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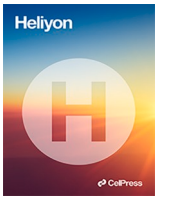

Research article

Evaluation of cadmium effects on the glucose metabolism on insulin resistance HepG2 cells

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ABSTRACT

Cadmium (Cd) is an environmental endocrine disruptor. Despite increasing research about the metabolic effects of Cd on HepG2 cells, information about the metabolic effects of Cd on insulin resistance HepG2 (IR-HepG2) cells is limited. Currently, most individuals with diabetes are exposed to Cd due to pollution. Previously, we reported that Cd exposure resulted in decreased blood glucose levels in diabetic mice, the underlying mechanism deserves further study. Therefore, we used palmitic acid (0.25 mM) to treat HepG2 cells to establish IR-HepG2 model. IR-HepG2 cells were exposed to $CdCl_2$ (1 μM and 2 μM). Commercial kits were used to measure glucose production, glucose consumption, ROS and mitochondrial membrane potential. Western blot and qRT-PCR were used to measure the proteins and genes of glucose metabolism. In the current study setting, we found no significant changes in glucose metabolism in Cd-exposed HepG2 cells, but Cd enhanced glucose uptake, inhibited gluconeogenesis and activated the insulin signaling pathway in IR-HepG2 cells. Meanwhile, we observed that Cd caused oxidative stress and increased the intracellular calcium concentration and inhibited mitochondrial membrane potential in IR-HepG2 cells. Cd compensatingly increased glycolysis in IR-HepG2 cells. Collectively, we found Cd ameliorated glucose metabolism disorders in IR-HepG2 cells. Furthermore, Cd exacerbated mitochondrial damage and compensatory increased glycolysis in IR-HepG2 cells. These findings will provide novel insights for Cd exposure in insulin resistant individuals.

1. Introduction

Cadmium (Cd) is an environmental contaminant with endocrine-disrupting effects. Due to rapid urbanization and industrialization in the last century, amount of farmland and water is polluted with Cd [1]. Non-occupational individuals are exposed to Cd through the consumption of contaminated food and water, leading to a gradual increase in the prevalence of Cd burden in human beings [1,2]. Cd is frequently detected in non-occupational individual biological samples, such as urine and serum, and has adverse health effects [3–5]. Besides the well-known osteotoxicity and nephrotoxicity, studies have shown that urinary Cd levels are associated with increased risk of diabetes [6–8]. Additionally, studies have reported that elevated blood Cd levels are closely associated with insulin resistance and hyperglycemia [9]. The animal study also demonstrated that Cd exposure increases blood glucose levels and induces diabetes in rats

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[10–12]. However, other studies found no significant association between Cd exposure and glucose metabolism [13–15]. All of the previous studies have focused on metabolic effects of Cd in healthy individuals or animals. However, with the increasing prevalence of diabetes and widespread Cd pollution, it is necessary to recognize the metabolic effects of Cd in diabetic individuals. Our previous study found that Cd played hypoglycemic effect in diabetic mice at low dosing exposure [16]. In addition, another study also reported that low dosing Cd exposure improved glucose tolerance in NAFLD mice [17].

Liver plays a critical role in glucose metabolism. Disrupted hepatic IRS-1/PI3K/AKT insulin signaling cascade, which is characterized by inhibited expression of PI3K and phosphorylation of AKT and promoted phosphorylation of IRS-1 (Ser307), induced hepatic insulin resistance. Hepatic insulin resistance led to decrease hepatic glucose uptake and glycogen synthesis and increased hepatic gluconeogenesis, ultimately contributing to hyperglycemia and type 2 diabetes [18–21]. Meantime, liver, as the largest detoxification organ of the organism, is one of the targets of Cd. Study reported that Cd disrupted hepatic IRS-1/PI3K/AKT insulin signaling cascade, leading to hepatic insulin resistance and hyperglycaemia in mice [22]. Additionally, study also reported that Cd increases hepatic gluconeogenesis and increases blood glucose levels in rats [23,24]. Besides Cd, other heavy metals, such as lead and mercury, have also been reported to induce hepatic gluconeogenesis and insulin resistance [25–27]. Intriguingly, different from these studies, our previous study found that Cd exposure resulted in decreased blood glucose levels in diabetic mice via inhibiting hepatic gluconeogenesis and promoting hepatic glycolysis [16].

HepG2 cells, a recognized model for the human hepatocyte, are widely applied for metabolic effects research [28]. Despite increasing research about the metabolic effects of Cd on HepG2 cells, information about the metabolic effects of Cd on insulin resistance HepG2 (IR-HepG2) cells is limited. Meanwhile, it is lacking confident evidence in our previous study which found that Cd played hypoglycemic effect in diabetic mice [16]. In the present study, we used palmitic acid (PA, 0.25 mM) treated HepG2 cells to establish classical IR-HepG2 model [29]. IR-HepG2 cells were exposed to Cd, exploring the detailed effects of Cd exposure on IR-HepG2 cells and the underlying mechanism. Currently, most individuals with diabetes are exposed to Cd due to pollution. Our findings will provide novel insights for Cd exposure in insulin resistant individuals.

2. Materials and methods

2.1. Cell culture

HepG2 cells (Procell, China) were cultured in DMEM (Procell, PM150210, China), 1 % penicillin/streptomycin (Beyotime, China) and 10 % FBS (Gibco, USA) at 37 °C in a humidified incubator of 5 % CO_2. When the HepG2 cells were grown to 80 %, we harvested the cells during the logarithmic phase of growth for the following experiments.

2.2. FFA-induced insulin resistance in HepG2 cells

HepG2 cells were seeded into a 96-well plate at a density of 1×10^5 cells/well overnight. After cell attachment, the medium was replaced by fresh complete DMEM. According to previous research for establishing IR-HepG2 model, cells (n = 3 for each concentration) were then exposed to PA (Sigma, P0500, USA) of varied concentrations 0.125 mM, 0.25 mM, 0.5 mM, and 1.0 mM for 24 h [29]. The glucose consumption and the proteins of insulin signaling pathways were determined to indicate the occurrence of insulin resistance.

2.3. Cell viability assay

HepG2 cells were seeded into a 96-well plate at a density of 1×10^5 cells/well. After overnight, cells (n = 3 for each concentration) were treated with cadmium chloride ($CdCl_2$, Sigma, 202908, USA) of a series of concentrations (0, 0.5, 1, 2, 5, 10, 20, 40 μM) and 0.25 mM PA and incubated for 24 h. Then the viability of cells was established by CCK-8 (Beyotime, C0037, China) assay following the manufacturer's instruction.

According to CCK8 results, cells were divided into six groups: Control group, 1 μM $CdCl_2$ group, 2 μM $CdCl_2$ group, IR-HepG2 cells group (PA, 0.25 mM), PA (0.25 mM) +1 μM $CdCl_2$ group, and PA (0.25 mM) +2 μM $CdCl_2$ group.

2.4. Glucose consumption assay

After treatment, following the manufacturer's instruction, the medium (n = 3 for each group) was determined for glucose content with glucose assay kit (Nanjing Jiancheng Bio-engineering Institute, F006-1-1, China). Glucose consumption was calculated as the difference of glucose concentration between the blank group and test groups (glucose consumption = glucose concentration of blank wells - glucose concentration of wells with cells).

2.5. Glucose production assay

Cells (n = 3 for each group) were washed three times with PBS to remove glucose, incubated for 5 h in 1 ml of glucose production medium (glucose and phenol red-free DMEM, containing gluconeogenic substrates, 20 mM sodium lactate, and 2 mM sodium pyruvate), and in the presence of 100 nM insulin (Solarbio, 11061-68-0, China) during the last 20 min. The medium was collected for the measurement of glucose concentration using a glucose assay kit.

2.6. Detection of lactic acid

The medium and cell pellets were collected to measure the contents of lactate (n = 3 for each group) using corresponding commercial kits (Nanjing Jiancheng Bio-engineering Institute, A020-2-2, China).

2.7. Detection of mitochondrial membrane potential

Detection of the mitochondrial membrane potential (n = 3 for each group) was performed using an enhanced mitochondrial membrane potential assay kit with JC-1 (Beyotime, C2003S China). 500,000 cells/sample were added with 500 μL JC-1 and analyzed for their fluorescence intensity using flow cytometry instrument (Beckman Coulter, USA).

2.8. Detection of Ca^{2+} levels

The collected cells (n = 3 for each group) were incubated in 5 μM Fluo-3 AM (Beyotime, S1056, China) at 37 °C for 20 min according to the instructions of manufacturer. Then, the cells were washed with PBS and incubated at 37 °C for 20 min. The intracellular Ca^{2+} levels were measured by flow cytometry instrument.

2.9. Measurement of reactive oxygen species (ROS)

The cells (n = 3 for each group) were washed with PBS and incubated with 10 μM DCFH-DA (Beyotime, S0033S, China) at 37 °C for 20 min. Cells were then washed twice with PBS and analyzed by flow cytometry instrument or microscope (Olympus, Japan).

2.10. Quantitative real-time PCR (qRT-PCR)

FastPure®Cell/Tissue Total RNA Isolation Kit V2 (Vazyme, China) was used to extract total RNA from cells (n = 3 for each group). RNA concentration was quantified by NanoDrop Ultra-Micro spectrophotometer (Thermo, USA). Total RNA (1000 ng/sample) was reverse-transcribed into cDNA using the PrimeScript RT reagent Kit (Takara, JAPAN), followed by qPCR with SYBR Green (Vazyme, China) using QuantStudio6 Flex Real-Time PCR System (Thermo, USA). β-actin was reference. The sequences of the forward (F) and reverse (R) primers such as glucose transporter 2 (GLUT2), pyruvate kinase M2 (PKM2), lactate dehydrogenase A (LDHA), glucose-6-phosphatase (G6pase), phosphoenolpyruvate carboxykinase 1 (PCK-1), glutathione peroxidase 4 (GPX4), catalase (CAT), interleukin6 (IL-6), interleukin1β (IL-1β), and tumor necrosis factor α (TNF-α) were presented in Table 1.

2.11. Western blot

The collected cells (n = 3 for each group) were lysed in RIPA buffer (Fudebio-tech, China), and the lysate was centrifuged at 12,000×*g* at 4 °C for 10 min. The levels of proteins in the supernatant were determined using a BCA kit (Beyotime, China). Equal amounts of cell lysate (30 μg protein) were separated by 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Beyotime, China) and were transferred to polyvinylidene difluoride (PVDF, Millipore, USA) membrane. The membranes were blocked with 5 % non-fat dry milk for 1.5 h, and then incubated with the different primary antibodies such as phosphorylation-insulin receptor substrate-1 (P-IRS-1, Ser307, 2381, 1:1000, CST), IRS-1 (3407S, 1:1000, CST), phosphoinositide 3-kinase (PI3K, 4249, 1:1000, CST), hexokinase-2(HK-2, 2867, 1:1000, CST), PKM-2 (4053, 1:1000, CST), LDHA (2012, 1:1000, CST), phosphorylation-glycogen synthase kinase-3β (P-GSK-3β, 5558, 1:1000, CST), GSK-3β (27C10, 1:1000, CST), glycogen phosphorylase (PYGL, A6710, 1:1000, Abcloanl), phosphorylation-protein kinase B (P-AKT, AP1453, 1:1000, Abcloanl), and AKT (A22533, 1:1000, Abcloanl) overnight. After washing the membrane with TBST, the membranes were incubated at room temperature with secondary antibodies for 1 h. The immunoblots were visualized by enhanced chemiluminescence (Fudebio-tech, China) and captured by Chemiluminescent Imaging System (Tanon Science & Technology, China). Relative levels of protein were quantified by ImageJ software.

Table 1
The primer sequences for RT-PCR.

Gene name	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
β-actin	CACAGAGCCTCGCCTTTGC	CCATCACGCCCTGGTGC
GLUT2	AATTGCTCCAACCGCTCTCA	CTAATAAGAATGCCCGACGAT
PKM-2	TGTCTGGAGAAACAGCCAAG	TCCTCGAATAGCTGCAAGTG
LDHA	ATGGCAACTCTAAAGGATCAGC	CCAACCCCAACAACTGTAATCT
G6pase	TACGTGATGGTCACATCTACTCT	TTCTGCAACAGCAATGCCTGA
PCK-1	GGCTACAACTTCGGCAAATACC	GGAAGATCTTGGGCAGTTTGC
IL-6	GCCAGAGCTGTGCAGATGAG	TCAGCAGGCTGGCATTTG
TNF-α	AGCCCTGGTATGAGCCCATCTATC	TCCCAAAGTAGACCTGCCCAGAC
IL-1β	AGCTACGAATCTCCGACCAC	CGTTATCCCATGTGTCGAAGAA
GPX4	CCGCTGTGGAAGTGGATGAAGATC	CTTGTCGATGAGGAACTGTGGAGAG
CAT	GTGCGGAGATTCAACACTGCCA	CGGCAATGTTCTCACACAGACG

2.12. Statistical analysis

All quantitative data were expressed as the mean ± SD. Comparisons between two groups were analyzed using Student's *t*-test, and comparisons between more than two groups were made using One-way ANOVA with Student–Newman–Keuls (SNK) multiple comparison test to identify differences among means. A value of $P < 0.05$ was considered statistically significant. Statistical analyses were performed by SPSS 25.0, and graphs were created using GraphPad Prism 8.

3. Results

3.1. IR-HepG2 cell model establishment and Cd treatment

HepG2 cells were exposed to varying concentrations of PA (0.125 mM, 0.25 mM, 0.5 mM, and 1 mM). The lowest glucose consumption levels were observed in HepG2 cells exposed to 0.25 mM PA ($P < 0.001$, Fig. 1A). Additionally, the phosphorylation level of AKT was significantly decreased ($P < 0.01$, Fig. 1B and C) in HepG2 cells exposed to 0.25 mM PA. The expression of *PCK-1* and *G6pase* was significantly up-regulated, and the level of glucose production was increased in HepG2 cells exposed to 0.25 mM PA ($P < 0.05$, Fig. 2C–E).

HepG2 cells were treated with different concentrations of $CdCl_2$ (0–40 μM) for 24 h. The viability of HepG2 cell was significant decreased when $CdCl_2$ concentration exceeded 10 μM ($P < 0.05$, Fig. 1D). Culturing cells with varying concentrations of $CdCl_2$ (0–20 μM) and 0.25 mM PA for 24 h revealed that Cd significantly influenced the viability of IR-HepG2 cells when the $CdCl_2$ concentration exceeded 5 μM ($P < 0.05$, Fig. 1E). So, 1 μM and 2 μM $CdCl_2$ were finally selected for subsequent experiments.

3.2. Cd improved glucose metabolism disorders

Compared to the control, the glucose consumption was no significant change in Cd-exposed HepG2 cells, IR-HepG2 cells showed lower glucose consumption levels ($P < 0.05$, Fig. 2A). However, Cd (1 μM and 2 μM) significantly increased glucose consumption levels in IR-HepG2 cells ($P < 0.01$, Fig. 2A). It was found that Cd (1 μM and 2 μM) had no effects on the expression of *GLUT2* in HepG2 cells. The expression of *GLUT2* in IR-HepG2 cells was lower than that in the control. Cd (1 μM and 2 μM) significantly up-regulated the expression of *GLUT2* in IR-HepG2 cells ($P < 0.05$, Fig. 2B).

Compared with the control, IR-HepG2 cells exhibited significantly increased glucose production levels and significant up-regulation of expression of *PCK-1* and *G6pase* ($P < 0.05$, Fig. 2C–E). In comparison with IR-HepG2, Cd (2 μM) significantly decreased glucose production levels and down regulated the expression of *PCK-1* and *G6pase* in IR-HepG2 cells ($P < 0.05$, Fig. 2C–E).

The protein expression of P-GSK-3β/GSK-3β in IR-HepG2 cells was significantly decreased compared with the control ($P < 0.01$,

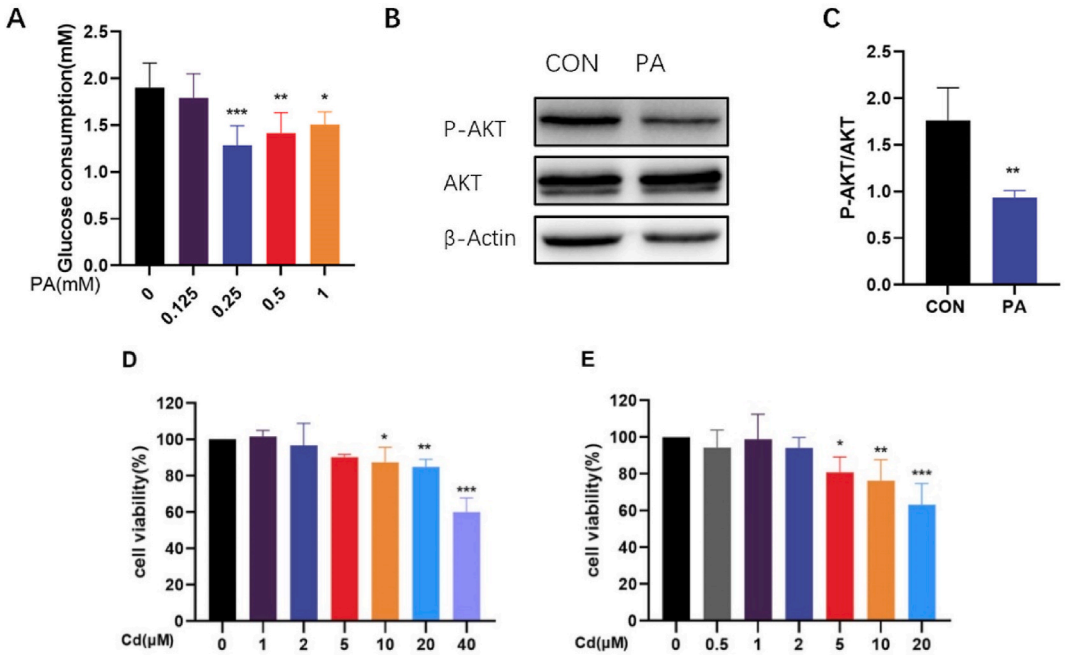

Fig. 1. IR-HepG2 cell model establishment and Cd treatment (n = 3 for each group). A: Glucose consumption levels; B: Protein expression of P-AKT and AKT in HepG2 cells treated with 0.25 mM PA; C: The quantization of P-AKT/AKT protein expression levels; D: Effect of Cd on HepG2 cell viability; E: Effect of Cd on IR-HepG2 cells viability. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, vs. CON group.

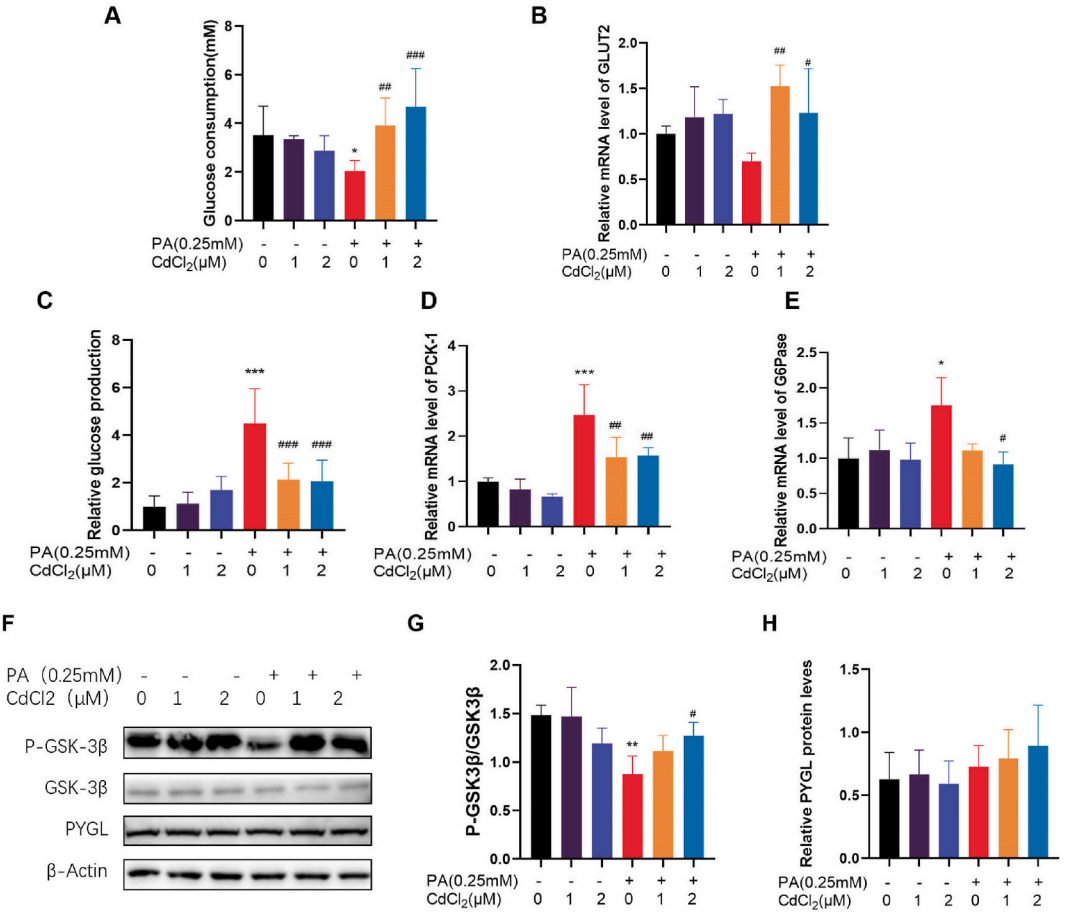

Fig. 2. The effect of Cd on glucose metabolism in IR-HepG2 cells (n = 3 for each group). A: Glucose consumption levels; B: The quantization of *GLUT2* relative expression level; C: Glucose production levels; D: The quantization of *PCK-1* relative expression levels; E: The quantization of *G6Pase* relative expression levels; F: The expression of glycogen synthesis and decomposition related protein; G: The quantization of P-GSK-3β/GSK-3β protein expression levels; H: The quantization of PYGL protein expression levels. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, vs. CON group. #$P < 0.05$, # #$P < 0.01$, # # #$P < 0.001$, vs. IR-HepG2 cells group.

Fig. 2F and G). Cd (2 μM) significantly promoted the protein expression of P-GSK-3β/GSK-3β in IR-HepG2 cells ($P < 0.05$, Fig. 2F and G). The expression of PYGL was not significant change in Cd (1 μM and 2 μM) exposed HepG2 cells, IR-HepG2 cells and Cd (1 μM and 2 μM) exposed IR-HepG2 cells ($P > 0.05$, Fig. 2H).

3.3. Cd activated IRS-1/PI3K/AKT signaling pathway

Compared with the control, it was found that Cd (1 μM and 2 μM) had no effects on the expression of phosphorylated IRS-1

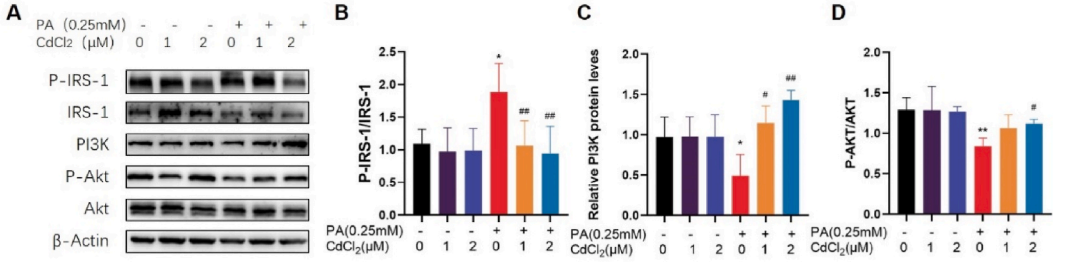

Fig. 3. The effect of Cd on IRS-1/PI3K/AKT pathway in IR-HepG2 cells (n = 3 for each group). A The expression of IRS-1/PI3K/AKT pathway related protein; B: The quantization of P-IRS-1 (Ser307)/IRS-1 protein expression levels; C: The quantization of PI3K protein expression levels; D: The quantization of P-AKT/AKT protein expression levels. *$P < 0.05$, **$P < 0.01$, vs. CON group; #$P < 0.05$, # #$P < 0.01$, vs. IR-HepG2 cells group.

(Ser307), PI3K and phosphorylated AKT in HepG2 cells. There was a significant increase in the phosphorylation level of IRS-1 (Ser307), a significant decrease in the expression of PI3K protein, and a significant decrease in the phosphorylation level of AKT in IR-HepG2 cells ($P < 0.05$, Fig. 3A–D). Compared with IR-HepG2, significantly decreased phosphorylation levels of IRS-1(Ser307) and increased expression of PI3K protein were found in Cd exposed (1 μM and 2 μM) IR-HepG2 cells, and significantly increased phosphorylation levels of AKT were only found in 2 μM Cd exposed IR-HepG2 cells ($P < 0.05$, Fig. 3A–D).

3.4. Cd enhanced glycolysis

Compared to the control, the protein expression of HK-2, PKM-2, and LDHA were not significantly change in IR-HepG2 cells. Cd (2 μM) significantly increased the protein expression of HK-2, PKM-2 and LDHA in IR-HepG2 cells ($P < 0.05$, Fig. 4A–F). The intracellular lactic acid level in IR-HepG2 cells was slightly higher than that in the control, but the difference was not statistically significant ($P = 0.48$, Fig. 4G). The lactate in the IR-HepG2 cell culture medium was significantly higher than that in the control, and Cd at dosing of 2 μM significantly increased the lactate in the IR-HepG2 cell culture medium ($P < 0.001$, Fig. 4H).

3.5. Cd aggravated mitochondria damage and oxidative stress

Mitochondrial membrane potential (MMP) levels in IR-HepG2 cells were significantly lower than those in the control ($P < 0.001$, Fig. 5A). Cd (1 μM and 2 μM) significantly reduced MMP levels in IR-HepG2 cells ($P < 0.001$, Fig. 5A). Compared with the control group, the intracellular calcium levels were significantly increased in Cd-exposed (1 μM and 2 μM) HepG2 cells and IR-HepG2 cells ($P < 0.001$, Fig. 5B). Cd (1 μM and 2 μM) substantially and significantly increased intracellular calcium levels in IR-HepG2 cells ($P < 0.001$, Fig. 5). Compared to the control group, increased ROS were found in IR-HepG2 cells, but it was not statistically significant. Cd (2 μM) significantly increased intracellular ROS levels in IR-HepG2 cells ($P < 0.05$, Fig. 5C and D). Significant inhibition of gene expression of antioxidant such as *GPX4* and *CAT* were found in IR-HepG2 cells, Cd (2 μM) significantly inhibited the gene expression of *GPX4* in IR-HepG2 cells ($P < 0.05$, Fig. 5E).

3.6. Cd promoted the inflammatory response

Compared with the control, IR-HepG2 cells exhibited a significant up-regulation in the expression of *TNF-α* and *IL-1β* ($P < 0.05$, Fig. 6B and C), while there was no significant alteration in the expression of *IL-6* ($P = 0.85$, Fig. 6A). Compared with IR-HepG2, Cd (1 μM) significantly up-regulated expression of *IL-6* in IR-HepG2 cells ($P < 0.05$, Fig. 6A). Cd (2 μM) significantly up-regulated expression of *TNF-α* and *IL-1β* in IR-HepG2 cells ($P < 0.05$, Fig. 6B and C).

4. Discussion

Several lines of evidence show that Cd is closely associated with insulin resistance and glucose metabolic disorders in epidemiological or experimental studies [6,11,30]. However, the glucose metabolic effects of Cd on insulin resistant individuals are scarce. Previously, we reported that Cd exposure resulted in decreased blood glucose levels in diabetic mice via inhibiting hepatic gluconeogenesis and promoting hepatic glycolysis [16]. In current study, we found that Cd (1 μM and 2 μM) had no obvious effect on glucose metabolism in HepG2 cells. However, Cd enhanced glucose uptake, inhibited gluconeogenesis and activated the insulin signaling pathway in IR-HepG2 cells.

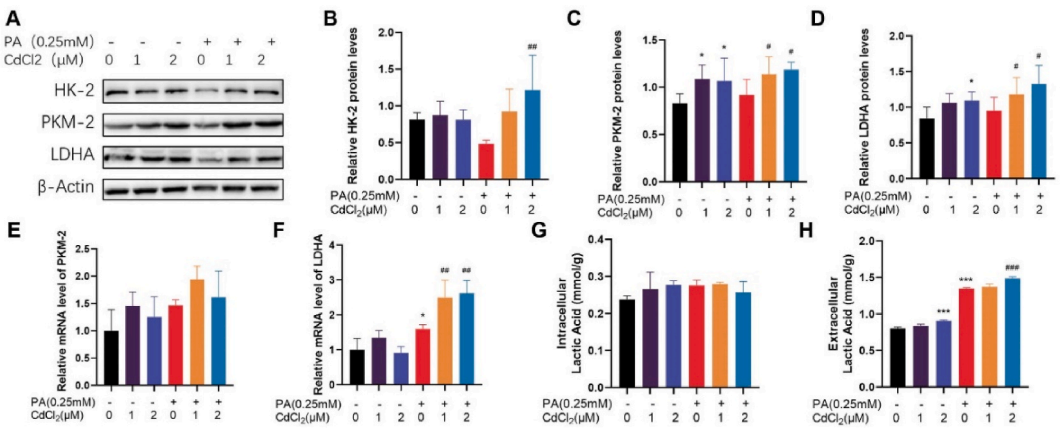

Fig. 4. The effect of Cd on glycolysis in IR-HepG2 cells (n = 3 for each group). A: The expression of glycolysis related protein; B: The quantization of HK-2 protein expression levels; C: The quantization of PKM-2 protein expression levels; D: The quantization of LDHA protein expression levels; E: The quantization of *PKM-2* relative expression levels; F: The quantization of *LDHA* relative expression levels; G: Intracellular lactate levels; H: Extracellular lactate levels.*$P < 0.05$, ***$P < 0.001$, vs. CON group; #$P < 0.05$, # #$P < 0.01$, # # #$P < 0.001$, vs. IR-HepG2 cells group.

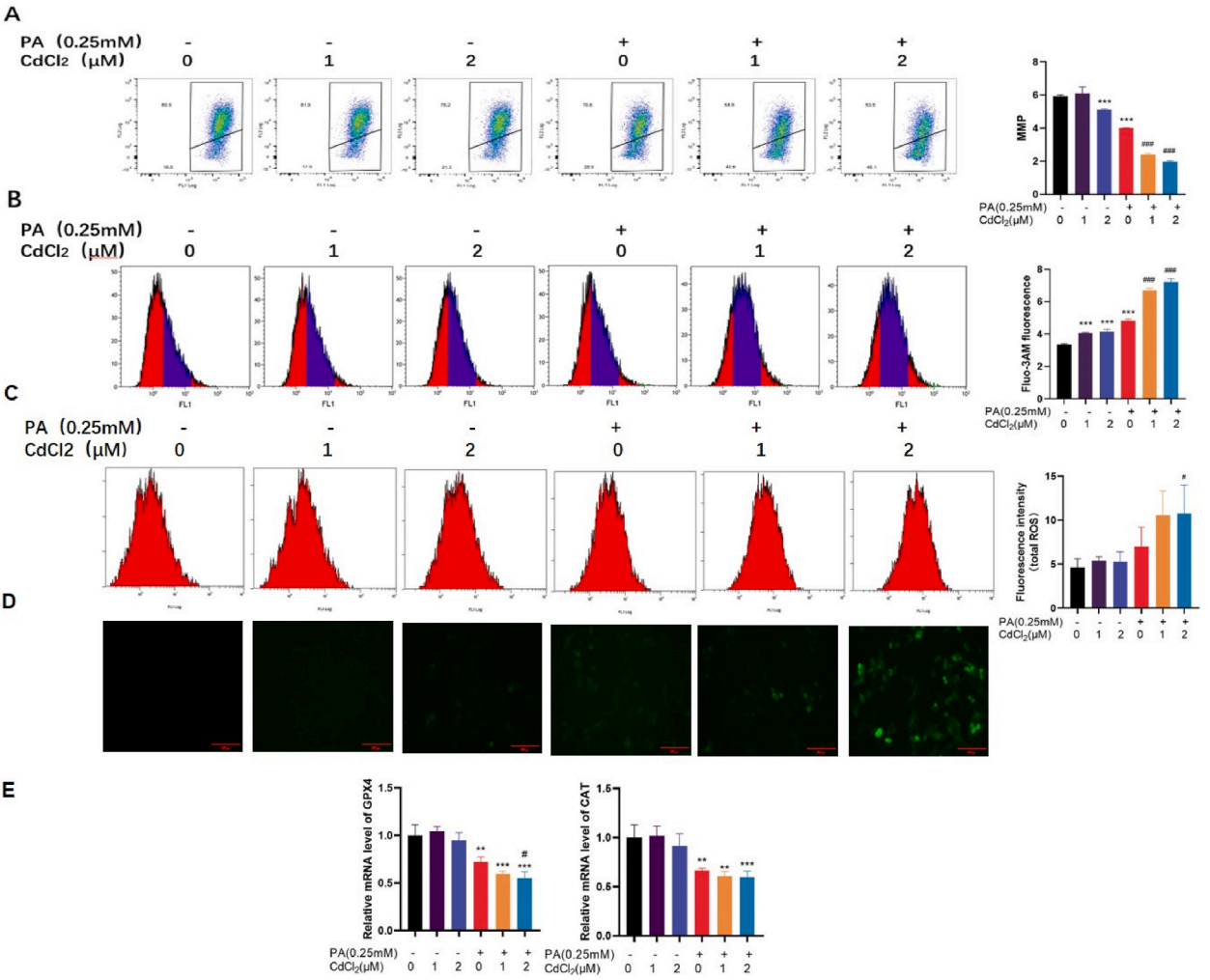

Fig. 5. The effect of Cd on mitochondrial damage and oxidative stress in IR-HepG2 cells (n = 3 for each group). A: Mitochondrial membrane potential levels; B: Calcium level; C: ROS levels; D: Intracellular ROS fluorescence levels; E: The quantization of *GPX4* and *CAT* relative expression levels. $^{**}P < 0.01$, $^{***}P < 0.001$, vs. CON group; $\#P < 0.05$, $\#\ \#\ \#P < 0.001$, vs. IR-HepG2 cells group.

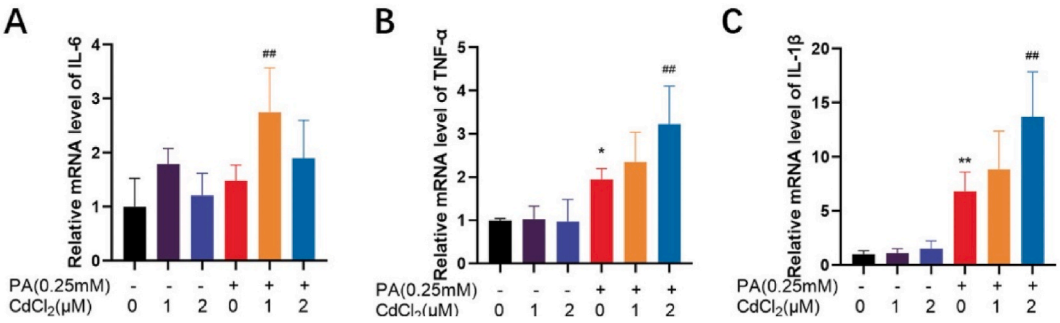

Fig. 6. The effect of Cd on inflammatory factors in IR-HepG2 cells (n = 3 for each group). A: The quantization of *IL-6* relative expression levels; B: The quantization of *TNF-α* relative expression levels C: The quantization of *IL-1β* relative expression levels. $^{*}P < 0.05$, $^{**}P < 0.01$, vs. CON group; $\#\ \#P < 0.01$, vs. IR-HepG2 cells group.

The effects of Cd exposure on HepG2 cells are different from IR-HepG2 cells. No obvious effects on glucose metabolism were found in HepG2 cells exposed Cd. It is reported that HepG2 cells were exposed to 3 μM Cd for 72 h resulted in reduced glucose uptake and increased gluconeogenesis. Human mature adipocytes were treated with 10 μM Cd for 48 h resulted in insulin resistance [31,32]. The

inconsistent results might be due to the low exposure dosing and short exposure period of Cd in current study. However, lack of detection of intracellular Cd concentration and metallothionein levels were the limits of the current study.

Free fatty acids, such as PA, can successfully induce insulin resistance in HepG2 cells [29,33,34]. Hepatic insulin resistance is characterized by suppressed glycogen synthesis, enhanced gluconeogenesis, compromised cellular glucose uptake, and disrupted insulin signaling cascade [35]. In the current study, we found that PA treated HepG2 cells exhibited reduced glucose uptake and increased gluconeogenesis. We also found disrupted IRS-1/PI3K/AKT insulin signaling cascade in PA-treated HepG2 cells, which was evidenced by significantly reduced expression of PI3K protein and phosphorylation of AKT, as well as significantly increased phosphorylation of IRS-1 (Ser307). These findings indicated that PA-treated HepG2 cells developed glucose metabolic disorder and insulin resistance [29].

Cd ameliorated the glucose metabolic disorder in IR-HepG2 cells. The improvement effect of Cd on glucose metabolic disorder in IR-HepG2 cells was evident by the activation of the insulin signaling pathway, which promoted glucose uptake and inhibited gluconeogenesis. In the current study, we found that Cd increased glucose consumption levels and significantly upregulated the expression of GLUT2 in IR-HepG2 cells, indicating that Cd enhanced glucose uptake. This may be related to that Cd activated insulin signaling pathway in IR-HepG2 cells. Our study observed that Cd promoted PI3K expression and induced AKT phosphorylation, suggesting that Cd activated the insulin signaling pathway. The activation of insulin signaling pathway facilitated GLUT2 translocation, which in turn improved glucose transport into hepatocytes to increase glucose uptake [36]. In parallel with our results, a study also reported that carboxylate of Cd enhanced glucose uptake in diabetic mice by activated insulin signaling pathway [37].

The activation of the insulin signaling pathway also promotes glycogen synthesis and suppresses hepatic gluconeogenesis. Our study found that Cd increased the phosphorylation of GSK-3β, a key enzyme in glycogen synthesis, suggesting a potential role for Cd in promoting glycogen synthesis in IR-HepG2 cells. Similar findings were also found that Cd increased GSK-3β activity and hepatic glycogen synthesis in Wistar rats [38]. Meanwhile, we found that Cd suppressed the expression of G6Pase and PCK-1, which are critical enzymes in hepatic gluconeogenesis, resulting in inhibited glucose production in IR-HepG2 cells. These findings provided clear support for our previous study that the inhibitory effect of Cd on hepatic gluconeogenesis, led to decreased blood glucose in diabetic mice [16].

A key observation of our current study is when Cd had no obvious effects on glucose metabolism in HepG2 cells, it had an ameliorated effect on glucose metabolism disorder in IR-HepG2 cells. We speculate that the result might be the hormesis effect of low-dose Cd in a state of metabolic disorder. Coincidentally, one study also reported that Cd improved hepatic metabolism disorder in NAFLD mice at low dosing exposure [17]. Likewise, some pollutants such as perfluorooctanoate and bisphenol F have been reported to improve metabolism disorder in metabolic syndrome animals at low dosing exposure [39,40]. However, the ameliorated effects of Cd on glucose metabolism under insulin resistance are never reported. This phenomenon deserves further exploration.

Currently, because of widespread Cd pollution, the effects of Cd exposure on glucose metabolism attract considerable attention. However, there is no consensus on the relationship between Cd and glucose metabolism disorder. Several cross-sectional studies have reported that Cd exposure induces insulin resistance, disturbs glucose metabolism in adolescents, and increases the prevalence of prediabetes in adults [30,41,42]. Likewise, chronic Cd exposure has also been linked to hepatic insulin resistance and increased blood glucose levels in rats [10,43]. In contrast, some studies have found no association between Cd exposure and glucose metabolism disorder [44–46]. One epidemiological investigation found that urinary Cd levels were not associated with diabetes [45]. Moreover, a study found that blood Cd levels were not linked to diabetes prevalence after adjusting for age, sex, BMI, region, smoking, alcohol consumption, and other factors [13]. In animal experiments, Cd exposure did not result in insulin resistance and hyperglycemia in mice [14]. In current study, we found that Cd (1 μM and 2 μM) had no significant effects on glucose metabolism in HepG2 cells, it had an ameliorated effect on glucose metabolism disorder in IR-HepG2 cells. The different roles of Cd in glucose metabolism might be influenced by multiple factors, including the toxicokinetics of Cd in vivo and in vitro, exposure duration or doses, nutritional condition, or other unknown factors.

In current study, we found that Cd caused mitochondrial impairment and oxidative stress in IR-HepG2 cells. Meanwhile, Cd significantly inhibited the expression of GPX4 in IR-HepG2 cells and reduced antioxidant capacity of IR-HepG2 cells. Cd substantially increased intracellular calcium content in IR-HepG2 cells, inducing mitochondrial calcium overload, which led to the decrease of mitochondrial membrane potential and induced mitochondria damage [47]. Additionally, damaged mitochondria produced ROS and inhibited antioxidant levels, which further damage mitochondria [48–50].

As an adaptive response to mitochondrial damage, cells may enhance glycolysis to meet energy needs [51,52]. Our study found that Cd increased the expression of key glycolytic enzymes, HK2 and PKM2 in IR-HepG2 cells, enhancing glycolytic capacity, which increased glucose consumption. These observations align with findings from our previous research, which Cd significantly promoted liver glycolysis, leading to decrease blood glucose in diabetic mice [16]. Nevertheless, our research found that Cd increased the expression of LDHA in IR-HepG2 cells and elevated lactic acid levels in the culture medium. Based on our results, we speculate that Cd-induced mitochondrial damage hinders pyruvate, a product of glycolysis, transport into the mitochondria. This interruption prevents pyruvate proceeding TCA cycle to generate energy, leading to compensatory increases in glycolysis in cytoplasm to meet energy needs. Moreover, it simultaneously promotes the conversion of pyruvate into lactic acid in the cytoplasm, resulting in elevated lactic acid levels. The elevation of lactic acid levels could then potentially induce an inflammatory response [53–55]. In current study, we found that Cd aggravated inflammation in IR-HepG2 cells. Therefore, despite Cd ameliorates glucose metabolism disorder in IR-HepG2 cells, it is also necessary to further study the negative effects caused by Cd in IR-HepG2 cells.

One study reported that HepG2 might not be the most accurate model to study liver insulin signaling [56]. In current study, the only HepG2 cell line was utilized, which was a major limit. Other hepatocyte cell line or primary hepatocyte cell should be test in the future study. Additionally, mechanism of Cd activates insulin signaling pathway in IR-HepG2 cells while causing mitochondrial damage and oxidative stress is not fully understood. We speculate that the two kinds of effects were through different pathways. However, it can not

be excluded other possibilities. There are evidence indicating that ROS are involved in activation of insulin signaling pathway [57,58]. Moreover, the long-term effects of Cd exposure on HepG2 and IR-HepG2 cells were unclear. Further study in this field would help us to understand glucose metabolism in diabetes under Cd pollution.

5. Conclusions

Our study is the first to find low dosing Cd improved glucose metabolism disorder in IR-HepG2. These findings are consistent with our previous animal study and provide novel insights into Cd exposure in insulin resistant individuals. Nevertheless, we can not ignore the toxicity of Cd. A number of studies have reported that Cd, as an environmental endocrine disruptor, can lead to hepatocyte glucose metabolism disorder.

Data and code availability

Data will be made available on request.

CRediT authorship contribution statement

Changhao Li: Writing – original draft, Investigation, Conceptualization. **Ke Lin:** Investigation, Data curation, Conceptualization. **Liang Xiao:** Data curation, Conceptualization. **Yilimilai Dilixiati:** Data curation, Conceptualization. **Yuan Huo:** Data curation, Conceptualization. **Zengli Zhang:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37325.

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