



Effects of cadmium and high-fat diet on essential metal concentration in the mouse testis

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ARTICLE INFO

Edited by Dr. A.M Tsatsaka

Keywords:

Cadmium
Testis
Obesity
Trace elements
Metal homeostasis

ABSTRACT

The effects of exposure to the environmental toxicant cadmium, in combination with obesity, on the metal content in mouse testis were evaluated. Starting in utero and continuing through to 10 or 24 weeks post-weaning, male mice were exposed to cadmium (0, 0.5 or 5 ppm), and fed either a low (LFD) or high fat diet (HFD) post-weaning. Testicular levels of cadmium and essential metals were determined 10 and 24 weeks post-weaning by ICP-MS. Similar to what has been previously observed in the liver, kidney, heart and brain, significant levels of cadmium accumulated in the testis under all exposure conditions. Additionally, HFD-fed animals accumulated more cadmium than did their LFD-treated counterparts. Both treatments affected essential metal homeostasis in the testis. These findings suggest that cadmium and obesity may compromise the reproductive potential in the male mouse by disrupting essential metal levels.

1. Introduction

Infertility affects approximately 12 % of couples of reproductive age and can often be attributed to combined female-male factors or individual female or male factors [1]. Male infertility, which has steadily increased over the past few decades, can be attributed to absent or low sperm production, production of abnormal sperm or and obstruction that prevents delivery of sperm. The spectrum of abnormalities in sperm production include gonadal failure, chromosomal defects, genetic disorders, endocrine disturbances and structural defects. Often an infertility etiology cannot be established, which has led to the proposal that it may in part be due to environmental factors including exposure to chemical toxicants and diet. This has fostered the exploration of a possible negative impact of obesity and exposure to transition metal toxicants on spermatogenesis or sperm parameters.

Cadmium is a stable, persistent transition metal toxicant that is

ubiquitous in the environment [2]. Humans are exposed to cadmium via food and inhalation of cigarette smoke with an average dietary intake between 8–25 µg [3–5]. Once absorbed, cadmium is primarily stored in the kidney and liver, with a predicted half-life between 10–30 years [3, 4]. It is listed as “a major health concern” by the World Health Organization and ranked number seven on the Agency for Toxic Substances and Disease Registry's list of environmental chemical hazards [6,7].

Although the major sites for cadmium accumulation and storage in mammals are the kidney followed by the liver, recent studies show cadmium also accumulates in the heart and brain [8,9]. Analysis of human autopsy tissue report significant levels of cadmium in testis, epididymis, prostate and seminal vesicles, which increases as a function of age [10]. Significant associations between cadmium exposure and male reproductive system pathologies have been reported, including cancer of the prostate and testis and decreased semen quality (i.e., sperm count, viability and mobility) and infertility [11,12]. Several

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<https://doi.org/10.1016/j.toxrep.2021.03.016>

Received 12 January 2021; Received in revised form 12 March 2021; Accepted 18 March 2021

Available online 20 March 2021

2214-7500/© 2021 The Author(s).

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mechanisms have been proposed to explain cadmium-induced reproductive pathologies. Cadmium exposure is associated with structural damage to the blood-testis barrier and vascular endothelium, cytotoxicity of Sertoli and Leydig cells and apoptosis, likely through the generation of reactive oxygen species. Additionally, cadmium may induce testicular inflammation and disrupt the hypothalamus-pituitary-gonadal axis [13,14].

Over the past several years there has been an epidemic rise in obesity with at least 65 % of Americans being overweight and of those 30 % are clinically obese. It is well known that overweight and obese women have difficulty conceiving, however the data for male obesity and infertility is not as robust [15,16]. Evidence suggests that there is also an inverse relationship between body mass index and sperm quality [15,17]. Additionally, obesity-associated comorbidities such as insulin dependent diabetes, hypertension and cardiovascular disease have all been associated with impaired sperm function [18].

Human data suggests that exposure to transition metals in the adult male affects male fertility [12,19]. There is a paucity of data, however on the effects of *in utero* exposure on the male reproductive system. Additionally, the impact of combined obesity and transition metal exposure on male factor infertility is unknown. To begin investigating this scenario on infertility, the effects of high fat diet (HFD) on the levels of cadmium in testis during a whole life metal exposure was investigated. Additionally, the impact of cadmium and/or HFD on the levels of essential transition metals was examined.

2. Methods

2.1. Animals and exposures

Six week old male and female C57BL/6 J mice were purchased from Jackson Laboratory (Bar Harbor) and then housed in a pathogen-free AAALAC-accredited facility. One week after the animals arrived, diets were changed from standard laboratory chow to AIN-76A purified diet to limit the confounding effects of metal contamination found in standard chow [20]. Food and deionized water were provided *ad libitum*. All procedures were approved by the University of Louisville’s Institutional Animal Care and Use Committee.

Cadmium exposure for parental mice (F₀) began at 10 weeks of age.

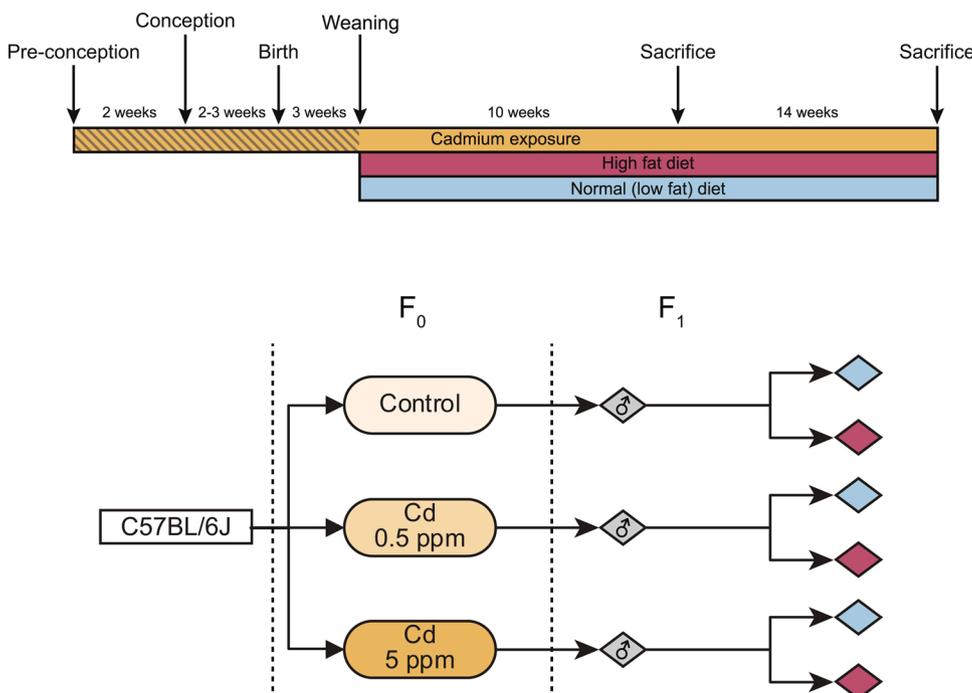


Fig. 1. Multigenerational exposure to cadmium in conjunction with diet. Adult male and female C57BL/6 J mice on defined, low-fat diets were exposed to 0, 0.5 or 5 ppm cadmium drinking water >2 weeks before being established into breeding groups (F₀, hatched lines). Pregnant dams and offspring (F₁) were continuously exposed to the same toxicant concentrations as their parents after weaning. At weaning, male offspring were fed either a low-fat (blue) or high-fat diet (pink), respectively for 10 or 24 weeks and then sacrificed.

Cadmium containing drinking water; 0, 0.5 and 5 ppm (final concentration); was prepared from stock solutions of cadmium chloride (Alfa Aesar), in deionized water and stored at –80 °C. Five ppm cadmium was used, as well as a ten times lower concentration of 0.5 ppm, which are approximately 1% and 0.1 % of the cadmium LD₅₀ [6]. At 12 weeks of age, mice were placed into breeding groups (1 male to 2 females) for each cadmium exposure group (Fig. 1). After weaning, offspring (F₁) were continuously exposed to the same concentration of cadmium as their parents until sacrifice. Additionally, offspring were fed either a low-fat (Envigo TD 160377 – 13% fat, Madison, WI) or high-fat (Envigo TD 09,682 – 42 % fat, Madison, MI) (Fig. 1). Offspring were sacrificed 10 or 24 weeks after weaning by first anesthetizing with ketamine/xylazine and then via exsanguination. Testis were harvested from each mouse and then snap-frozen in liquid nitrogen for future analyses.

2.2. Metal analysis

Testes were digested by incubating in 70 % nitric acid at 85 °C for 4 h. Samples were then cooled to room temperature, centrifuged to remove undigested debris and then diluted to 2% nitric acid (final concentration) with Milli-Q deionized water. Element quantification was performed using an X Series II quadrupole inductively coupled plasma mass spectrometry (ICP-MS). During sample injection, internal standards including Bi, In, ⁶Li, Sc, Tb and Y were mixed with each sample for instrument calibration. Each sample was analyzed three times (technical replicates).

2.3. Statistical analyses

Descriptive statistics for F₀ mice are summarized as mean, standard deviation (SD), and sample size (i.e., mean ± SD (n)), stratified by cadmium concentration. One-way ANOVA was applied to examine whether cadmium concentrations were significant for each metal concentration in parental mice. The F-test for the main effect was significant if the corresponding p-value was less than 0.05. For example, small p-values for F₀ generation indicate that the cadmium or essential metal concentrations in the testes are significantly different among the three cadmium exposure doses.

Descriptive statistics for cadmium and essential metals in the F₁ mice

are summarized as mean ± SD and stratified by diet (LFD vs. HFD), cadmium concentration (0, vs. 0.5 vs. 5 ppm) and exposure time (10 vs. 24 weeks). Three-way ANOVA with two-way and three-way interactions was applied to examine whether diet, cadmium concentration and exposure time are significant for each metal. The main effect or interaction was significant if the corresponding p-value was less than 0.05. For example, small p-values for F₁ animals for cadmium concentration in testes indicates that the metal was significantly different between the three cadmium exposure levels; a significant exposure time effects indicates that metal levels were significantly different between 10 and 24 weeks and a significant diet effect indicates that metal concentration were significantly different between HFD and LFD fed mice. Two-way interaction term indicates whether the difference of metal concentration in F₁ mice between two levels of one factor was significantly different among the different levels of the other factor. Three-way interactions indicated that the effects of the three factors were quite complex. The effect of each factor was carried out using post-hoc t-tests with the other two factors fixed at certain levels. Specifically, group comparisons due to cadmium exposure, if either cadmium or its interaction in the three-way ANOVA was significant, were further examined using post-hoc t-tests. The effect due to diet or exposure time was also examined in a similar manner. All the statistical analyses were carried out in the statistical software R version 3.6.2 (<https://www.r-project.org/>).

3. Results

3.1. Effects of cadmium concentration, diet and exposure time on cadmium levels in the testis

Concentrations of cadmium in testis were measured using ICP-MS. In F₀ mice, exposure to 5, but not 0.5 ppm, cadmium resulted in a significant increase in the metal (Tables 1 and 2). For F₁ mice, exposure to 5 ppm cadmium resulted in significant increases in cadmium levels under all treatment conditions, ranging from ~14–30-fold (Fig. 2, Table 1). Additionally, there were significantly higher levels of cadmium in 5 ppm exposed mice, compared to 0.5 ppm mice (Table 1). Exposure time did not significantly affect the levels of cadmium for 0.5 ppm treated animals, but resulted in significant, ~two-fold increases in cadmium levels in 5 ppm exposed mice (Fig. 2, Table 1). High fat diet combined with a 5 ppm cadmium exposure resulted in a significant increase in cadmium following 10-week exposure. Diet did not significantly affect metal levels under other treatment conditions (Table 1).

3.2. Effect of cadmium concentrations, exposure times and diet on essential metal levels in testis

ICP-MS was used to measure the effects of cadmium dose, exposure time and/or HFD on the concentration of the following essential metals: ²³Na, ²⁴Mg, ³⁹K, ⁴⁴Ca, ⁵²Cr, ⁵⁵Mn, ⁵⁷Fe, ⁵⁹Co, ⁶⁵Cu, ⁶⁶Zn, ⁸²Se, and ⁹⁵Mo. In F₀ mice, exposures to either 0.5 or 5 ppm cadmium lead to small, but significantly higher levels of Na, Mg, K, Co and Zn in the testis, compared to non-exposed animals. Additionally, exposure to 5 ppm cadmium lead to higher levels of Cu and Mo (Table 2, Suppl. Table 1).

For F₁ mice, in the absence of cadmium, the HFD caused significant decreases in Fe and Co, and an increase in Cu (Table 3, Suppl. Tables 1 and 2). Similarly, in the presence of cadmium, HFD-fed mice had small, but significant decreases in the levels of Na, Fe and Co (Table 3, Suppl. Table 1). The levels of Cu were significantly different between 0.5 and 5 ppm cadmium, with exposure to the higher cadmium concentration yielding high testicular Cu levels. Exposure time to cadmium and/or HFD affected the testicular levels of some essential metals. Iron levels significantly increased under all experimental conditions (Table 3, Suppl. Table 1). Levels of Na, Ca, Mn, Co and Mo were also affected under a variety of conditions (Table 3, Suppl. Table 1).

Table 1
Paired Comparisons for Cadmium.

Generation:diet:exposure time	Cd (ppm)	μCd/g testis (n)	p-values
F0:LFD	0	0.8 ± 1.73(5)	<0.001 ^a
	0.5	0.8 ± 0.7(5)	0.998 ^b
	5.0	14.8 ± 2.63(5)	<0.001 ^{c,d}
F1:LFD:10	0	4.7 ± 4.84(7)	<0.001
	0.5	5.6 ± 5.73(8)	0.756
	5.0	17.7 ± 4.51(7)	<0.001
F1:LFD:24	0	0.8 ± 1.75(5)	<0.001
	0.5	2.1 ± 2.53(4)	0.863
	5.0	40 ± 18.15(5)	<0.001
F1:HFD:10	0	1.4 ± 2.42(7)	<0.001
	0.5	4.4 ± 8.19(7)	0.538
	5.0	33.6 ± 12.65(8)	<0.001
F1:HFD:24	0	2.4 ± 2.02(6)	<0.001
	0.5	1.6 ± 2(6)	0.732
	5.0	60.3 ± 8.04(3)	<0.001

Generation:Cd (ppm):exposure time	Diet	p-values ^e
F1:0:10	HFD	0.130
	LFD	
F1:0:24	HFD	0.198
	LFD	
F1:0.5:10	HFD	0.76
	LFD	
F1:0.5:24	HFD	0.753
	LFD	
F1:5:10	HFD	0.008
	LFD	
F1:5:24	HFD	0.124
	LFD	

Generation:Cd (ppm):diet	Exposure time(weeks)	p-values ^f
F1:0:HFD	10	0.445
	24	
F1:0:LFD	10	0.116
	24	
F1:0.5:HFD	10	0.434
	24	
F1:0.5:LFD	10	0.284
	24	
F1:5:HFD	10	0.009
	24	
F1:5:LFD	10	0.01
	24	

^a p-value is based on F-test, indicating significant differences among the three exposure groups.

^b p-value indicates the comparison between 0.5 ppm Cd versus 0 ppm Cd.

^c p-value indicates the comparison between 5 ppm Cd versus 0 ppm Cd.

^d p-value indicates the comparison between 0.5 ppm versus 5 ppm.

^e p-value indicates the comparison between LFD versus HFD.

^f p-value indicates the comparison between 10 versus 24 weeks.

4. Discussion

The ability of the transition metal cadmium to impact human health is well established. Analyses of human data from several large cohort studies find significant associations between cadmium levels and other human diseases, including metabolic syndrome, type II diabetes, hypertension, hearing loss and neurological abnormalities [21–23]. Although significant associations have been established, there is a paucity of information on the link between cadmium exposure and male infertility.

To begin understanding the contribution of cadmium to male infertility, the effects of chronic whole-life, low-dose exposure to cadmium on essential metals levels in the testis were examined. In F₀ animals only exposure to the higher concentration resulted in a significant increase of testicular cadmium. Additionally, this level of metal significantly affected the levels of Na, Mg, K, Co, Cu, Zn and Mo. In contrast, the higher level of cadmium only affected Na and Cu in 24 week old F₁ animals (Fig. 3). This suggests that mice may need to be reproductively active for cadmium to affect other metals.

Table 2
Effects of Cadmium on Essential Metal Concentrations^a.

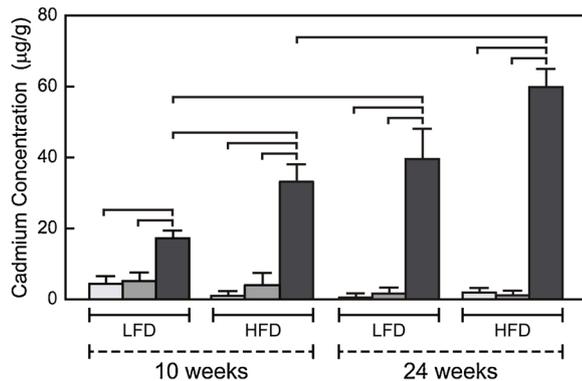
Generation:diet: exposure time	Cadmium (ppm)	Na	Mg	K	Ca	Cr	Mn	Fe	Co	Cu	Zn	Se	Mo
F0:LFD	0	0.004 ^b	0.009	0.012	0.564	0.55	0.857	0.326	0.06	0.084	0.017	0.415	0.048
F0:LFD	0.5	0.006 ^c	0.005	0.009	0.36	0.629	0.586	0.145	0.038	0.073	0.015	0.453	0.277
F0:LFD	5.0	0.002 ^d	0.009	0.008	0.362	0.545	0.78	0.551	0.04	0.041	0.01	0.562	0.016
F1:LFD:10	0	0.336	0.725	0.958	0.142	0.372	0.167	0.141	0.424	0.288	0.897	0.334	0.101
F1:LFD:10	0.5	0.495	0.996	0.958	0.056	0.221	0.144	0.238	0.409	0.133	0.757	1	0.083
F1:LFD:10	5.0	0.408	0.489	0.823	0.555	0.236	0.08	0.052	0.203	0.713	0.653	0.197	0.767
F1:LFD:24	0	0.041	0.045	0.137	0.864	0.322	0.304	0.377	0.439	0.027	0.289	0.441	0.634
F1:LFD:24	0.5	0.32	0.191	0.107	0.881	0.175	0.889	0.864	0.297	0.55	0.128	0.293	0.62
F1:LFD:24	5.0	0.014	0.144	0.756	0.705	0.909	0.2	0.201	0.261	0.031	0.328	0.266	0.632
F1:HFD:10	0	0.451	0.552	0.596	0.254	0.36	0.063	0.495	0.055	0.085	0.497	0.469	0.1
F1:HFD:10	0.5	0.653	0.316	0.511	0.902	0.224	0.925	0.973	0.273	0.615	0.337	0.524	0.197
F1:HFD:10	5.0	0.436	0.859	0.748	0.177	1	0.048	0.306	0.018	0.102	0.284	0.224	0.034
F1:HFD:24	0	0.014	0.157	0.112	0.048	0.207	0.517	0.115	0.553	0.004	0.169	0.711	0.513
F1:HFD:24	0.5	0.005	0.151	0.267	0.052	0.109	0.282	0.046	0.294	0.012	0.066	0.419	0.78
F1:HFD:24	5.0	0.388	0.522	0.206	0.026	1	0.892	0.193	0.539	0.129	0.335	0.736	0.368

^bp-value is based on F-test, indicating significant differences among the three exposure groups.

^cp-value indicates the comparison between 0.5 ppm Cd versus 0 ppm Cd.

^dp-value indicates the comparison between 5 ppm Cd versus 0 ppm Cd.

^a p values; highlighted values indicate p ≤ 0.05.



SUMMARY OF 2 AND 3-WAY ANOVA FOR CADMIUM

Treatments	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Parental Mice (F0 Generation)					
Cadmium Concentration ^{a,d}	2	649.526	324.763	93.694	0
Residuals	12	41.594	3.466	-	-
Offspring (F1 Generation)					
Cadmium Concentration ^a	2	14700.9	7350.4	125.655	0
Diet	1	225.4	225.4	3.853	0.054
Exposure Time	1	397.5	397.5	6.796	0.011
Cadmium Concentration : Diet ^b	2	1048.7	524.4	8.964	0
Cadmium Concentration : Exposure Time ^b	2	2508.3	1254.2	21.440	0
Diet : Exposure Time	1	46.4	46.4	0.793	0.377
Cadmium Concentration : Diet : Exposure Time	2	15.8	7.9	0.135	0.874
Residuals	61	3568.3	58.5	-	-

^{a,d} indicates whether Cd concentration in F0 was significantly, Pr(>F) < 0.05, different among the three Cd exposure levels

^b indicates whether Cd concentration in F1 was significantly different among the three Cd exposure levels

^c two-way interaction term indicates whether the difference of Cd concentration in F1 between two levels of one factor was significantly different among the different levels of the other factor;

Fig. 2. Effects of metal concentration, exposure time and diet on cadmium levels in mouse testis. Animals were exposed to 0 (light gray), 0.5 (medium gray) or 5 ppm (dark gray) cadmium and fed either a LF or HF diet for 10 or 24 weeks. Cadmium levels in testis were then measured by ICP-MS. Brackets indicate a significant difference (p > 0.05) in cadmium concentrations between two treatment groups.

In F1 mice, exposure to 5 ppm cadmium resulted in a time-dependent, significant accumulation of the metal, compared to 0.5 ppm and non-exposed animals. These levels however, were much lower than that present in the livers, kidneys and hearts of the same animals. They were however greater than the levels found in the brain (Suppl. Table 3).

Cadmium and obesity interact to exacerbate human pathologies

including prediabetes, metabolic syndrome and hypertension [24–26]. Additionally, in identical animals, HFD increases the levels of cadmium in the heart, liver, kidney and brain [8,9]. Therefore, the impact of HFD on cadmium levels in the testis was also examined. Similar to other tissue, HFD caused a significant increase in the amount of cadmium in the testis. In the absence of cadmium, obesity alone also caused significance changes in Fe, Co and Cu levels.

Activities that led to cadmium exposure including cigarette smoking and mining are associated with male infertility [27–29]. Metabolic syndrome and obesity are also associated with male infertility [18,30]. A recent study reports that both smoking and metabolic syndrome are independent risk factors for male infertility [30]. The combined effects of cadmium and obesity in mice results in significant accumulation of cadmium and disruption of essential metal homeostasis in the testis. These results suggest that both factors may synergistically disrupt spermatogenesis leading to male factor infertility. The mechanism for cadmium-induced disruption of spermatogenesis is presently unknown. Increases in the levels of the redox active metals Cu and Zn (Table 2) may lead to elevated levels of oxidative stress. Cadmium induced increases in reactive oxygen species has been hypothesized as a contributor to altered spermatogenesis and male infertility [31,32]. Future studies to evaluate the possible hormonal changes (serum testosterone, gonadotropins), that may occur during toxicant exposure, as well as evaluation of semen parameters (sperm count, motility, and morphology) will provide further insight to the possible causal physiological alterations seen in this study. This information could provide a link between environmental cadmium exposures and ongoing unexplained male infertility.

Author contributions

J.H.F., M.K., K.P., W.H.W. and L.C. designed the study. B.Z., A.G. and J.L.Y. performed the experiments including organ harvest and sample preparation for ICP-MS. M.K., Q.X. and X.Y. performed statistical analyses of the data. All of the authors contributed to the preparation of the manuscript.

Funding

This study was funded in part by National Institute of Environmental Health Sciences, National Institutes of Health (R01ES028102 to JHF; P30 ES030283 to LC and P20 GM113226 to WHW). Jamie Young was

Table 3
Effects of Diet and Exposure Time on Essential Metal Concentrations in F₁ mice^a.

Diet													
Cd (ppm): exposure time	Diet	Na	Mg	K	Ca	Cr	Mn	Fe	Co	Cu	Zn	Se	Mo
0:10	HFD	0.799	0.743	0.821	0.232	0.343	0.223	0.006	0.081	0.738	0.319	NaN	0.098
	LFD												
0:24	HFD	0.081	0.605	0.956	0.181	NA	0.174	0.996	0.049	0.018	0.966	0.744	0.536
	LFD												
0.5:10	HFD	0.43	0.361	0.51	0.446	0.302	0.25	0.447	0.128	0.475	0.799	0.302	0.32
	LFD												
0.5:24	HFD	0.028	0.739	0.62	0.663	0.25	0.48	0.041	0.889	0.621	0.556	0.447	0.496
	LFD												
5:10	HFD	0.125	0.581	0.611	0.075	NA	0.054	0.392	0.008	0.186	0.825	0.524	0.745
	LFD												
5:24	HFD	0.171	0.987	0.054	0.178	0.482	0.409	0.303	0.953	0.079	0.549	0.22	0.483
	LFD												

Exposure Time													
Cd (ppm):diet	Exposure time (weeks)												
0:HFD	10	0.035	0.29	0.2	0.495	NA	0.119	0	0.387	0.877	0.236	0.3	0.768
	24												
0:LFD	10	0.761	0.544	0.291	0.717	0.429	0.292	0	0.062	0.151	0.163	0.22	0.003
	24												
0.5:HFD	10	0.546	0.645	0.637	0.121	0.526	0.314	0	0.003	0.52	0.671	0.923	0.044
	24												
0.5:LFD	10	0.901	0.471	0.481	0.103	0.159	0.469	0	0.541	0.598	0.954	NA	0.318
	24												
5:HFD	10	0.953	0.255	0.241	0.014	NA	0.819	0.016	0.001	0.555	0.449	0.614	0.428
	24												
5:LFD	10	0.015	0.094	0.279	0.728	0.255	0.013	0.005	0.139	0.978	0.416	0.424	0.172
	24												

^a Highlighted values indicate $p \leq 0.05$.

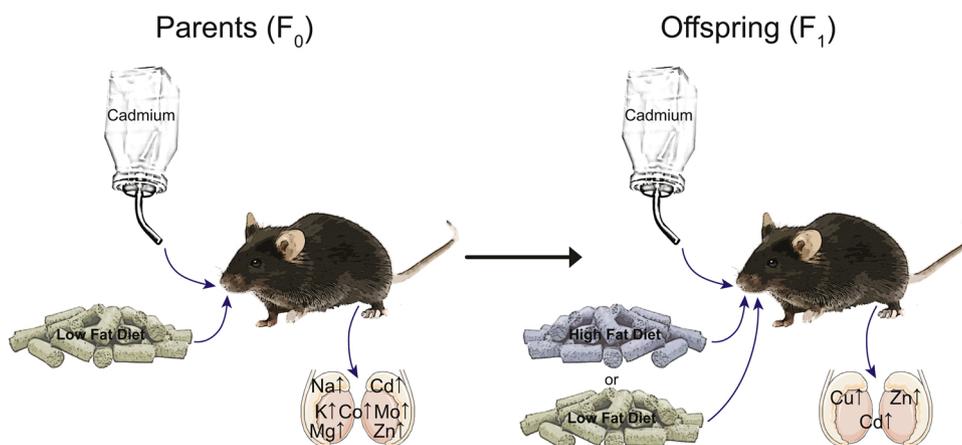


Fig. 3. Summary results of the effects of cadmium and diet on essential metal homeostasis. Mice were exposed to cadmium and/or HFD as diagrammed (Fig. 1). After 10 and 24 weeks testis were collected and then cadmium and essential metal levels measured by ICP-MS. Metals whose levels significantly changed under any condition for both the parent (F₀) and offspring (F₁) are presented.

the supported by NIH grant T32-ES011564. This work was part of the University of Louisville-China Pediatric Research Exchange Program (to LC). The personnel expenses and partial research-related expenses for Dr. Bin Zhou were provided by the Affiliated Children’s Hospital of Nanchang University, Nanchang, China. All basic science experiments were completed at the University of Louisville, School of Medicine, Louisville, KY, USA.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

We are grateful to Dr. Jianxiang (Jason) Xu for his assistance in metal measurements and Tom Burke for his assistance with the animal studies.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.toxrep.2021.03.016>.

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