, J. Biol. Inorg. Chem. 11 (2006) 1049–1062.
[11] W. Maret, J.M. Moulis, The bioinorganic chemistry of cadmium in the context of its toxicity, Met. Ions Life Sci. 11 (2013) 1–29.
[12] Y.S. Lin, J.L. Caffrey, M.H. Chang, N. Dowling, J.W. Lin, Cigarette smoking, cadmium exposure, and zinc intake on obstructive lung disorder, Respir. Res. 11 (2010) 53.
[13] C.A. Raguso, C. Luthy, Nutritional status in chronic obstructive pulmonary disease: role of hypoxia, Nutrition 27 (2011) 138–143.
[14] R. Herzog, S. Cunningham-Rundles, Immunologic impact of nutrient depletion in chronic obstructive pulmonary disease, Curr. Drug Targets 12 (2011) 489–500.
[15] F. Karadag, O. Cildag, M. Altinisik, L.D. Kozaci, G. Kiter, C. Altun, Trace elements as a component of oxidative stress in COPD, Respirology 9 (2004) 33–37.
[16] P.F. Collins, R.J. Stratton, M. Elia, Nutritional support in chronic obstructive pulmonary disease: a systematic review and meta-analysis, Am. J. Clin. Nutr. 95 (2012) 1385–1395.
[17] G.T. Motz, B.L. Eppert, G. Sun, S.C. Wesselkamper, M.J. Linke, R. Deka, M.T. Borchers, Persistence of lung CD8 T cell oligoclonal expansions upon smoking cessation in a mouse model of cigarette smoke-induced emphysema, J. Immunol. 181 (2008) 8036–8043.
[18] L. He, B. Wang, E.B. Hay, D.W. Nebert, Discovery of ZIP transporters that participate in cadmium damage to testis and kidney, Toxicol. Appl. Pharmacol. 238 (2009) 250–257.
[19] B. Wang, S.N. Schneider, N. Dragin, K. Girijashanker, T.P. Dalton, L. He, M.L. Miller, K.F. Stringer, M. Soleimani, D.D. Richardson, D.W. Nebert, Enhanced cadmium-induced testicular necrosis and renal proximal tubule damage caused by gene-dose increase in a Slc39a8-transgenic mouse line, Am. J. Physiol., Cell Physiol. 292 (2007) C1523–1535.
[20] B.E. Clausen, C. Burkhardt, W. Reith, R. Renkawitz, I. Forster, Conditional gene targeting in macrophages and granulocytes using LysMcre mice, Transgenic Res. 8 (1999) 265–277.
[21] J. Rennolds, S. Butler, K. Maloney, P.N. Boyaka, I.C. Davis, D.L. Knoell, N.L. Parinandi, E. Cormet-Boyaka, Cadmium regulates the expression of the CFTR chloride channel in human airway epithelial cells, Toxicol. Sci. 116 (2010) 349–358.
[22] N.S. Rajasekaran, S. Varadharaj, G.D. Khanderao, C.J. Davidson, S. Kannan, M.A. Firpo, J.L. Zweier, I.J. Benjamin, Sustained activation of nuclear erythroid 2-related factor 2/antioxidant response element signaling promotes reductive stress in the human mutant protein aggregation cardiomyopathy in mice, Antioxid. Redox Signal. 14 (2011) 957–971.
[23] O.J. Lucis, R. Lucis, Distribution of cadmium 109 and zinc 65 in mice of inbred strains, Arch. Environ. Health 19 (1969) 334–336.
[24] J.R. Napolitano, M.J. Liu, S. Bao, M. Crawford, P. Nana-Sinkam, E. Cormet-Boyaka, D.L. Knoell, Cadmium-mediated toxicity of lung epithelia is enhanced through NF-kappaB-mediated transcriptional activation of the human zinc transporter ZIP8, Am. J. Physiol. Lung Cell Mol. Physiol. 302 (2012) L909–918.
[25] L. He, K. Girijashanker, T.P. Dalton, J. Reed, H. Li, M. Soleimani, D.W. Nebert, ZIP8, member of the solute-carrier-39 (SLC39) metal-transporter family: characterization of transporter properties, Mol. Pharmacol. 70 (2006) 171–180.
[26] T.M. Vance, O.K. Chun, Zinc intake is associated with lower cadmium burden in US adults, J. Nutr. (2015).
[27] M.A. Spruit, S.J. Singh, C. Garvey, R. ZuWallack, L. Nici, C. Rochester, K. Hill, A.E. Holland, S.C. Lareau, W.D. Man, F. Pitta, L. Sewell, J. Raskin, J. Bourbeau, R. Crouch, F.M. Franssen, R. Casaburi, J.H. Vercoulen, I. Vogiatzis, R. Gosselink, E.M. Clini, T.W. Effing, F. Maltais, J. van der Palen, T. Troosters, D.J. Janssen, E. Collins, J. Garcia-Aymerich, D. Brooks, B.F. Fahy, M.A. Puhan, M. Hoogendoorn, R. Garrod, A.M. Schols, B. Carlin, R. Benzo, P. Meek, M. Morgan, M.P. Rutten-van Molken, A.L. Ries, B. Make, R.S. Goldstein, C.A. Dowson, J.L. Brozek, C.F. Donner, E.F. Wouters, Rehabilitation AETFoP. An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation, Am. J. Respir. Crit. Care Med. 188 (2013) e13–64.
[28] L. Ezzell, G.L. Jensen, Malnutrition in chronic obstructive pulmonary disease, Am. J. Clin. Nutr. 72 (2000) 1415–1416.
[29] M. Tellez-Plaza, A. Navas-Acien, K.L. Caldwell, A. Menke, P. Muntner, E. Guallar, Reduction in cadmium exposure in the United States population, 1988-2008: the contribution of declining smoking rates, Environ. Health Perspect. 120 (2012) 204–209.
[30] A.G. Davison, P.M. Fayers, A.J. Taylor, K.M. Venables, J. Darbyshire, C.A. Pickering, D.R. Chettle, D. Franklin, C.J. Guthrie, M.C. Scott, et al., Cadmium fume inhalation and emphysema, Lancet 1 (1988) 663–667.
[31] D.M. Mannino, F. Holguin, H.M. Greves, A. Savage-Brown, A.L. Stock, R.L. Jones, Urinary cadmium levels predict lower lung function in current and former smokers: data from the Third National Health and Nutrition Examination Survey, Thorax 59 (2004) 194–198.
[32] B.A. Hart, R.J. Potts, R.D. Watkin, Cadmium adaptation in the lung - a double-edged sword? Toxicology 160 (2001) 65–70.
[33] E. Tatrai, Z. Kovacikova, A. Hudak, Z. Adamis, G. Ungvary, Comparative in vitro toxicity of cadmium and lead on redox cycling in type II pneumocytes, J. Appl. Toxicol. 21 (2001) 479–483.
[34] P. Chowdhury, D.B. Louria, L.W. Chang, P.L. Rayford, Cadmium-induced pulmonary injury in mouse: a relationship with serum antitrypsin activity, Bull. Environ. Contam. Toxicol. 28 (1982) 446–451.
[35] D.F. Church, W.A. Pryor, Free-radical chemistry of cigarette smoke and its toxicological implications, Environ. Health Perspect. 64 (1985) 111–126.
[36] W.A. Pryor, K. Stone, Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite, Ann. N. Y. Acad. Sci. 686 (1993) 12–27 discussion 27-18.
[37] B.W. Willemse, N.H. ten Hacken, B. Rutgers, I.G. Lesman-Leegte, D.S. Postma, W. Timens, Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers, Eur. Respir. J. 26 (2005) 835–845.