CrossMark

# Alleviation Mechanisms of Selenium on Cadmium-Spiked in Chicken Ovarian Tissue: Perspectives from Autophagy and Energy Metabolism

Shuang Wang[1] · Zhe Xu[1] · Hang Yin[1] · Yahong Min[1] · Shu Li[1]

## Abstract

Cadmium (Cd) is a kind of toxic heavy metal and it can cause damage to organs and tissues. Selenium (Se) can antagonize some metal element toxicity including Cd. The present study was designed to investigate Cd-induced damage to chicken ovary by autophagy and the protective mechanism of Se on Cd-induced damage. Administration of Cd for 12 weeks led to energy metabolism disorder of the chicken ovarian tissues, which resulted in autophagy. In addition, the mRNA expression of glucose-related genes including hexokinase II (HK2), pyruvate kinase (PK), pyruvate dehydrogenase complex (PDHX), and succinate dehydrogenase (SDH) and the activities of ATPase, including $Na^+$-$K^+$-ATPase, $Ca^{2+}$-ATPase, $Mg^{2+}$-ATPase, were all downregulated remarkably compared with the control. However, combined with oral administration of Se at 2 mg/kg, the mRNA expression of glucose-related genes and the activities of ATPase increased. The mRNA expression of the autophagy-related genes by Cd treatment, including microtubule-associated protein light chain 3 (LC3), dynein, autophagy-related gene 5 (Atg5), and Beclin 1, was remarkably enhanced, whereas mammalian target of rapamycin (mTOR) was downregulated. However, besides mTOR, their levels displayed a downregulated trend beyond simultaneous Se treatment. The protein expression of autophagy genes was similar to those of mRNA. In conclusion, Cd toxicity affect energy metabolism and induce autophagy, which causes damage to chicken ovary, whereas Se could protect effectively this injury induced by Cd.

**Keywords** Cadmium · Selenium · Autophagy · Energy metabolism · Chicken ovary

## Abbreviations

| | |
|---|---|
| Cd | Cadmium |
| Se | Selenium |
| HK2 | Hexokinase II |
| PK | Pyruvate kinase |
| PDHX | Pyruvate dehydrogenase complex |
| SDH | Succinate dehydrogenase |
| LC3 | Microtubule-associated protein light chain 3 |
| mTOR | Mammalian target of rapamycin |
| Atg5 | Autophagy-related gene 5 |
| q-PCR | Quantitative real-time polymerase chain reaction |

All of the authors have read the manuscript and agreed to submit it in its current form for consideration for publication in the *Biological Trace Element Research*.

✉ Shu Li
lishu@neau.edu.cn

[1] College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, People's Republic of China

## Introduction

Cd is an environmental contaminant that has been recognized to be a risk factor in organism. It can enter human and animal bodies via different industrial products, environmental pollution, and different contaminated food. Several animal studies have revealed that Cd accumulates preferentially in the hepatic and renal tissue and causes distinct pathological changes to the liver and the kidney [1, 2] even in very low concentrations. Besides, Cd exposure can inhibit production of testosterone, reduce sperm motility, and cause testicular damage [3]. Haouem et al. have indicated that Cd has toxic effects on the testicular function of rats and leads to a significant decline in epididymal sperm count [4]. Cd has obvious toxic effects on estrogen synthesis, oocyte, ovary, and uterus of female animals; it can affect the expression of estrogen receptor [5]. Zhang et al. have injected different doses of Cd to Wistar rats for 6 weeks in order to find the effects of Cd on their reproductive system; they found that Cd could cause the decrease of progesterone and estradiol in the ovary of rats [6]. Höfer et al. have reported that an induction of estrogen-sensitive uterine

gene expression was observed after treatment with Cd [7]. All these evidences showed that reproduction toxicity induced by Cd.

Autophagy is a conserved evolutionary lysosomal pathway for degrading cytoplasmic proteins, macromolecules, and organelle [8]. LC3, mTOR, dynein, Beclin 1, and Atg5 are involved in autophagy. This process helps maintain cellular homeostasis. However, a persistent and massive autophagy can also result in tissue damage. Xue et al. have shown that in superior cervical ganglia neurons, HeLa or CHO cells treated with a cell death inducer in the presence of caspase inhibitors, the majority of the mitochondria were destroyed by autophagy, cells became smaller, and they were eventually irreversibly committed to death [9]. Several studies have reported that the toxic metals can cause autophagy including Cd [10, 11]. One of the major regulators of autophagy is the mTOR, which promotes autophagy due to poor nutrients [12]. Cd induces ROS and activates mTOR pathways, and then resulting in autophagy [13, 14]. Wang et al. have reported that the MES-13 mesangial cells were determined to have undergone autophagy after treatment with Cd [15]. Dong et al. have illustrated that low concentrations of Cd promoted autophagy and their results suggested that autophagy played a key role in Cd-induced endothelial dysfunction [16]. Besides, some studies have reported that autophagy was related to energy metabolism [17, 18]. Lock et al. demonstrated an unexpected connection between autophagy and glucose metabolism that facilitates adhesion-independent transformation driven by mutationally active Ras [19].

Cd is harmful to organism, it is very important to prevent and/or attenuate Cd toxicity. One strategy to attenuate Cd exposure in animal is the use of Se. Se is an organism essential trace element, displaying an antioxidant effective oxygen free radical scavenging [20], protecting the organs and tissues from oxidative damage [21, 22]. Several studies have indicated that the treatment with Se protects various organs and tissues against harmful elements [23, 24] including Cd [25, 26]. Some studies found a protective effect of Se against the Cd toxicity in rodents and humans [27, 28]. Other studies have shown that organic Se-administered could protect broilers from Cd toxicity [29, 30]. Besides, Se is closely related to energy metabolism. Pinto et al. indicated that dietary Se oversupply may affect expression and activity of proteins involved in energy metabolism of major insulin target tissues [31]. At the same time, Se can also exert some influences on autophagy. Liu et al. have indicated that autophagy was involved in the development of liver injury (pathological processes), which is induced by Se deficiency [32]. Frustaci et al. have shown that Se deficiency can cause decline of myocardial antioxidant reserve and enhance cell autophagy [33].

Although Cd toxicity and the antagonism of Se to Cd in mammals have been reported, the effects of Cd on the autophagy of avian ovarian tissue are not clear. In the study, we investigated the ovarian tissue autophagy in chickens after an exposure to Cd and the simultaneous administration of Cd and Se by a dietary route. We tested the ATPase activities, the mRNA expression of glucose metabolism-related genes, and the expression of autophagy-related genes in ovarian tissue of chickens. We evaluated the ovarian tissue autophagy caused by Cd exposure in chickens, as well as the ability of Se to reduce the toxicity of Cd. These results will not only add new and important data on Cd-induced autophagy but also enrich detoxification effect of Se on Cd in chicken ovary.

## Material and Methods

### Preparation of Animals

All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. Forty-eight 50-day-old Isa Brown female chickens were divided randomly into four groups ($n = 12$ per group). The animal room was maintained at 18–26 °C; the chickens were kept under a 16-h light/8-h dark cycle and were given free access to standard food and water about 12 weeks. According to the LC50 of Cd (218.44 mg/kg diet $CdCl_2$), the exposure doses of Cd (150 mg/kg diet $CdCl_2$) were used in this study. In regard to Se doses, we added and supplied 2 mg/kg Se (super nutritional Se but not Se toxicity) with $Na_2SeO_3$ supplement. Control group was fed a basic diet containing 0.2 mg/kg Se; Se-treated group was fed with the basic diet supplemented with $Na_2SeO_3$ and the total Se content was 2 mg/kg; Se/Cd-treated group was fed with the basic diet supplemented with $Na_2SeO_3$; the total Se content was 2 mg/kg and supplemented with 150 mg/kg $CdCl_2$. Cd-treated group was fed with the basic diet supplemented with 150 mg/kg $CdCl_2$ [34]. The total rearing period was 12 weeks; in the end of experiment, ovarian tissues of chickens were collected.

### ATPase Activity

The activities of $Na^+$-$K^+$-ATPase, $Ca^{2+}$-ATPase, and $Mg^{2+}$-ATPase were determined according to the manufacturer's instructions of the appropriate assay kits (Nanjing Jiancheng Bioengineering Institute, China). The activities of the $Na^+$-$K^+$-ATPase, the $Ca^{2+}$-ATPase, and the $Mg^{2+}$-ATPase were measured by quantifying the inorganic phosphorus (Pi) production from the conversion of ATP to ADP at 660 nm using the molybdenum blue spectrophotometric method, and were expressed as μmol/mg.pr. When one type of ATPase was tested, the inhibitors of other types of ATPase were added to depress the hydrolysis of phosphate radicals.

## RNA Isolation and Quantitative Real-time Polymerase Chain Reaction (q-PCR)

Total RNA was dissociated from the ovarian tissue using Trizol reagent according to the manufacturer's protocol. The concentration and purity of the total RNA were determined spectrophotometrically at 260/280 nm (Gene Quant 1300/100, General Electric Company, USA). First-strand cDNA was synthesized from 5 lg of total RNA using oligo dT primers and superscript II reverse transcriptase according to the manufacturer's instructions (Roche, USA). Synthesized cDNA was diluted five times with sterile water and stored at − 80 °C before using.

All of the primers (Table 1) were designed by Premier Software (PREMIER Biosoft International, USA) for q-PCR. Detected via q-PCR, gene expression levels were performed on a LightCycler® 480 System (Roche, Basel, Switzerland) using fast Universal SYBR Green Master Mix (Roche, Basel, Switzerland). The reactions were performed in a 20 μL reaction mixture containing 10 μL of 2 × SYBR Green I PCR Master Mix (TaKaRa, China), 2 μL of either diluted cDNA, 0.4 μL of each primer (10 μM), 0.4 μL of 50 × ROX reference Dye II, and 6.8 μL of PCR-grade water. The PCR procedure for all of the primers consisted of heating the reaction mixture was 95 °C for 30 s followed by 40 cycles

**Table 1** Gene-special primers used in the present study

| Gene | Primer (5′-3′) |
|------|----------------|
| PK | *Up: GAACTGCGATGAGAATGTGC* |
| | *Down: ACCAGCAAGGAAATGAGACC* |
| SDH | *Up: GCTGCGGCCGATCTGT* |
| | *Down: GCTTGTCCCCAGGCTTATCA* |
| PDHX | *Up: GCAAGGGAAGCAGAAGGATA* |
| | *Down: GGATAAGCAGAAGGCACAGC* |
| HK2 | *Up: CCCAGATAGAAAGCGACTGC* |
| | *Down: CAGAGTCGTAGAGCCCGATG* |
| LC3-I | *Up: TTACACCCATATCAGATTCTTG* |
| | *Down: ATTCCAACCTGTCCCTCA* |
| LC3-II | *Up: AGTGAAGTGTAGCAGGATGA* |
| | *Down: AAGCCTTGTGAACGAGAT* |
| Atg5 | *Up: GGCACCGACCGATTTAGT* |
| | *Down: GCTGATGGGTTTGCTTTT* |
| mTOR | *Up: GGACTCTTCCCTGCTGGCTAA* |
| | *Down: TACGGGTGCCCTGGTTCTG* |
| Dynein | *Up: CGGCTTGACCTATGGAATCT* |
| | *Down: CATCACTGCGAGGAACTGC* |
| Beclin1 | *Up: CGACTGGAGCAGGAAGAAG* |
| | *Down: TCTGAGCATAACGCATCTGG* |
| β-actin | *Up: CACCACAGCCGAGAGAGAAAT* |
| | *Down: TGACCATCAGGGAGTTCATAGC* |

of 95 °C for 15 s, 60 °C for 30 s, and 60 °C for 30 s. The melting curve analysis showed only one peak for each PCR product. Electrophoresis was performed with the PCR products to verify primer specificity and product purity. The relative mRNA abundance was calculated according to the method of $2^{-\Delta\Delta Ct}$, accounting for gene-specific efficiencies was normalized to the mean mRNA expressions of $\beta$-actin.

## Western Blot

Total protein was extracted from ovarian tissues of chickens after cell lyses. SDS-polyacrylamide gel electrophoresis was used to extract protein from 12% gel for western blot. Protein emergent was obtained on nitrocellulose membranes through transfer tank method. Membranes were blocked in 5% skim milk for 2 h and incubated with diluted primary chicken antibodies: autophagy LC3-II, dynein, Beclin 1, Atg5, and mTOR for 1 h at 37 °C, respectively. PBST was used to wash the membrane for four times, 5 min each. After that, membranes were incubated in horseradish peroxidase (HRP)-conjugated secondary antibody against rabbit Ig G (1:500, Santa Cruz, CA, USA) for 1 h at 37 °C. After washing with PBST for four times, 5 min each, the signal was detected by Chemi Scope 5300 (Clinx Science Instruments, Shanghai, China). The optical density (OD) of each band was determined by Image VCD gel imaging system. Monoclonal $\beta$-actin antibody content was analyzed as a control by means of a rabbit polyclonal antibody (Sigma, USA).

## Electron Microscopy Detection

Cells were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h at 4 °C and then post-fixed in 0.1 M phosphate buffer containing 1% osmium tetroxide for 1 h. Ultrathin sections were stained with uranyl acetate-lead citrate and examined by a transmission electron microscopy (TEM).

## Statistical Analyses

Statistical analyses of all data were performed using GraphPad Prism (version 5.0, GraphPad Software Inc., San Diego, CA, USA). All the data were analyzed by Student's *t* test or one-way ANOVA, had a normal distribution, and passed equal variance testing. Quantitative data are shown as the mean ± SD. Differences of $P < 0.05$ were considered to be significant.

# Results

## ATPase Activities in Chicken Ovarian Tissues

In order to explore the toxic mechanism of Cd on chicken ovary, we detected the Na$^+$-K$^+$-ATPase, Ca$^{2+}$-ATPase, and

$Mg^{2+}$-ATPase in ovarian tissue of chickens. We did not find the chickens died because of Cd poisoning or other infections. As shown in Fig. 1, there was little difference between control group and Se-treated group for the activity of $Na^+$-$K^+$-ATPase in ovarian tissue. Compared with the control group, the activity of $Na^+$-$K^+$-ATPase in corresponding Se/Cd-treated group and Cd-treated group was lower, but the activities of ATPase in Se/Cd-treated group were higher than Cd-treated group. For $Ca^{2+}$-ATPase, we found that both Se/Cd-treated group and Cd-treated group were lower than corresponding control group; in addition, the activity of $Ca^{2+}$-ATPase in Se/Cd-treated group was higher than that of Cd-treated group. There were significant differences between control group and Se/Cd-treated group or Cd-treated group ($P < 0.05$). The activity of $Mg^{2+}$-ATPase in Se/Cd-treated group and Cd-treated group was significantly lower compared to corresponding control group. There was no significant difference between Se/Cd-treated group and Cd-treated group ($P > 0.05$). However, the activity of $Mg^{2+}$-ATPase in Se/Cd-treated group was higher compared to that of Cd-treated group.

## The mRNA Expression of Glucose Metabolism-Related Genes in Chicken Ovarian Tissues

As shown in Fig. 2, we found that the mRNA expression levels of HK2, PK, SDH, and PDHX for Se/Cd-treated group and Cd-treated group were lower than corresponding control group and Se-treated group. Compared with control group, the mRNA expression level of SDH for Cd-treated group decreased the most. For the Se/Cd-treated group, the mRNA expression level of HK2 decreased the least and the mRNA expression level of SDH decreased the most compared to the corresponding control group. Besides that, we found that the mRNA expression levels of HK2 and PDHK had almost no difference in Cd-treated group. Our results showed that the



Fig. 2 The mRNA expression of glucose metabolism-related genes in ovarian tissue of chickens. Different letters indicate significant differences ($P < 0.05$) in different treated groups at the same glucose metabolism-related genes expression. The data are shown as the means ± SD ($n = 12$)

mRNA expression of all the glucose metabolism-related genes for Se/Cd-treated group and Cd-treated group was significantly higher than that of corresponding control group and Se-treated group ($P < 0.05$).

## The mRNA Expression of Autophagy-Related Genes in Chicken Ovarian Tissues

As shown in Fig. 3, the mRNA expression levels of Beclin 1, dynein, Atg5, LC3-I, and LC3-II for Se/Cd-treated group and Cd-treated group were significantly higher than corresponding control group and Se-treated group. In the Se/Cd-treated group and Cd-treated group, the mRNA expression level of LC3-II increased the most and the mRNA expression level of LC3-I increased the least compared to that of the control group. Whereas the expression of mTOR in Cd-treated group
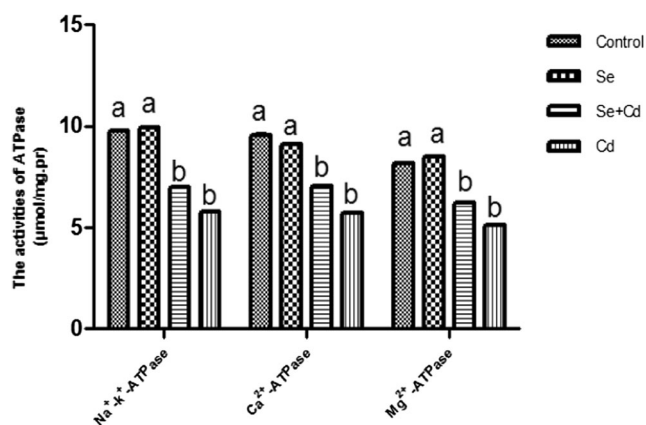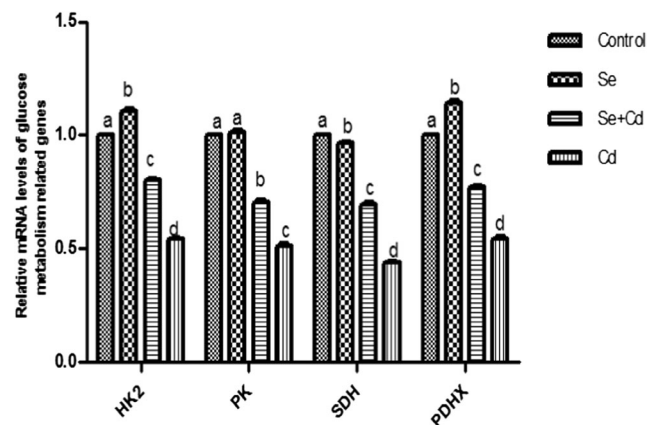


Fig. 1 The activities of ATPase in ovarian tissue in ovarian tissue of chickens. Bars with different letters represented statistically significant difference ($P < 0.05$) between the different treated groups at the same ATPase. Each value represents the mean ± SD ($n = 12$)
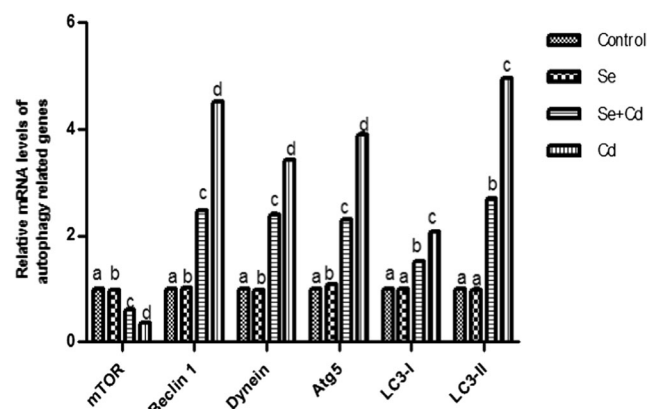


Fig. 3 The mRNA expression of autophagy-related genes in ovarian tissue of chickens. Different letters indicate significant differences ($P < 0.05$) at the same autophagy-related genes expression. Bars with different letters represented statistically significant difference ($P < 0.05$) in the different treated groups at the same autophagy-related gene. The data are shown as the means ± SD ($n = 12$)

was lower than other three groups. There was a significant difference between control group and Se/Cd-treated group or Cd-treated group ($P < 0.05$). Additionally, the ratio of LC3-I and LC3-II for Se/Cd-treated group was higher than that of the Cd-treated group.

## The Protein Expression of Autophagy-Related Genes in Chicken Ovarian Tissues

As shown in Fig. 4, the protein expression levels of Beclin 1, dynein, ATG5, LC3-I, and LC3-II for Se/Cd-treated group and Cd-treated group were significantly higher than corresponding control group and Se-treated group. The expression of LC3-II
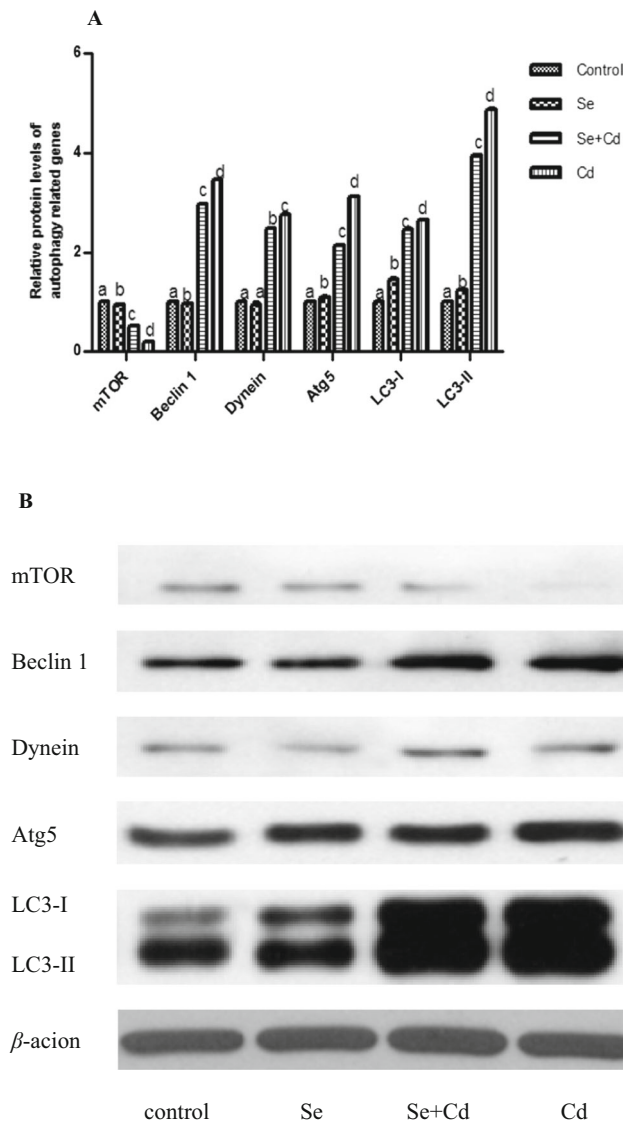


**Fig. 4** The protein expression of autophagy-related genes in ovarian tissue of chickens. **a** Different letters indicate significant differences ($P < 0.05$) in different treated groups at the same autophagy-related genes expression. The data are shown as the means ± SD ($n = 12$). **b** The immunoblotting of mTOR, Beclin 1, dynein, Atg5, LC3-I, LC3-II, and $\beta$-actin at various treated groups in chicken ovarian tissues

for Cd-treated group increased the most compared to control group. Whereas the protein expression of mTOR for Se/Cd-treated group and Cd-treated group was lower than corresponding control group and Se-treated group. Moreover, we could find there was a significant difference between control group and Se/Cd-treated group or Cd-treated group about the protein expression level of the autophagy-related genes ($P < 0.05$). Besides this, the ratio of LC3-I and LC3-II in Se/Cd-treated group was higher than that of Cd-treated group.

## Electron Microscopic Observation of Chicken Ovarian Tissues

To determine whether Se and Cd affect autophagy in the ovarian tissue, TEM was used to perform ultrastructural analysis. From the control group (Fig. 5a), we found the ovarian cell structure integrity, nuclear membrane was clear, and the structure of the mitochondria in the cytoplasm was integrated. From Cd-treated group (Fig. 5d), we found that Cd led to ultrastructure changes, including nuclear membrane shrinkage, variation of chondriosome's structure. In addition, there were several double-membrane vesicles appeared in Cd-treated cells; similar vesicles were rarely seen in the normal cells. The result showed the formation of autophagosomes and autophagolysosomes in Cd-treated cells, suggesting that Cd promotes autophagy in ovarian cells. From the Se/Cd-treated group (Fig. 5c), we also found the ultrastructure changes like the Cd-treated group; however, the degree was smaller.

## Discussion and Conclusion

From the experiment investigation, we found Cd exposure can cause mitochondrial damage. In eukaryotic cell, mitochondrion is the main site of energy metabolism, whereas mitochondrial damage can cause energy metabolism disorder [35]. We found that the activities of ATPase and the mRNA expression of glucose metabolism enzyme including HK2, PK, PDHX, and SDH decreased when the ovarian tissue of chickens were treated with Cd. Krstić et al. found some metals including Cd-inhibited $Na^+$-$K^+$-ATPase and $Mg^{2+}$-ATPase activities [36]. Kim et al. found a significant increase in $Na^+$-$K^+$-ATPase activity after exposure to Cd and Pb in a concentration-dependent manner [37]. Both HK2 and PK are the important enzymes in the anaerobic glycolysis process. HK2 can catalyze the conversion of glucose to glucose-6-phosphate; it is a key enzyme in the process of glycolysis. ATP is formed in the payoff phase of glycolysis by two reactions, one of which promoted by PK. PDHX and SDH are all involved in aerobic glycolysis pathway in the mitochondrial inner membranes. PDHX can oxidize pyruvate to acetyl coenzyme A and SDH is an essential enzyme in the citric acid cycle. Canesi et al. found a significant decrease in HK2 activity in Cu-exposed

**Fig. 5** The results of the ovarian tissue ultrastructure observations by electron microscopy. In control, Se-treated, Se/Cd-treated, and Cd-treated groups are shown respectively in **a**, **b**, **c**, and **d**. Magnification was 30,000 for all parts. Red arrow represents mitochondria. Green arrow represents chromatin aggregation. Yellow arrow represents autophagy vacuole
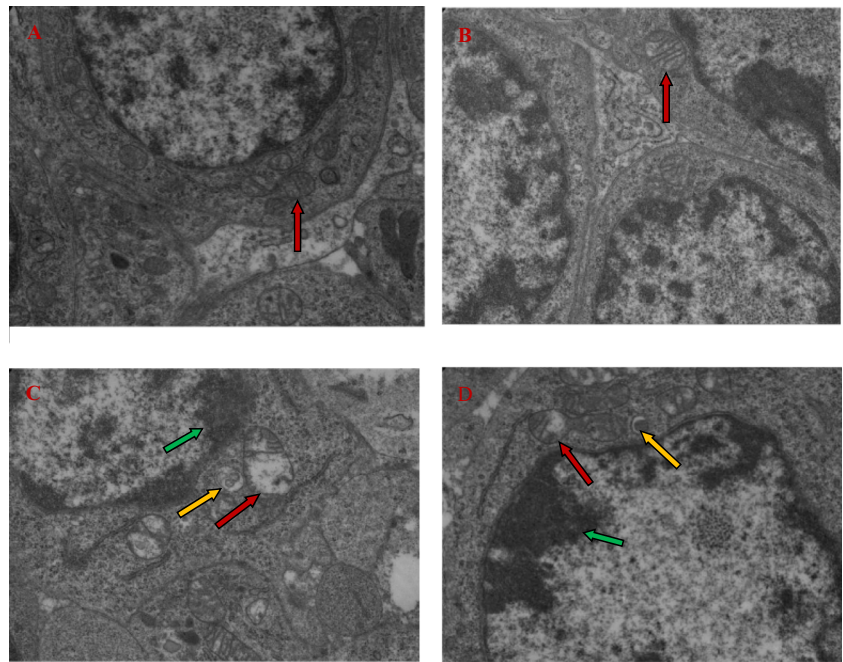


mussels [38]. Pysher et al. suggested that alterations in renal HK2 expression may be involved in arsenic-induced pathological conditions involving the kidney [39]. Our results are similar to those reported above. It is suggested that Cd toxicity could cause mitochondrial damage and then cause the disorder of energy metabolism.

Autophagy is an evolutionarily conserved lysosomal pathway of degrading cytoplasmic proteins and organelles. There is a close relationship between autophagy and energy metabolism. mTOR, a receptor of amino acids and ATP, is a key component that coordinately regulates the balance between growth and autophagy in response to cellular physiological conditions and environmental stress. The expression of mTOR is inhibited under nutrient starvation, which has been known as a crucial step for autophagy induction in eukaryotes. Lin et al. authenticated that during autophagy induced in cancer cells by (i) starvation through serum and amino acid deprivation or (ii) treatment with PI-103, a class I PI3K/mTOR inhibitor, glycolytic metabolism, would be affected [40]. Liu et al. found that the autosis (a unique type of autophagic cell death) is inhibited both in vitro and in vivo by cardiac glycosides, which are Na$^+$-K$^+$-ATPase antagonists used in clinical medicine [41]. The regulation of autophagy is a very complex process, including mTOR, LC3-I, LC3-II, Beclin 1, Atg5, and dynein. LC3 are considered important markers of autophagy; the amount of LC3-II or the LC3-II/LC3-I ratio correlates with the number of autophagosomes. Beclin 1 is a critical component of mammalian autophagy [42]; it is engaged in the initial stages of autophagosome formation and promotes the nucleation of the autophagosome. Dynein also has an essential role in the process of autophagy, because it brings

autophagosomes closer to lysosomes for subsequent fusion [43]. Increased levels of ATG can maintain stressful conditions during the autophagic process. If Atg5 gene was knocked out, autophagy would be blocked. Any changes in these genes can cause changes in autophagy. Some studies have indicated that Cd can induce autophagy in different organs and tissues of animals. Chargui et al. have suggested that rats' kidney cortex adapts to subtoxic Cd dose by activating autophagy [44]. Zou et al. found that autophagy can occur in rat liver cells treated with Cd; the mRNA expression of autophagy-related genes Atg5, Beclin 1, and LC3 increased [45]. Our results were consistent with the above reports; the expression of all autophagy-related genes increased except mTOR after Cd exposure in ovarian tissue of chickens. Therefore, we inferred Cd exposure can cause energy metabolism disorder by damaging mitochondria, at the same time, the expression of autophagy-related genes changed. This finally led to autophagy in ovarian tissue of chickens. The results of electron microscopy proved our conjecture. Taken together, we suggested that autophagy was involved in the damage process of chicken ovarian tissue induced by Cd.

Se can antagonize many heavy metals including Cd to reduce their toxic effects on organs and tissues of animals and alleviate the damage of mitochondria. Several studies have reported that Se can antagonize Cd, which reduce the toxicity of Cd to organs or tissues of animals. Banni et al. have suggested that the well-established protective effect Se against Cd-induced toxicity in rat liver passes through non-MT gene expression mechanisms [46]. Taskin et al. have indicated that Se could restore the functions of liver mitochondria after dysfunction induced by adriamycin in rats [47]. Our results

showed that both the activities of ATPase and the mRNA expression of glucose metabolism enzyme in the Se-treated group were higher than that of the Cd-treated group. This indicated that Se improved the energy metabolism disorder caused by Cd. Becker et al. found that the mRNA levels of the PK increased in diabetic rats after Se treatment [48]. Besides, results from our experiment found that the expression of autophagy-related genes (LC3-I, LC3-II, Beclin 1, Atg5, and dynein) was lower in Se antagonistic group than in Cd-treated group while mTOR increased significantly in the Cd-treated group. Therefore, we think that Se reduces the damage caused by Cd-induced autophagy in ovarian tissue of chickens. Wang et al. found that the expression of autophagy-related genes (Atg5, LC3-I, LC3-II, Beclin1, and dynein) increased when Se deficiency happened [49]. Santosh et al. found that Se could prevent glutamate-activating autophagy markers Beclin 1 and LC3-II [50]. As mentioned above, our study also demonstrated that Se can reduce the toxicity of Cd to the ovarian tissue of chickens by reducing autophagy.

In conclusion, our results emphasized that Cd exposure can cause energy metabolism disorder by mitochondrial injury and then induce autophagy, which cause damage to ovarian tissue of chickens. Whereas Se had an ability to inhibit Cd-induced autophagy in ovarian cells of chickens and provided mechanistic evidence that cytoprotective effects may mediate through improving energy metabolism and decreasing autophagy.

## Compliance with Ethical Standards

**Conflict of Interest**    The authors declare that they have no conflict of interest.

## References

1. Koyu A, Gokcimen A, Ozguner F, Bayram DS, Kocak A (2006) Evaluation of the effects of cadmium on rat liver. Mol Cell Biochem 284(1):81–85

2. Thijssen S, Maringwa J, Faes C, Lambrichts I, Van KE (2007) Chronic exposure of mice to environmentally relevant, low doses of cadmium leads to early renal damage, not predicted by blood or urine cadmium levels. Toxicology 229(1–2):145–156

3. Saksena SK, Dahlgren L, Lau IF, Chang MC (1977) Reproductive and endocrinological features of male rats after treatment with cadmium chloride. Biol Reprod 16(5):609–613

4. Haouem S, Hmad N, Najjar MF, El HA, Sakly R (2007) Accumulation of cadmium and its effects on liver and kidney functions in rats given diet containing cadmium-polluted radish bulb. Exp Toxicol Pathol Off J Gesellschaft Für Toxikologische Pathologie 59(1):77–80

5. Kluxen FM, Höfer N, Kretzschmar G, Degen GH, Diel P (2012) Cadmium modulates expression of aryl hydrocarbon receptor-associated genes in rat uterus by interaction with the estrogen receptor. Arch Toxicol 86(4):591–601

6. Zhang W, Pang F, Huang Y, Yan P, Lin W (2008) Cadmium exerts toxic effects on ovarian steroid hormone release in rats. Toxicol Lett 182(1–3):18–23

7. Höfer N, Diel P, Wittsiepe J, Wilhelm M, Degen GH (2009) Dose- and route-dependent hormonal activity of the metalloestrogen cadmium in the rat uterus. Toxicol Lett 191(2–3):123–131

8. Sheng Y, Sun B, Guo WT, Zhang YH, Liu X, Xing Y, Dong DL (2013) 3-Methyladenine induces cell death and its interaction with chemotherapeutic drugs is independent of autophagy. Biochem Biophys Res Commun 432(1):5–9

9. Xue L, Fletcher GC, Tolkovsky AM (1999) Autophagy is activated by apoptotic signalling in sympathetic neurons: an alternative mechanism of death execution. Mol Cellu Neurosci 14(3):180–198

10. Lim SC, Hahm KS, Lee SH, Oh SH (2010) Autophagy involvement in cadmium resistance through induction of multidrug resistance-associated protein and counterbalance of endoplasmic reticulum stress WI38 lung epithelial fibroblast cells. Toxicology 276(1):18–26

11. Chen M, Li X, Fan R, Yang J, Jin X, Hamid S, Xu S (2017) Cadmium induces BNIP3-dependent autophagy in chicken spleen by modulating miR-33-AMPK axis. Chemosphere 194:396–402

12. Eisenberglerner A, Kimchi A (2009) The paradox of autophagy and its implication in cancer etiology and therapy. Apoptosis 14(4): 376–391

13. Woods A, Heath R, Dickerson K, Hong SP, Momcilovic M, Johnstone SR, Carlson M, Carling D (2005) $Ca^{2+}$/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. Cell Metab 2(1):21–33

14. Mizushima N (2004) Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 6(6):1221–1228

15. Wang SH, Shih YL, Kuo TC, Ko WC, Shih CM (2009) Cadmium toxicity toward autophagy through ROS-activated GSK-3β in mesangial cells. Toxicol Sci 108(1):124–131

16. Dong Z, Wang L, Xu J, Li Y, Zhang Y, Zhang S, Miao J (2009) Promotion of autophagy and inhibition of apoptosis by low concentrations of cadmium in vascular endothelial cells. Toxicol in Vitro 23(1):105–110

17. Ramírez-Peinado S, León-Annicchiarico C, Iurlaro R, Muñoz-Pinedo C (2012) Cell death induced by inhibition of glucose metabolism: role of Bcl-2 proteins and autophagy. Exp Clin Endocrinol Diabetes 120(10):567–638

18. Moruno-Manchón JF, Pérez-Jiménez E, Knecht E (2013) Glucose induces autophagy under starvation conditions by a p38 MAPK-dependent pathway. Biochem J 449(2):497–506

19. Lock R, Roy S, Kenific CM, Su JS, Salas E, Ronen SM, Debnath J (2011) Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. Mol Biol Cell 22(2):165–178

20. Yao HD, Wu Q, Zhang ZW, Zhang JL, Li S, Huang JQ, Ren FZ, Xu SW, Wang XL, Lei XG (2013) Gene expression of endoplasmic reticulum resident selenoproteins correlates with apoptosis in various muscles of se-deficient chicks. J Nutr 143(5):613–619

21. El-Sharaky AS, Newairy AA, Badreldeen MM, Eweda SM, Sheweita SA (2007) Protective role of selenium against renal toxicity induced by cadmium in rats. Toxicology 235(3):185–193

22. Yao H, Liu W, Zhao W, Fan R, Zhao X, Khoso PA, Zhang Z, Xu S (2014) Different responses of selenoproteins to the altered expression of selenoprotein W in chicken myoblasts. RSC Adv 4(109): 64032–64042

23. Li X, Xing M, Chen M, Zhao J, Fan R, Xia Z, Cao C, Jie Y, Zhang Z, Xu S (2017) Effects of selenium-lead interaction on the gene expression of inflammatory factors and selenoproteins in chicken neutrophils. Ecotoxicol Environ Safety 139:447–453

24. Jin X, Xu Z, Zhao X, Chen M, Xu S (2017) The antagonistic effect of selenium on lead-induced apoptosis via mitochondrial dynamics pathway in the chicken kidney. Chemosphere 180:259–266

25. Yao HD, Wu Q, Zhang ZW, Shu L, Wang XL, Lei XG, Xu SW (2013) Selenoprotein W serves as an antioxidant in chicken myoblasts. Biochim Biophys Acta 1830(4):3112–3120

26. Messaoudi I, El HJ, Hammouda F, Saïd K, Kerkeni A (2009) Protective effects of selenium, zinc, or their combination on cadmium-induced oxidative stress in rat kidney. Biol Trace Elem Res 130(2):152–161

27. Ognjanovic B, Markovic S, Pavlovic S, Zikic R, As SZ (2008) Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium. Physiol Res 57(3):403–411

28. Lazarus M, Orct T, Jurasoviæ J, Blanuša M (2009) The effect of dietary selenium supplementation on cadmium absorption and retention in suckling rats. Biometals 22(6):973–983

29. Pappas AC, Zoidis E, Georgiou CA, Demiris N, Surai PF, Fegeros K (2011) Influence of organic selenium supplementation on the accumulation of toxic and essential trace elements involved in the antioxidant system of chicken. Food Addit Contam Part A Chem Anal Control Exposure Risk Assess 28(4):446–454

30. Alwaeli A, Zoidis E, Pappas AC, Demiris N, Zervas G, Fegeros K (2013) The role of organic selenium in cadmium toxicity: effects on broiler performance and health status. Animal 7(3):386–393

31. Pinto A, Juniper DT, Sanil M, Morgan L, Clark L, Sies H, Rayman MP, Steinbrenner H (2012) Supranutritional selenium induces alterations in molecular targets related to energy metabolism in skeletal muscle and visceral adipose tissue of pigs. J Inorg Biochem 114(9):47–54

32. Liu C, Fu J, Liu C, Li S (2015) The role of nitric oxide and autophagy in liver injuries induced by selenium deficiency in chickens. RSC Adv 5(62):50549–50556

33. Frustaci A, Sabbioni E, Fortaner S, Farina M, Torchio RD, Tafani M, Morgante E, Ciriolo MR, Russo MA, Chimenti C (2012) Selenium- and zinc-deficient cardiomyopathy in human intestinal malabsorption: preliminary results of selenium/zinc infusion. Eur J Heart Fail 14(2):202–210

34. Tan S, Chi Q, Liu T, Sun Z, Min Y, Zhang Z, Shu L (2017) Alleviation mechanisms of selenium on cadmium-spiked neutrophil injury to chicken. Biol Trace Elem Res 178:301–309

35. Wang L, Li J, Li J, Liu Z (2010) Effects of lead and/or cadmium on the oxidative damage of rat kidney cortex mitochondria. Biol Trace Elem Res 137(1):69–78

36. Krstić D, Krinulović K, Vasić V (2005) Inhibition of Na+-K+-ATPase and Mg2+-ATPase by metal ions and prevention and recovery of inhibited activities by chelators. J Enzyme Inhib Med Chem 20(5):469–476

37. Kim H, Lim B, Kim BD, Lee YM (2016) Effects of heavy metals on transcription and enzyme activity of Na+-K+-ATPase in the monogonont rotifer, Brachionus koreanus. Toxicol Environ Heal Sci 8(2):128–134

38. Canesi L, Ciacci C, Piccoli G, Stocchi V, Viarengo A, Gallo G (1998) In vitro and in vivo effects of heavy metals on mussel digestive gland hexokinase activity: the role of glutathione. Comp Biochem Physiol C: Pharmacol Toxicol Endocrinol 120(2):261–268

39. Pysher MD, Sollome JJ, Regan S, Cardinal TR, Hoying JB, Brooks HL, Vaillancourt RR (2007) Increased hexokinase II expression in the renal glomerulus of mice in response to arsenic. Toxicol Appl Pharmacol 224(1):39–48

40. Lin G, Andrejeva G, Ac WTF, Hill DK, Orton MR, Parkes HG, Koh DM, Robinson SP, Leach MO, Eykyn TR (2014) Reduced Warburg effect in cancer cells undergoing autophagy: steady-state 1H-MRS and real-time hyperpolarized 13C-MRS studies. PLoS One 9(3):e92645

41. Liu Y, Shoji-Kawata S, Jr SR, Wei Y, Ginet V, Zhang L, Posner B, Tran KA, Green DR, Xavier RJ (2013) Autosis is a Na+-K+-ATPase-regulated form of cell death triggered by autophagy-inducing peptides, starvation, and hypoxia-ischemia. Proc Natl Acad Sci U S A 110(51):20364–20371

42. Z Y SJ, C Y LAJ, Heintz N (2003) Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc Natl Acad Sci U S A 100(25):15077–15082

43. Cui J, Gong Z, Shen HM (2013) The role of autophagy in liver cancer: molecular mechanisms and potential therapeutic targets. Biochim Biophys Acta 1836(1):15–26

44. Chargui A, Zekri S, Jacquillet G, Rubera I, Ilie M, Belaid A, Duranton C, Tauc M, Hofman P, Poujeol P (2011) Cadmium-induced autophagy in rat kidney: an early biomarker of subtoxic exposure. Toxicol Sci 121(1):31–42

45. Zou H, Zhuo L, Han T, Hu D, Yang X, Wang Y, Yuan Y, Gu J, Bian J, Liu X (2015) Autophagy and gap junctional intercellular communication inhibition are involved in cadmium-induced apoptosis in rat liver cells. Biochem Biophys Rese Commun 459(4):713–719

46. Banni M, Messaoudi I, Said L, Heni JE, Kerkeni A, Said K (2010) Metallothionein gene expression in liver of rats exposed to cadmium and supplemented with zinc and selenium. Arch Environ Contam Toxicol 59(3):513–519

47. Taskin E, Dursun N (2015) Recovery of adriamycin induced mitochondrial dysfunction in liver by selenium. Cytotechnology 67(6):977–986

48. Becker DJ, Reul B, Ozcelikay AT, Buchet JP, Henquin JC, Brichard SM (1996) Oral selenate improves glucose homeostasis and partly reverses abnormal expression of liver glycolytic and gluconeogenic enzymes in diabetic rats. Diabetologia 39(1):3–11

49. Wang W, Zhang T, Lin H, Chang Y, Xing J (2016) Role of hydrogen sulfide on autophagy in liver injuries induced by selenium deficiency in chickens. Biol Trace Elem Res 175(1):194–203

50. Kumari S, Mehta SL, Li PA (2012) Glutamate induces mitochondrial dynamic imbalance and autophagy activation: preventive effects of selenium. PLoS One 7(6):e39382