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Inhibition of DNA Methylation Attenuates Low-Dose Cadmium-Induced Cardiac Contractile and Intracellular Ca²⁺ Anomalies

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Abstract

1. Cadmium is a human carcinogen with unfavorable health impact probably associated with its DNA methylation property. Recent data suggest that environmental cadmium exposure is associated with incidence of myocardial infarction and peripheral arterial disease. Nonetheless, the effect of chronic cadmium exposure on cardiac contractile function remains elusive.
2. This study was designed to examine the impact of low-dose cadmium exposure on cardiac contractile function and intracellular Ca²⁺ homeostasis. Adult male mice were exposed to cadmium for 4 weeks with or without the DNA methylation inhibitor (5-aza-2'-deoxycytidine, 5-AZA). Cardiac contractile and intracellular Ca²⁺ properties were analyzed including echocardiographic left ventricular parameters, fractional shortening (FS), peak shortening amplitude (PS), maximal velocity of shortening/relengthening ($\pm$ dL/dt), time-to-PS (TPS), time-to-90% relengthening (TR₉₀), electrically-stimulated increase of intracellular Ca²⁺ and intracellular Ca²⁺ decay.
3. Our results revealed that cadmium exposure depressed FS, PS, $\pm$ dL/dt and electrically-stimulated rise in intracellular Ca²⁺ without affecting TPS, TR₉₀, intracellular Ca²⁺ level and decay rate, the effects of which were significantly attenuated or nullified by 5-AZA. Cadmium exposure led to overt interstitial fibrosis (collagen deposition), the effect of which was mitigated by 5-AZA. Western blot analysis showed unchanged expression of ICAM-1, TNF- α and Cleaved caspase-3 in response to cadmium exposure and/or 5-AZA treatment, suggesting a relatively minor role of pro-inflammatory cytokines and apoptosis in cadmium- and 5-AZA-induced cardiac responses.
4. Taken together, our data demonstrated for the first time direct cardiac depressant effect following cadmium exposure, which may be rescued by DNA methylation inhibition.

Keywords

Cadmium; cardiac; contraction; intracellular Ca²⁺

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INTRODUCTION

Cadmium is a ubiquitous environmental toxin with the major known sources of exposure encompassing emissions from industrial activity and waste management, intake of foods (e. g. leafy vegetables, grains, organ meats and crustaceans) and exposure to cigarette smoke.¹⁻³ Accumulation of cadmium leads to tissue toxicity exerting a broad spectrum of biological actions possibly through induction of inflammation and apoptosis. Cadmium toxicity and carcinogenesis has been extensively examined in organs such as testis, liver, lung and kidney.⁴ For instance, our laboratory recently demonstrated persistent liver and renal damage at more than 40 weeks after a 4-week exposure to low-dose cadmium in rodents.⁵⁻⁷ Nonetheless, cardiac toxicity, if any, and the underlying mechanisms remain poorly elucidated.

It has been reported that cadmium exerts its adverse cardiovascular effects by promoting atherosclerosis as well as disadvantageous functional and metabolic changes in the heart.¹ In particular, high susceptibility of heart to cadmium was reported in a human 15-year following up study,⁸ in line with the fact that heart is a relatively sensitive organ due to its low antioxidant capacity. Similarly, high susceptibility of the heart to cadmium was also found in experimental animals.^{9, 10} Prenatal exposure to cadmium is known to lead to change of cardiovascular function during adulthood.^{1, 11, 12} Environmental exposure to cadmium has been found to be associated with an increased prevalence of heart failure¹ although the precise toxic response of cadmium exposure on myocardial function remains elusive. More recent data from the National Health and Nutrition Examination Survey have strongly suggested that cadmium, at substantially low levels of exposure, remains a rather important determinant of all-cause cardiovascular mortality in certain populations of U.S. adults.¹³ It is thus pertinent to examine the toxic effect of cadmium on myocardial function, if any, and the mechanism of action involved.

Traditional concepts for the mechanisms for cadmium toxicity include its generation of reactive oxygen or nitrogen species (ROS and RNS), inhibition of antioxidants, and impairment of DNA repair enzymes.¹⁴⁻¹⁶ However, a recent study has shown that even though a soy-based diet was capable of ameliorating cadmium-induced oxidative stress and damage, it failed to prevent against cadmium-elicited cardiac damage,¹⁷ suggesting possible involvement of other mechanisms including epigenetic modulating properties in cadmium-elicited toxicity.¹⁸⁻²⁴

Epigenetics refers to the heritable regulation of gene expression via modification of chromosomal components without an alteration in the nucleotide sequence of the genome. Such modifications include methylation of genomic DNA as well as acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation of core histone proteins. Epigenetic changes in the heart following environmental stress and life style changes have been implicated in the pathogenesis of cardiac remodeling and dysfunction.²⁵⁻²⁹ Recently we have demonstrated the induction of global DNA hypermethylation in the liver of rats and mice exposed to low-dose cadmium.⁷ Accordingly, we hypothesize that exposure to low-dose cadmium may cause cardiac global DNA methylation to modify certain key gene expression, leading to cardiac dysfunction.

Therefore, the present study was designed to address the following novel and important questions: (1) whether exposure of low-dose cadmium elicits cardiac functional toxicity and involvement of DNA methylation in such pathological process; (2) the effect of DNA methylation inhibition on cadmium exposure-induced unfavorable cardiac responses, if any.

MATERIALS AND METHODS

Experimental animal model

The animal experimental procedure described in the present study was approved by our Institutional Animal Care and Use Committee (University of Wyoming, Laramie, WY). In brief, forty adult male C57BL/6 mice (6-week-old, ~25 g) were randomly divided into four groups (10 each) as the following: (1) Control group: Mice were given i.p. injection of saline every other day for 4 weeks; (2) Cd treatment group: Mice were given i.p. injection of CdCl₂ (20 nmol/kg in saline) every other day for 4 weeks; (3) 5-AZA treatment group: Mice were given i.p. injection of 5-aza-2'-deoxycytidine (5-AZA, Sigma-Aldrich, 0.25 mg/kg in saline) twice weekly for 6 weeks; and (4) CdCl₂/5-AZA group: Mice were given both CdCl₂ and 5-AZA (20 nmol/kg and 0.25 mg/kg, respectively, in saline, i.p.). All mice were sacrificed at the 56th week following the 4-week Cd exposure (i.e.: 60th week since the initiation of the study) as early appearance of hepatic steatosis and preneoplasia were reported to develop at ~ 60 weeks, leading to induction of hepatic cancers at more advanced stages.^{30–32}

Echocardiographic assessment

Cardiac geometry and function were evaluated in anesthetized mice using a 2-D guided M-mode echocardiography (Sonos 5500, Phillips Medical System, Andover, MA) equipped with a 15-6 MHz linear transducer. Left ventricular (LV) wall thickness, diastolic and systolic dimensions were recorded from the M-mode images. Fractional shortening was calculated from end-diastolic diameter (EDD) and end-systolic diameter (ESD) using the equation of (EDD-ESD)/EDD x100%. Heart rate was measured in 10 consecutive cardiac cycles.³³

Isolation of murine cardiomyocytes

After ketamine/xylazine sedation (ketamine 80 mg/kg and xylazine 12 mg/kg, i.p.), hearts were removed from mice and perfused with Ca²⁺-free Tyrode's solution containing (in mM): NaCl 135, KCl 4.0, MgCl₂ 1.0, HEPES 10, NaH₂PO₄ 0.33, glucose 10, butanedione monoxime 10, and the solution was gassed with 5% CO₂/95% O₂. Hearts were digested with Liberase Blendzyme 4 (Hoffmann-La Roche Inc., Indianapolis, IN) for 15 min. Left ventricles were removed and minced before being filtered. Tissue pieces were gently agitated and pellet of cells was resuspended. Extracellular Ca²⁺ was added incrementally back to 1.20 mM. Cardiomyocytes were used within 8 hrs of isolation. A yield of ~ 60% viable rod-shaped cardiomyocytes with clear sarcomere striations was achieved. Only rod-shaped cells with clear edges were selected for mechanical study.³³

Cardiomyocyte shortening and relengthening

Mechanical properties of cardiomyocytes were assessed using an IonOptix Myocam system (IonOptix Inc., Milton, MA). Cells were placed in a chamber mounted on the stage of an inverted microscope and superfused (at 25°C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl₂, 1 MgCl₂, 10 glucose, 10 HEPES, at pH of 7.4. The cells were field stimulated at a frequency of 0.5 Hz. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-PS (TPS) and time-to-90% relengthening (TR₉₀), maximal velocities of shortening (+ dL/dt) and relengthening (– dL/dt).³³

Intracellular Ca²⁺ fluorescence measurement

A cohort of cardiomyocytes was loaded with fura-2/AM (0.5 μM) for 10 min at 25°C, and fluorescence measurements were recorded with a dual-excitation fluorescence

photomultiplier system (Ionoptix) as described.³³ Myocytes were imaged through an Olympus IX-70 Fluor oil objective. Cells were exposed to light emitted by a 75W lamp and passed through either a 360 or a 380 nm filter (bandwidths were ± 15 nm), while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480–520 nm by a photomultiplier tube after first illuminating the cells at 360 nm for 0.5 sec then at 380 nm for the duration of the recording protocol (333 Hz sampling rate). The 360 excitation scan was repeated at the end of the protocol and qualitative changes in intracellular Ca^{2+} concentration was inferred from the ratio of the fura-2 fluorescence intensity (FFI) at the two wavelengths. Fluorescence decay time (single and bi-exponential) was also measured as an indication of intracellular Ca^{2+} clearing rate.

Western blot analysis

Western blotting was performed as described.^{34, 35} In brief, tissue samples from mouse ventricles were removed and homogenized in a lysis buffer containing 10% 10 × RIPA, 1% NaF, 1% Na_3VO_4 and 1% protease inhibitor cocktail. Samples were sonicated on ice for 15 sec and centrifuged at $13,000 \times \text{rpm}$ for 20 min at 4°C . Murine cardiomyocytes were collected and sonicated in a lysis buffer. The protein concentration of the supernatant was evaluated using a Protein Assay Reagent (500-0006; Bio-Rad Laboratories, Hercules, CA). Protein samples were then mixed 1:2 with Laemmli sample buffer with 5% 2-mercaptoethanol and heated at 95°C for 5 min. Equal amounts (50 μg protein/lane) of proteins were separated on 7%, 10% or 15% SDS-polyacrylamide gels in a minigel apparatus (Mini-PROTEAN II, Bio-Rad) and transferred electrophoretically to nitrocellulose membranes (0.2 μm pore size, Bio-Rad). Membranes were incubated for 1 hr in a blocking solution containing 5% milk in Tris-buffered saline (TBS). Membranes were washed briefly in TBS and incubated overnight at 4°C with the anti-intercellular adhesion molecule 1 (ICAM-1, 1:500), anti-TNF- α (1:500, Abcam), anti-Cleaved caspase-3 (1:500, Cell Signaling) and anti- α -actin (1:2000, loading control) antibodies. All primary antibodies were purchased from Santa Cruz Biotech Inc. (Santa Cruz, CA) unless otherwise stated. After washing blots to remove excessive primary antibody binding, blots were incubated for 1 h with horseradish peroxidase (HRP)-conjugated secondary antibody (1:2,500). Antigens were detected by the luminescence method. Quantification of band density was determined using Quantity One software (Bio-Rad, version 4.4.0, ChemiDoc XRS) and reported in optical density per square millimeter.

Histopathology examination

Mouse hearts were collected and immersion-fixed in 10% neutral formalin, embedded in paraffin and sectioned into 5- μm -thick sections onto glass slides. After deparaffinization, tissue sections were rehydrated and stained with 0.1% Sirius-red F3BA and 0.25% Fast green FCF. Sirius-red stained sections were assessed for the proportion of fibrosis (collagen) in the heart tissues, as described previously.³⁶

Data analysis

Data were expressed as Mean $\pm$ SEM. Statistical significance ($p < 0.05$, two-sided) for each variable was estimated by two-way analysis of variance (ANOVA) followed by Tukey's test for *post hoc* analysis.

RESULTS

Effect of cadmium exposure and 5-AZA treatment on cardiomyocyte contractile function

Neither chronic cadmium exposure nor 5-AZA treatment significantly affected blood glucose levels, body and organ (heart, liver, kidney and testis) weights, as well as organ size

when normalized to body weight. Our data depicted that cadmium exposure overtly increased LVESD and suppressed fractional shortening without affecting heart rate, left ventricular wall thickness and LVEDD. While 5-AZA itself did not elicit any overt effect on echocardiographic parameters tested, it mitigated cadmium exposure-induced changes in echocardiographic indices (Table 1). Neither cadmium exposure nor 5-AZA significantly affected resting cell length. Chronic cadmium exposure significantly suppressed peak shortening (PS) and maximal velocity of shortening/relengthening ($-dL/dt$) without affect duration of shortening (TPS) and relengthening (TR_{90}) in murine cardiomyocytes. Although 5-AZA did not elicit any effect on these cardiomyocyte contractile parameters, it significantly attenuated or mitigated cadmium exposure-induced cardiomyocyte contractile defects (Fig. 1).

Effect of cadmium exposure and 5-AZA treatment on intracellular Ca^{2+} handling

To explore the possible mechanism(s) of action behind chronic cadmium exposure-induced cardiomyocyte contractile anomalies, the membrane permeable intracellular Ca^{2+} fluorescent dye fura-2 was employed to evaluate intracellular Ca^{2+} handling in cardiomyocytes. Our results shown in Fig. 2 depicted comparable resting intracellular Ca^{2+} levels and intracellular Ca^{2+} transient decay rates (either single or bi-exponential) in cardiomyocytes from mice exposed to cadmium with or without 5-AZA treatment. Interestingly, chronic cadmium exposure overtly suppressed electrically-stimulated rise in intracellular Ca^{2+} in cardiomyocytes, the effect of which was obliterated by 5-AZA treatment. 5-AZA did not exert any effect on intracellular Ca^{2+} handling properties. These data favored a role of intracellular Ca^{2+} handling underscoring chronic cadmium exposure- and 5-AZA-induced cardiac contractile responses.

Effect of cadmium exposure and 5-AZA treatment on myocardial histology

Myocardial histological examination using Sirius-red staining revealed overt interstitial fibrosis manifested as enhanced collagen deposition following cadmium exposure, the effect of which was negated by 5-AZA treatment. 5-AZA treatment itself did not elicit any notable effect on myocardial histology and collagen content (Fig. 3).

Protein expression of pro-inflammatory cytokines and apoptosis following chronic cadmium exposure and 5-AZA treatment

To evaluate if pro-inflammatory cytokines and apoptosis contribute to chronic cadmium exposure and/or 5-AZA-induced responses in the heart, Western blot analysis was performed to assess the expression of ICAM-1, TNF- α and cleaved caspase-3 in murine hearts following cadmium exposure and/or 5-AZA treatment. Our data shown in Fig. 4 indicated that neither cadmium exposure nor 5-AZA treatment, or both, significantly affected the protein expressions of ICAM-1, TNF- α and cleaved caspase-3, not favoring a major role of these pro-inflammatory cytokines and caspase-3-mediated apoptosis in chronic cadmium exposure and 5-AZA-induced cardiac mechanical responses.

DISCUSSION

The salient findings from our present study provided evidence for the first time that the human carcinogen cadmium exposure alters myocardial geometry, depresses cardiac contractile function and compromises intracellular Ca^{2+} handling in murine hearts. More strikingly, our results indicated that inhibition of DNA methylation improves chronic cadmium exposure-induced cardiac mechanical function, favoring a likely role of DNA methylation in cadmium exposure-induced cardiomyocyte mechanical anomalies.

Data from our present study revealed that chronic cadmium exposure led to myocardial and cardiomyocyte contractile dysfunction, altered myocardial geometry (LVESD but not LVEDD or LV wall thickness), intracellular Ca^{2+} mishandling and overt interstitial fibrosis (as evidenced by collagen deposition), the effect of which was mitigated by 5-AZA. Although the mechanism(s) of action underscoring compromised cardiomyocyte contractile function in response to chronic low-dose cadmium exposure is not clear at this time, several speculations may be considered. First, the observation that cadmium exposure inhibits the electrically-stimulated intracellular Ca^{2+} release depicts that cadmium exposure may directly interfere with intracellular Ca^{2+} handling. The reduced intracellular Ca^{2+} release in response to electrical stimuli is consistent with the compromised myocardial and cardiomyocyte contractile capacity (fractional shortening, $\pm \text{dL/dt}$ and PS) following chronic cadmium exposure. Second, global DNA methylation caused by chronic exposure to cadmium may play a key role. It is known that the carcinogenic mechanism of cadmium include its abnormal effect on DNA methylation. Three *in vitro* studies have indicated that prolong exposure to low-dose cadmium results global DNA methylation through stimulation of DNA methyltransferase (MeTase) activity.^{20, 21, 37} Our very recent *in vivo* study indicated that chronic cadmium exposure may promote DNA methylation in the liver.⁷ Ample evidence has suggested a unique role of abnormal DNA methylation in the etiology of cardiomyopathies *en route* to heart failure.^{28, 38–43} Movassagh and colleagues have identified at least three loci of angiogenesis-related genes where methylation of which may be correlated with overt differential expression of corresponding genes in myopathic hearts in patients with end-stage heart failure. These findings suggest that altered DNA methylation may be responsible for, at least in part, characteristic gene expression changes in heart failure.²⁸

It is known that sarcoplasmic reticulum (SR) Ca^{2+} -ATPase (SERCA2a) plays an essential role in intracellular Ca^{2+} homeostasis and maintenance of cardiac function. The promoter region of SERCA2a possesses a high level of CpG islands. Therefore, epigenetic modification through inhibition of methylation can enhance SERCA2a expression in cardiomyocytes. Recent study has demonstrated that hydralazine, a potential DNA methylation inhibitor used for the management of heart failure, decreased the expression of MeTase 1 in the heart in association with increased intracellular Ca^{2+} transients and SR Ca^{2+} contents along with a decrease in SERCA2a promoter region methylation and an increase the RNA and protein expressions of SERCA2a.⁴⁴ Moreover, treatment with hydralazine in isoproterenol-induced heart failure suppressed SERCA2a promoter methylation and increased SERCA2a RNA expression. These data favor the notion that hydralazine improves cardiac function through induction of SERCA2a promoter demethylation to increase SERCA2a function and intracellular Ca^{2+} handling in cardiomyocytes.⁴⁴ Along the same line, our data indicated comparable resting intracellular Ca^{2+} levels and intracellular Ca^{2+} transient decay rates (single or bi-exponential) in cardiomyocytes from mice exposed to cadmium with or without 5-AZA treatment. However, cadmium exposure overtly suppressed electrically-stimulated rise in intracellular Ca^{2+} in cardiomyocytes, the effect of which was obliterated by 5-AZA, suggesting the likelihood involvement of intracellular Ca^{2+} homeostasis in cadmium and 5-AZA-induced cardiac mechanical and geometric responses. Nonetheless, whether SERCA2a was reduced by cadmium-induced hypermethylation of SERCA2a promoter, an effect that was prevented by 5-AZA, warrants further investigation.

We did not see any increased level of cleaved caspase-3 in the present study. It is possible that the caspase-3 dependent apoptosis may not be the predominant form of cell death induced by cadmium in the current experimental setting. Earlier studies depicted that cadmium is capable of promoting apoptosis, necrosis, programmed necrosis, or autophagy. For instance, a recent study has indicated that cadmium may turn on a programmed,

lysosomal membrane permeabilization-dependent necrosis under certain conditions.⁴⁵ Data from our current study revealed unchanged levels of ICAM-1 and TNF- α which are expectable since these measurements are often observed in the acute cardiac injury.^{46–48} These measurements from the heart collected at 56 weeks after mice were exposed to chronic cadmium may be abnormally changed at the time period during cadmium exposure, but not right now. For instance, TNF- α is a common marker in the heart in response to stress such as cadmium.⁴⁷ An increase in TNF- α may play an important role in cadmium-induced DNA methylation as TNF- α may decrease the SERCA2a RNA and protein levels in the heart with an increased methylation in the SERCA2a promoter region, both of which were attenuated by 5-AZA. In addition, TNF- α may upregulate the expression of MeTase 1 in the heart. Taken together our finding with these previous studies, it suggests that inhibition of hypermethylation may be a novel treatment strategy for cardiac dysfunction.

In conclusion, our study has provided evidence, for the first time, at the levels of whole heart and cardiomyocytes to confirm clinical observations that the carcinogen cadmium may trigger myocardial contractile depression possibly through intracellular Ca^{2+} dysregulation. These findings not only reveal an essential role of the basic myocardial working element, cardiomyocytes, in chronic cadmium exposure-induced cardiac anomalies but also provide new insights for better therapeutic remedy against chronic cadmium exposure-induced myopathic changes. Assessment of cardiac electromechanical response in multicellular preparations may suffer from the limitation of heterogeneity in the heart, which may not accurately represent changes in the cardiomyocytes. For example, compromised myocardial contractile function seen at our whole heart or *in vivo* settings may reflect increased ventricular stiffness or poor ventricular compliance due to interstitial fibrosis and a shift in collagen content (as seen in our present study) rather than a loss of mechanical properties of cardiomyocytes. It is noteworthy that the precise nature behind chronic cadmium exposure-induced depression on cardiac contractile function still remains not fully clear. Awaiting future studies include cardiac excitation-contraction coupling and membrane ion channels in response to cadmium exposure. These approaches will be crucial to further our understanding of the pathological profiles of cadmium exposure.

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Fig. 1. Murine cardiomyocyte contractile function in response to chronic cadmium exposure with or without 5-AZA treatment. A: Resting cell length; B: Peak shortening (% of cell length); C: Maximal velocity of shortening (+ dL/dt); D: Maximal velocity of relengthening (- dL/dt); E: Time-to-PS (TPS); and F: Time-to-90% relengthening (TR₉₀). Mean ± SEM, n = 79–80 cells per group, * p < 0.05 vs. Control, # p < 0.05 vs. Cadmium group.

Fig. 2. Cardiomyocyte intracellular Ca^{2+} property in response to chronic cadmium exposure with or without 5-AZA treatment. A: Baseline intracellular Ca^{2+} fura-2 fluorescence intensity (FFI); B: Electrically-stimulated increase in FFI (Δ FFI); C: Intracellular Ca^{2+} decay rate (single exponential); and D: Intracellular Ca^{2+} decay rate (bi-exponential). Mean $\pm$ SEM, n = 60 cells per group, * p < 0.05 vs. control, # p < 0.05 vs. cadmium group.

Fig. 3. Interstitial fibrosis and collagen content in myocardium from mice exposed to cadmium with or without 5-AZA treatment. A: Representative Sirius-red stained myocardial sections used to assess fibrosis (collagen); and B: Collagen content. Mean $\pm$ SEM, $n = 5-6$ sections per group, * $p < 0.05$ vs. control, # $p < 0.05$ vs. cadmium group.

Fig. 4.

Levels of ICAM-1, TNF- α and cleaved caspase-3 in myocardium from mice exposed to cadmium with or without 5-AZA treatment. A: Representative gel blots depicting ICAM-1, TNF- α and cleaved caspase-3 using specific antibodies (β -actin used as loading control); B: Quantitative analysis based on gel profiles were presented for ICAM-1 (B), TNF- α (C), and cleaved caspase-3 (D). Mean $\pm$ SEM, n = 7 – 8 hearts per group.

Table 1

Biometric parameters of adult mice exposed to cadmium (CdCl_2 , 20 nmol/kg, every other day for 4 weeks) in the absence or presence of the DNA methylation inhibitor 5-AZA treatment (0.25 mg/kg, i.p. twice weekly for 6 weeks)

Parameter	Control	Cadmium	5-AZA	Cadmium-5-AZA
Body Weight (g)	33.3 ± 2.6	33.2 ± 3.7	29.8 ± 1.3	35.7 ± 1.8
Heart Weight (mg)	138 ± 10	148 ± 10	156 ± 4	166 ± 8
Heart/Body Weight (mg/g)	4.23 ± 0.42	4.57 ± 0.34	5.26 ± 0.16	4.71 ± 0.37
Liver Weight (g)	1.84 ± 0.11	1.90 ± 0.16	1.64 ± 0.10	1.64 ± 0.07
Liver/Body Weight (mg/g)	55.8 ± 2.7	58.1 ± 3.5	55.0 ± 3.0	46.0 ± 1.9
Kidney Weight (mg)	450 ± 29	438 ± 44	383 ± 29	440 ± 6
Kidney/Body Weight (mg/g)	13.6 ± 0.4	13.3 ± 0.8	12.8 ± 0.4	12.4 ± 0.5
Testis Weight (mg)	191 ± 15	221 ± 10	229 ± 11	236 ± 8
Testis/Body Weight (mg/g)	5.88 ± 0.65	6.90 ± 0.58	7.69 ± 0.11	6.63 ± 0.20
Blood Glucose (mg/dl)	116.5 ± 5.5	125.2 ± 5.9	118.3 ± 7.4	115.2 ± 6.6
Heart Rate (bpm)	503 ± 25	492 ± 14	497 ± 17	492 ± 24
LV Wall Thickness (mm)	1.01 ± 0.04	0.98 ± 0.04	1.03 ± 0.05	1.00 ± 0.04
LV EDD (mm)	2.30 ± 0.06	2.23 ± 0.08	2.29 ± 0.07	2.29 ± 0.08
LV ESD (mm)	1.15 ± 0.04	1.36 ± 0.05 [*]	1.20 ± 0.06	1.23 ± 0.06 [#]
Fractional Shortening (%)	49.8 ± 2.0	38.9 ± 2.1 [*]	47.7 ± 1.8	46.5 ± 1.6 [#]

LV: left ventricular; LV ESD: LV end systolic diameter; LV EDD: LV end diastolic diameter; Mean ± SEM, n = 5 mice per group

^{*} p < 0.05 vs. Control group

[#] p < 0.05 vs. Cadmium group.