Nutrition Research and Practice 2016;10(1):19-25

http://e-nrp.org

Protective effect of dietary chitosan on cadmium accumulation in rats

Mi Young Kim[1*], Woo-Jeong Shon[1*], Mi-Na Park[1], Yeon-Sook Lee[1] and Dong-Mi Shin[1,2§]

[1]Department of Food and Nutrition, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Korea.
[2]Research institution of human ecology, Seoul National University,1 Gwanak-ro, Gwanak-gu, Seoul 08826, Korea

BACKGROUND/OBJECTIVES: Cadmium is a toxic metal that is an occupational and environmental concern especially because of its human carcinogenicity; it induces serious adverse effects in various organs and tissues. Even low levels of exposure to cadmium could be harmful owing to its extremely long half-life in the body. Cadmium intoxication may be prevented by the consumption of dietary components that potentially reduce its accumulation in the body. Dietary chitosan is a polysaccharide derived from animal sources; it has been known for its ability to bind to divalent cations including cadmium, in addition to other beneficial effects including hypocholesterolemic and anticancer effects. Therefore, we aimed to investigate the role of dietary chitosan in reducing cadmium accumulation using an *in vivo* system.
MATERIALS/METHODS: Cadmium was administered orally at 2 mg (three times per week) to three groups of Sprague-Dawley rats: control, low-dose, and high-dose (0, 3, and 5%, respectively) chitosan diet groups for eight weeks. Cadmium accumulation, as well as tissue functional and histological changes, was determined.
RESULTS: Compared to the control group, rats fed the chitosan diet showed significantly lower levels of cadmium in blood and tissues including the kidneys, liver, and femur. Biochemical analysis of liver function including the determination of aspartate aminotransferase and total bilirubin levels showed that dietary chitosan reduced hepatic tissue damage caused by cadmium intoxication and prevented the associated bone disorder.
CONCLUSIONS: These results suggest that dietary chitosan has the potential to reduce cadmium accumulation in the body as well as protect liver function and bone health against cadmium intoxication.

Nutrition Research and Practice 2016;10(1):19-25; doi:10.4162/nrp.2016.10.1.19; pISSN 1976-1457 eISSN 2005-6168

Keywords: Chitosan, cadmium, liver function, bone disorder, mineral metabolism

INTRODUCTION

Cadmium, a toxic heavy metal was first reported as an occupational health hazard; however, recently, environmental exposure to cadmium has become a concerning issue as well. In the general population, the main sources of cadmium exposure are air pollution, tobacco, and food [1]. Cadmium is a contaminant that is often present in varying concentrations in different food sources. Its content is usually higher in plant-derived food sources than in animal sources of food [2]. Increased cadmium contamination of the soil, air, and water accounts for its accumulation in plants. Rice, wheat, green leafy and root vegetables, as well as potato, are ranked high in cadmium contamination [3,4]. The average cadmium intake from food varies from 8 to 25 μg per day but may be higher in some countries in Asia owing to the rapid increase in industrial pollution and high intake of rice grown locally on contaminated soil [1,5]. Even low levels of exposure to cadmium could be of concern because it has an extremely long biological half-life, reported to be 10-30 years in humans [6]. Therefore, serious health problems can potentially develop even from chronic low-level cadmium exposure.

Exposure to cadmium causes various adverse effects in humans such as kidney dysfunction, liver injury, osteoporosis, and cancer [7-10]. It has been reported that exposure to cadmium primarily damages the liver, bones, lungs, pancreas, placenta, and testis, as well as the kidneys [11,12], which are a critical target organ. The long-term exposure to cadmium even at low concentrations could lead to kidney tubular damage. A number of studies from different countries have reported the dose-effect or dose-response relationship between urinary cadmium level and makers for kidney damages in human [13-16]. In addition to renal damage, high cadmium levels also lead to bone disease. The causal relationship between cadmium exposure and bone disease was first reported in Japan in the case of itai-itai disease caused by the consumption of cadmium contaminated rice [17]. The disease exhibits a mixed combination of mainly osteomalacia symptoms as well as osteoporosis, which are accompanied by kidney damage. This association has been confirmed by recent studies by Gallagher *et al.* [18] and Schutte *et al.* [19] showing that exposure to cadmium was positively associated with incidences

§ Corresponding Author: Dong-Mi Shin, Tel. 82-2-880-6832, Fax. 82-2-884-0305, Email. shindm@snu.ac.kr
Received: May 12, 2015, Revised: May 29, 2015, Accepted: August 13, 2015
* These authors contributed equally to the manuscript

of osteoporosis [18,19]. Cadmium has also been reported as carcinogenic in human and animal models [7,20,21]. In animal models, cadmium strongly induces cancers in different tissues through consumption or injection, although the underlying mechanism is poorly understood.

Chitosan is a natural occurring polysaccharide from the shells of crustaceans such as crab, shrimp, and crawfish. It is both chemically and physiologically considered a dietary fiber because it cannot be degraded by human and animal digestive enzymes [22]. It has a various biological activities including hypocholesterolemic, antiosteoarthritic, and anticarcinogenic activities [23-27]. Dietary chitosan has been shown to increase cholesterol excretion and decrease its plasma levels in humans and animals [24,28]. Chitosan has been also reported to be possibly beneficial in preventing arthritis. The excess production of nitric oxide (NO) by inducible NO synthase (iNOS) mediates the pathogenesis of arthritis, and the chitosan monomer was shown to inhibit iNOS expression [29]. Furthermore, hydrolyzed products of chitosan showed antitumor and antimetastatic activities in both *in vitro* and *in vivo* studies [30]. Chitosan contains reactive functional groups with structures and physicochemical properties that correlate with their chelation and flocculation functions [31,32]. Chitosan has been extensively studied for its cation binding ability, especially divalent cations [33]. The high hydrophilicity of chitosan, which is due to the large number of hydroxyl groups in its active sites, enables it to attract those cations [34,35]. For this reason, chitosan has been used as a natural chelator of metal ions.

There are currently no proven effective treatments for cadmium intoxication [36] and, therefore, its prevention would be an important control strategy. For preventative strategies to be effective, it would be necessary to find ways to minimize cadmium absorption particularly through food since that is the major exposure source. There have been numerous suggestions that natural products such as green tea catechins, daidzein, quercetin compounds, and curcumin are beneficial in protecting against cadmium toxicity. In this present study, we investigated the possible role of chitosan in alleviating cadmium intoxication or lowering its accumulation in the body. The dietary effects of chitosan on cadmium accumulation *in vivo* were determined by administering cadmium to rats fed with different concentrations of a chitosan-containing diet. Then, we determined and compared the blood and tissue concentrations of cadmium in rats fed the chitosan diet, and those fed a regular diet.

MATERIALS AND METHODS

Animals, cadmium administration and diets

Male Sprague-Dawley rats weighing approximately 160 g (Institute of Laboratory Animal Resources, Seoul National University, Seoul, Korea) were individually housed in cages in a controlled environment (22°C, 65% relative humidity, 12-hour light-dark cycle with lighting from 6AM to 6PM). All animal procedures were approved by the Animal Care and Use Committee of Seoul National University (SNU 110412-3). In the preliminary experiment, to determine the experimental condition for cadmium intoxication, two different doses of cadmium-low (2 mg/day) or high (4 mg/day) were orally administrated to rats (n = 8 in each group). The control group of animals was administrated with saline replaced cadmium. The treatment in all the groups was carried on for 4 weeks (three times per week). The rats had free access to drinking water and normal AIN-93G diet [37]. In the second experiment, to investigate the effect of dietary chitosan on cadmium intoxication, the animals were randomly divided into three groups (n = 8): control (Cd + CS0), low chitosan diet (Cd + CS3), and high chitosan diet group (Cd + CS5). The rats in control group received AIN-93G [37] based diet with 5% cellulose but no chitosan (Table 1). The other two groups were fed the same diet as the control except the cellulose replaced by 3% chitosan (YongDuck, Chito Co., Seoul, Korea; Cd + CS3) or 5% chitosan (Cd + CS5) for 8 weeks. 2 ml of cadmium solution (1mg/ml) (Cadmium chloride, Sigma-Aldrich, St. Louis, MO, USA) was orally given to all animals three times per week.

Table 1. Composition of experimental diets[1)]

Ingredients	Cd + CS0	Cd + CS3	Cd + CS5
Corn starch	529.5	549.5	529.5
Casein	200	200	200
Sucrose	100	100	100
Soy oil	70	70	70
Cellulose	50	-	-
Chitosan[2)]	-	30	50
Mineral[3)]	35	35	35
Vitamin[4)]	10	10	10
Methionine	3	3	3
Choline	2.5	2.5	2.5
t-butylhydroquinone	0.014	0.014	0.014
Total	1000	1000	1000

[1)] Rats were randomly divided into three groups (n = 8 per group) and fed AIN-93 diet (Cd + CS0) or modified AIN-93 diet with replacement of cellulose by 3% chitosan (Cd + CS3), or 5% chitosan (Cd + CS5) for 8 weeks. Numbers are weight in gram (g).
[2)] Chitosan was from YongDuck Chito, Co., Korea.
[3)] Mineral mix were prepared according to AIN-93G-MX.
[4)] Vitamin mix (AIN-93-VX) was purchased from ICN Pharmaceuticals, USA.

Determination of Cd and other minerals in blood and tissues

At the end of 8 weeks, all animals were given Ketalar (100 mg/kg, Yuhan Co., Seoul, Korea) after fasting for 12 hours. Blood was collected from abdominal aorta in heparinized sterile tube and liver, kidneys, femur were harvested. Samples were wet-digested with concentrated nitric acid and submitted to microwave oven heating (Milestone Inc., Monroe, CT, USA). All samples were stored at -80°C until used for further analysis. Concentrations of cadmium and calcium in each digested sample were determined by atomic absorption spectrophotometry (Buck Scientific Co., East Norwalk, CT, USA).

Measurement of biochemical analysis of liver, kidney and femur functions, and histopathological examination

Blood was collected from each rat and stored at 4°C overnight. Serum was obtained by centrifugation of the blood at 3,000 g for 15 minutes. Those levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin for liver function and blood urinary nitrogen (BUN) and

creatinine for kidney function in serum were measured by automatic blood analyzer (Spotchem SP-4410, KDK Co., Kyoto, Japan). The breaking force of the femur was measured with a three-point bending test using an Instron 1000 (Instron Co., Canton, MA, USA) with a 50 kg load transducer and 10 mm per minute crosshead speed. Formalin-fixed liver and kidney samples were sectioned, mounted on a slide, stained with hematoxylin and Eosin, and then examined for tissue damages by light microscopy.

Statistical analysis

All data were analyzed with SAS version 8.0 (SAS institute Inc., Cary, NC, USA) and values were presented as means ± SE. Differences between control group and experimental group were analyzed by one-way ANOVA followed by Duncan's multiple range test.

RESULTS

Determination of experimental condition for cadmium intoxication in rats

To determine the experimental condition for cadmium intoxication, two different doses of cadmium-low (2 mg/day) or high (4 mg/day) were orally administrated to rats. Saline was used for control group and all rats were provided with normal AIN-93G diet. After four weeks, cadmium concentrations in blood and tissues were measured. Fig. 1A shows that cadmium administration caused dramatic increases in blood cadmium levels, in both low and high treatment groups. With a dose dependent manner, rats in high treatment group showed 45% more cadmium levels in the blood than those in low treatment group. The significant cadmium accumulations were also observed in tissues including liver, kidneys, and femur (Fig. 1A). Biochemical analysis of serum in the high treatment group showed that there were significant increases in both serum ALT and AST, which are indicators for liver tissue damages (Fig. 1B). Renal tissue damages were examined by measuring levels of BUN and creatinine in the serum. Rats in high cadmium group had significantly higher levels of BUN and serum creatinine than those in the control and low dose group (Fig. 1B). Immunohistochemistry analysis demonstrated that both low and high dose of cadmium treatment caused necrosis in liver, with more severe necrosis found in high cadmium group (Fig. 1C). Cell swelling and degeneration were also observed in both groups. Substantial tissue damages were found in kidneys. Fig. 1C shows there were cell hypertrophy and vacuolization, tubular degeneration, and congestion of renal cortex in low cadmium treatment group. The rats with high cadmium administration showed more aggressive tubular degeneration in kidneys. Although high cadmium treatment induced more severe conditions of intoxication in rats, the low dose of cadmium treatment turned out to be sufficient enough to accumulate cadmium in blood and tissue, thereby resulting in tissue damages in liver and kidneys histologically. Therefore, we concluded in using low dose of cadmium for the following experiments, to study the effect of dietary chitosan in cadmium-intoxicated rats.

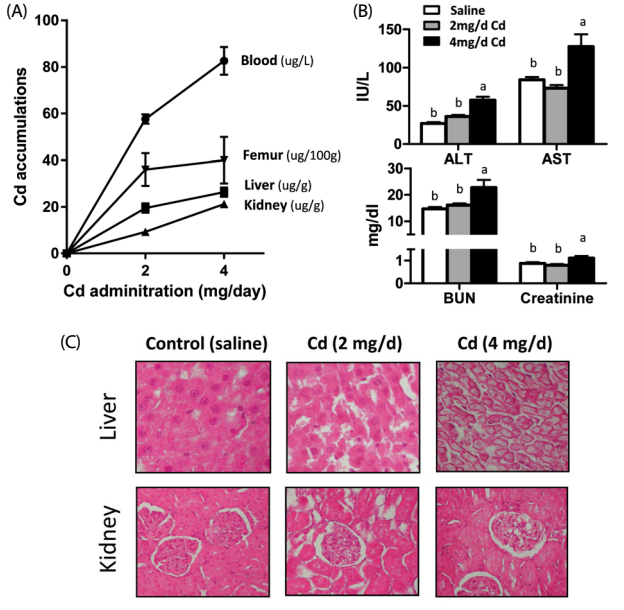


Fig. 1. Cadmium accumulations and biochemical analysis for tissue damages of rats. Values are mean ± SE of 8 rats per group. 2 mg/day or 4 mg/day of cadmium was given to rats three times per week for four weeks. The levels of cadmium in blood and tissues were presented (A). Serum ALT, AST, BUN, and creatinine were measured (B) and representative H&E staining images for liver and kidney of rats administrated saline, low (2 mg/day), or high (4 mg/day) of cadmium were shown in panel (C). Values with different superscript are significantly different as determined by Duncan's multiple test.

Food intake, body weight and organ weights

To investigate the effect of dietary chitosan on cadmium intoxication, low (3%) and high (5%) chitosan diet were provided to rats with cadmium administration (2 mg/day) for 8 weeks. There were no significant differences in food intake, body weight gain, and food efficiency ratios (FER) among control (0% chitosan; Cd + CS0), low (Cd + CS3) and high (Cd + CS5) chitosan diet groups, as shown in Table 2. The weight of liver, kidneys, testes, lung and brain did not show any

Table 2. Body weight, food intake and organ weights

Experimental groups	Cd + CS0	Cd + CS3	Cd + CS5
Final body weight (g)	323.4 ± 6.2$^{NS1)}$	327.7 ± 6.9	328.9 ± 10.3
Body weight gain (g/day)	2.9 ± 0.1^{NS}	3.0 ± 0.1	3.0 ± 0.2
Food intake (g/day)	15 ± 0.2^{NS}	15.9 ± 0.2	16.5 ± 0.6
FER$^{2)}$	0.19 ± 0.01^{NS}	0.19 ± 0.01	0.18 ± 0.02
Liver weight (g/ 100 g body weight)	2.43 ± 0.15^{NS}	2.44 ± 0.19	2.30 ± 0.13
Kidney weight (g/ 100 g body weight)	0.62 ± 0.02^{NS}	0.65 ± 0.04	0.61 ± 0.03
Testes weight (g/ 100 g body weight)	1.1 ± 0.05^{NS}	1.1 ± 0.04	1.07 ± 0.05
Lung weight (g/ 100 g body weight)	0.39 ± 0.02^{NS}	0.41 ± 0.02	0.41 ± 0.02
Brain weight (g/ 100 g body weight)	0.53 ± 0.02^{NS}	0.52 ± 0.02	0.52 ± 0.02

[1] Statistical significance; NS = not significant
[2] Food efficiency ratio = body weight gain/ food intake
Values are mean ± SE of 8 rats per group. Cd + CS0 indicates control group in which rats were fed normal diet containing 5% cellulose with 2 mg cadmium administration for 8 weeks; Cd + CS3, chitosan 3% diet + 2 mg cadmium administration; Cd + CS5, chitosan 5% diet + 2 mg cadmium administration.

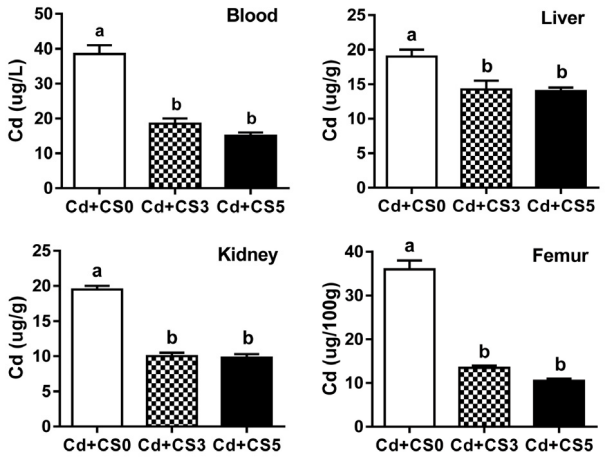


Fig. 2. The levels of cadmium in blood and tissues of rats. Values are mean ± SE of 8 rats per group. Cd + CS0 indicates control group in which rats were fed normal diet containing 5% cellulose with 2 mg cadmium administration for 8 weeks; Cd + CS3, chitosan 3% diet + 2 mg cadmium administration; Cd + CS5, chitosan 5% diet + 2 mg cadmium administration. Values with different superscript are significantly different as determined by Duncan's multiple tests.

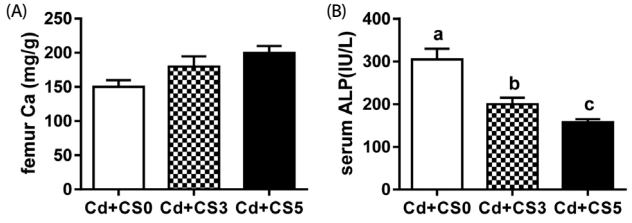
significant differences among the rats fed different levels of chitosan in diet (Table 2).

Cadmium levels in blood and tissues

Cadmium levels in blood and tissues were determined as shown in Fig. 2. Blood cadmium levels in rats were dramatically reduced by chitosan consumption. Compared to control, rats in Cd + CS3 and Cd + CS5 group showed the significant reduction in blood cadmium level by 50% and 60%, respectively ($P < 0.001$). Liver and kidneys have been reported to be the primary tissues in which cadmium accumulates [38]. In liver tissues, the cadmium contents in rats that were fed low (Cd + CS3) and high (Cd + CS5) chitosan were significantly lower than those in the control group (Fig. 2). Cadmium levels in kidneys were also dramatically decreased in both Cd + CS3 and Cd + CS5 groups, although there was no dose-dependent manner. The observation that cadmium has been notably deposited in bones as well as liver and kidneys (Fig. 1A) prompted us to study the effect of chitosan in bones. Rats in both Cd + CS3 and Cd + CS5 groups had significantly less cadmium accumulation in femur than the control as shown in Fig. 2.

Analysis of bone function

In our preliminary study, cadmium administration to rats resulted in significant decrease in the level of bone calcium. Therefore, we studied if dietary chitosan could be beneficial in preventing the loss of calcium in bone or any defect in bone function caused by cadmium intoxication. With chitosan consumption, the level of calcium tended to be increased (Fig. 3A) by chitosan administration, although there was no statistical significance. In our study, we also found that serum alkaline phosphatase (ALP), which is known to be a well-known marker for bone disorder [39], was increased in rats upon cadmium administration (data not shown). Interestingly, chitosan consumption in diet resulted in dramatic decrease in serum ALP with



(C)

Group	Weight (g)	Breaking force (kg/g)
Cd + CS0	0.84 ± 0.02	5.55 ± 0.23
Cd + CS3	0.86 ± 0.03	5.99 ± 0.17
Cd + CS5	0.87 ± 0.02	5.97 ± 0.12

Fig. 3. The levels of calcium, alkaline phosphates, and breaking force in bones of rats. Values are mean ± SE of 8 rats per group. Cd + CS0 indicates control group in which rats were fed normal diet containing 5% cellulose with 2 mg cadmium administration for 8 weeks; Cd + CS3, chitosan 3% diet + 2 mg cadmium administration; Cd + CS5, chitosan 5% diet + 2 mg cadmium administration. Values with different superscript are significantly different as determined by Duncan's multiple tests.

Table 3. Biochemical and histological analysis for liver and kidney functions

Experimental groups	Cd + CS0	Cd + CS3	Cd + CS5
Liver function			
AST (IU/L)	131.75 ± 5.26[a]	131.40 ± 6.83[a]	118.00 ± 4.31[b]
ALT (IU/L)	48.13 ± 2.65	46.00 ± 2.68	53.00 ± 2.52
Total bilirubin (mg/dL)	0.51 ± 0.04[a]	0.40 ± 0.00[b]	0.39 ± 0.01[b]
Kidney function			
BUN (mg/dL)	20.50 ± 0.88	21.20 ± 0.56	22.88 ± 0.61
Creatinine (mg/dL)	1.13 ± 0.04	1.10 ± 0.05	1.09 ± 0.04
Liver histological damages			
Cell swelling and degeneration	++ (4/4)	+ (3/4)	+ (2/4)
Mononuclear cell infiltration	+ (3/4)	+ (2/4)	+ (2/4)

Values are mean ± SE of 8 rats per group. Cd + CS0 indicates control group in which rats were fed normal diet containing 5% cellulose with 2 mg cadmium administration for 8 weeks; Cd + CS3, chitosan 3% diet + 2 mg cadmium administration; Cd + CS5, chitosan 5% diet + 2 mg cadmium administration. Values with different superscript within the same row are significantly different as determined by Duncan's multiple test. Degree of liver tissue damage is represented as follow: - (none), + (mild), ++ (moderate), +++ (severe) (n = 4 rats/groups)

dose dependent manner as shown in Fig. 3B. In addition, breaking force of femur was measured to evaluate bone function. Rats in both Cd + CS3 and Cd + CS5 groups had a tendency to increase in breaking force of femur compared to control group (Fig. 3C).

Analysis of liver and kidneys function

Since we observed cadmium treatment deteriorated liver and kidney functions, we studied the effect of dietary chitosan on preventing hepatic and renal tissues from being damaged by cadmium intoxication. Average AST levels in serum was 131.75 IU/L in rats fed no chitosan, which was significantly reduced to 118.00 IU/L in rats fed high chitosan diet (Cd + CS5). However, levels of serum ALT were not changed by dietary chitosan. Heightened levels of total bilirubin resulted from cadmium intoxication were consistently decreased in both Cd + CS3 and Cd + CS5 groups (Table 3). Biochemical analysis for kidney function was performed to evaluate the effect of dietary chitosan on restoration of tissue damages in kidneys caused by cadmium intoxication. Table 3 shows level of BUN tended to be elevated in rats fed chitosan diet, but there was no

statistical significance. On the other hand, level of creatinine showed some decrease without significance.

Since the dietary chitosan significantly affects preventive effect on liver functions compared with the control, we further studied histopathological evaluation of liver sections under a light microscope. Obvious morphological changes such as necrosis in liver tissue were observed in the cadmium treated group in our preliminary study (data not shown). In the cadmium treated group (Cd + CS0), cell swelling and degeneration and mononuclear cell infiltration were observed in liver. However, after co-administration of chitosan, the histological liver damage caused by cadmium was markedly ameliorated compared to the Cd + CS0 group (Table 3). These data suggest that chitosan could improve cadmium-induced histopathological damage of the liver, and could be a potential protectant of liver damage. Overall, dietary chitosan played the helpful roles in recovering from liver tissue damages induced by cadmium intoxication, but no significant effect was observed in the recovery form renal tissue damages.

DISCUSSION

Cadmium is a well-known potent environmental pollutant that induces severe organ and tissue damage in human and animals. This study revealed the protective effects of dietary chitosan against cadmium intoxication in rats. The level of cadmium accumulated in the blood was decreased by 50 and 60% more in the low and high chitosan diet groups, respectively, than it was in the control group. In addition, cadmium accumulation in the tissues including liver, kidneys, and bones was reduced by 30-50% in the chitosan diet groups, with a dramatic reduction observed in bones. However, the dietary chitosan showed only minimal preventive effects on the liver and bone functions, and even lower effects on kidney function compared with the control. Collectively, the dietary chitosan reduced cadmium accumulation in the body.

Cadmium acts similarly to essential divalent minerals like zinc, iron, and calcium, and has an equally low absorption rate in the gastrointestinal tract, which was reported to be around 5% of the oral dose [36,40]. Cadmium shares absorption pathways with other divalent minerals through the divalent metal transporter-1 (DMT-1). Leazer *et al.* [41] demonstrated that DMT-1 was responsible for the high accumulation of cadmium during conditions of iron deficiency. Following absorption, cadmium accumulates in a range of tissues, and in this study, we showed its dose-dependent accumulation in the liver and kidneys. Metallothionein (MT) is a metal-binding protein that can be induced by and bind to cadmium. Therefore, since the liver and kidneys produce high levels of MT, this might explain why cadmium mainly accumulates in these two tissues [36]. In addition, we also showed that the bones stored a considerable amount of cadmium, although the level was approximately 1/50 of that in the liver. The determination of the expression and activity of MT in bone tissues reveals the route by which cadmium accumulates there.

Numerous studies have been reported on bone disorders caused by cadmium intoxication [15,18,19,42,43], which causes a dramatic loss of bone mineral. This is mainly attributable to the associated kidney failure but cadmium also directly affects cell differentiation in the bones and, as a result, increases bone resorption [44]. In our preliminary study, rats treated with cadmium showed a 35% higher loss of calcium from their femurs than the untreated rats did. However, dietary chitosan did not prevent the loss of calcium from the bones or improve the breaking propensity of the femurs. This observation correlated with the results showing that dietary chitosan had little effect on kidney damage. However, the level of ALP that is known to increase in osteoporotic conditions [45,46] and was abnormally elevated following cadmium intoxication, was reduced by dietary chitosan in this study.

Contaminated food is one of the main sources of cadmium exposure. However, there have not been many studies examining the preventative effects of dietary components in cadmium intoxication. Catechin in green tea has been reported to facilitate the normalization of bone metabolic disorders caused by chronic exposure to cadmium [44]. Chlorella, which is a marine alga has been reported to counteract cadmium poisoning in rats. However, the beneficial effect of dietary chlorella was abrogated when the cadmium supply stopped after the intoxication was established [47,48].

Previous studies have shown that chitosan is a metal chelating agent as well as a natural, safe, and effective reactive oxygen species (ROS) scavenger [49,50]. Therefore, chitosan might inhibit cadmium-induced intoxication via multiple mechanisms. The first may involve direct chelation, which is likely facilitated by the chemical structure of chitosan that has reactive functional groups. In particular, the large number of hydroxyl groups confers a high hydrophilicity, and these structural features may provide more interactive sites for metallic ion. The next possible preventative mechanism may involve antioxidant activity against cadmium-induced oxidative stress. Recent studies have implicated ROS in cadmium toxicity and it has been suggested that chitosan has powerful and direct ROS radical scavenging ability, *in vitro* [50]. There are several possible mechanisms by which chitosan might protect against cadmium-induced intoxication. However, the precise underlying mechanisms remain unclear, and further studies designed to identify the specific molecular mechanism are required.

Recently, Korean National Health and Nutrition Examination Survey (KNHANES) reported that the overall geometric mean of the blood cadmium levels from Korean general adult population was 0.86-0.89 ug/L [51]. Although the effective dose of chitosan in the diet should be tested by a range of clinical studies, we assume that consumption of 0.7-1.3 g/day chitosan would be beneficial for reducing the level of blood cadmium based upon our observation that consumption of 3-5% chitosan diet efficiently reduced the level of blood cadmium in rats.

To the best of our knowledge, this is the first study to show the preventative effects of dietary chitosan in cadmium intoxication in an *in vivo* system. These findings reveal that chitosan might be an effective functional material in alleviating the toxic effects of cadmium. Our further studies will include the determination of changes in the transcriptional expression and protein activities of MT and DMT-1, to provide greater insights into the molecular mechanism of the prevention of cadmium accumulation by dietary chitosan.

REFERENCES

1. Järup L, Akesson A. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol 2009;238:201-8.
2. Clemens S, Aarts MG, Thomine S, Verbruggen N. Plant science: the key to preventing slow cadmium poisoning. Trends Plant Sci 2013; 18:92-9.
3. European Food Safety Authority. Cadmium dietary exposure in the European population. EFSA J 2012;10:2551.
4. Sharma A. Evaluation of certain food additives and contaminants, Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives. Indian J Med Res 2012;135:446-7.
5. Olsson IM, Bensryd I, Lundh T, Ottosson H, Skerfving S, Oskarsson A. Cadmium in blood and urine--impact of sex, age, dietary intake, iron status, and former smoking--association of renal effects. Environ Health Perspect 2002;110:1185-90.
6. Järup L, Rogenfelt A, Elinder CG, Nogawa K, Kjellström T. Biological half-time of cadmium in the blood of workers after cessation of exposure. Scand J Work Environ Health 1983;9:327-31.
7. Adams SV, Passarelli MN, Newcomb PA. Cadmium exposure and cancer mortality in the Third National Health and Nutrition Examination Survey cohort. Occup Environ Med 2012;69:153-6.
8. Engström A, Michaëlsson K, Vahter M, Julin B, Wolk A, Åkesson A. Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. Bone 2012;50:1372-8.
9. Nordberg G, Jin T, Wu X, Lu J, Chen L, Liang Y, Lei L, Hong F, Bergdahl IA, Nordberg M. Kidney dysfunction and cadmium exposure--factors influencing dose-response relationships. J Trace Elem Med Biol 2012;26:197-200.
10. Wu KC, Liu JJ, Klaassen CD. Nrf2 activation prevents cadmium-induced acute liver injury. Toxicol Appl Pharmacol 2012;263:14-20.
11. Satarug S, Garrett SH, Sens MA, Sens DA. Cadmium, environmental exposure, and health outcomes. Cien Saude Colet 2011;16: 2587-602.
12. Amamou F, Nemmiche S, Meziane RK, Didi A, Yazit SM, Chabane-Sari D. Protective effect of olive oil and colocynth oil against cadmium-induced oxidative stress in the liver of Wistar rats. Food Chem Toxicol 2015;78:177-84.
13. Thomas LD, Hodgson S, Nieuwenhuijsen M, Jarup L. Early kidney damage in a population exposed to cadmium and other heavy metals. Environ Health Perspect 2009;117:181-4.
14. Noonan CW, Sarasua SM, Campagna D, Kathman SJ, Lybarger JA, Mueller PW. Effects of exposure to low levels of environmental cadmium on renal biomarkers. Environ Health Perspect 2002;110: 151-5.
15. de Burbure C, Buchet JP, Leroyer A, Nisse C, Haguenoer JM, Mutti A, Smerhovsky Z, Cikrt M, Trzcinka-Ochocka M, Razniewska G, Jakubowski M, Bernard A. Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. Environ Health Perspect 2006;114:584-90.
16. Kobayashi E, Suwazono Y, Dochi M, Honda R, Nishijo M, Kido T, Nakagawa H. Estimation of benchmark doses as threshold levels of urinary cadmium, based on excretion of β2-microglobulin in cadmium-polluted and non-polluted regions in Japan. Toxicol Lett 2008;179:108-12.
17. Inaba T, Kobayashi E, Suwazono Y, Uetani M, Oishi M, Nakagawa H, Nogawa K. Estimation of cumulative cadmium intake causing Itai-itai disease. Toxicol Lett 2005;159:192-201.
18. Gallagher CM, Kovach JS, Meliker JR. Urinary cadmium and osteoporosis in U.S. Women >or= 50 years of age: NHANES 1988-1994 and 1999-2004. Environ Health Perspect 2008;116:1338-43.
19. Schutte R, Nawrot TS, Richart T, Thijs L, Vanderschueren D, Kuznetsova T, Van Hecke E, Roels HA, Staessen JA. Bone resorption and environmental exposure to cadmium in women: a population study. Environ Health Perspect 2008;116:777-83.
20. Yuan G, Dai S, Yin Z, Lu H, Jia R, Xu J, Song X, Li L, Shu Y, Zhao X. Toxicological assessment of combined lead and cadmium: acute and sub-chronic toxicity study in rats. Food Chem Toxicol 2014;65: 260-8.
21. Eriksen KT, Halkjær J, Sørensen M, Meliker JR, McElroy JA, Tjønneland A, Raaschou-Nielsen O. Dietary cadmium intake and risk of breast, endometrial and ovarian cancer in Danish postmenopausal women: a prospective cohort study. PLoS One 2014;9:e100815.
22. Harish Prashanth KV, Tharanathan RN. Chitin/chitosan: modifications and their unlimited application potential--an overview. Trends Food Sci Technol 2007;18:117-31.
23. Samarasinghe RM, Kanwar RK, Kanwar JR. The effect of oral administration of iron saturated-bovine lactoferrin encapsulated chitosan-nanocarriers on osteoarthritis. Biomaterials 2014;35:7522-34.
24. Zhang J, Liu J, Li L, Xia W. Dietary chitosan improves hypercholesterolemia in rats fed high-fat diets. Nutr Res 2008;28:383-90.
25. Yamada S, Ganno T, Ohara N, Hayashi Y. Chitosan monomer accelerates alkaline phosphatase activity on human osteoblastic cells under hypofunctional conditions. J Biomed Mater Res A 2007;83:290-5.
26. Liao FH, Shieh MJ, Chang NC, Chien YW. Chitosan supplementation lowers serum lipids and maintains normal calcium, magnesium, and iron status in hyperlipidemic patients. Nutr Res 2007;27:146-51.
27. Qin C, Du Y, Xiao L, Li Z, Gao X. Enzymic preparation of water-soluble chitosan and their antitumor activity. Int J Biol Macromol 2002;31:111-7.
28. Gallaher DD, Gallaher CM, Mahrt GJ, Carr TP, Hollingshead CH, Hesslink R Jr, Wise J. A glucomannan and chitosan fiber supplement decreases plasma cholesterol and increases cholesterol excretion in overweight normocholesterolemic humans. J Am Coll Nutr 2002;21:428-33.
29. Meininger CJ, Kelly KA, Li H, Haynes TE, Wu G. Glucosamine inhibits inducible nitric oxide synthesis. Biochem Biophys Res Commun 2000;279:234-9.
30. Shen KT, Chen MH, Chan HY, Jeng JH, Wang YJ. Inhibitory effects of chitooligosaccharides on tumor growth and metastasis. Food Chem Toxicol 2009;47:1864-71.
31. Renault F, Sancey B, Badot PM, Crini G. Chitosan for coagulation/flocculation processes-an eco-friendly approach. Eur Polym J 2009; 45:1337-48.
32. Gamage A, Shahidi F. Use of chitosan for the removal of metal ion contaminants and proteins from water. Food Chem 2007;104: 989-96.
33. Bravo-Osuna I, Millotti G, Vauthier C, Ponchel G. In vitro evaluation of calcium binding capacity of chitosan and thiolated chitosan poly (isobutyl cyanoacrylate) core-shell nanoparticles. Int J Pharm 2007;338:284-90.
34. Lima IS, Airoldi C. Interaction of copper with chitosan and succinic anhydride derivative-a factorial design evaluation of the chemi-

sorption process. Colloids Surf A Physicochem Eng Asp 2003;229: 129-36.
35. Crini G, Badot PM. Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: a review of recent literature. Prog Polym Sci 2008;33:399-447.
36. Waalkes MP. Cadmium carcinogenesis. Mutat Res 2003;533:107-20.
37. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939-51.
38. Klaassen CD, Liu J, Choudhuri S. Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu Rev Pharmacol Toxicol 1999;39:267-94.
39. Bikle DD. Biochemical markers in the assessment of bone disease. Am J Med 1997;103:427-36.
40. Nordberg GF, Nogawa K, Nordberg M, Friberg LT. Cadmium. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, editors. Handbook on the Toxicology of Metals. 3rd ed. Amsterdam: Academic Press; 2007. p.446-86.
41. Leazer TM, Liu Y, Klaassen CD. Cadmium absorption and its relationship to divalent metal transporter-1 in the pregnant rat. Toxicol Appl Pharmacol 2002;185:18-24.
42. Alfvén T, Elinder CG, Carlsson MD, Grubb A, Hellström L, Persson B, Pettersson C, Spång G, Schütz A, Järup L. Low-level cadmium exposure and osteoporosis. J Bone Miner Res 2000;15:1579-86.
43. Staessen JA, Roels HA, Emelianov D, Kuznetsova T, Thijs L, Vangronsveld J, Fagard R. Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Public Health and Environmental Exposure to Cadmium (PheeCad) Study Group. Lancet 1999;353:1140-4.
44. Choi JH, Rhee IK, Park KY, Park KY, Kim JK, Rhee SJ. Action of green tea catechin on bone metabolic disorder in chronic cadmium-poisoned rats. Life Sci 2003;73:1479-89.
45. Chan EL, Swaminathan R. Calcium metabolism and bone calcium content in normal and oophorectomized rats consuming various levels of saline for 12 months. J Nutr 1998;128:633-9.
46. Sims NA, Morris HA, Moore RJ, Durbridge TC. Parathyroidectomy does not prevent bone loss in the oophorectomized rat. J Bone Miner Res 1994;9:1859-63.
47. Shim JA, Son YA, Park JM, Kim MK. Effect of Chlorella intake on Cadmium metabolism in rats. Nutr Res Pract 2009;3:15-22.
48. Kim YJ, Kwon S, Kim MK. Effect of Chlorella vulgaris intake on cadmium detoxification in rats fed cadmium. Nutr Res Pract 2009;3:89-94.
49. Je JY, Kim SK. Reactive oxygen species scavenging activity of aminoderivatized chitosan with different degree of deacetylation. Bioorg Med Chem 2006;14:5989-94.
50. Mendis E, Kim MM, Rajapakse N, Kim SK. An in vitro cellular analysis of the radical scavenging efficacy of chitooligosaccharides. Life Sci 2007;80:2118-27.
51. Seo JW, Kim BG, Kim YM, Kim RB, Chung JY, Lee KM, Hong YS. Trend of blood lead, mercury, and cadmium levels in Korean population: data analysis of the Korea National Health and Nutrition Examination Survey. Environ Monit Assess 2015;187:146.