Drug and Chemical Toxicology

ISSN: 0148-0545 (Print) 1525-6014 (Online) Journal homepage: www.tandfonline.com/journals/idct20

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To cite this article: Wenxin Wang, Shuhua Xi & Yue Wang (03 Jan 2026): N-nitrosodimethylamine increased glucose production by promoting hyperglycemia in hepatocyte via AMPK signaling pathway in vivo and in vitro, Drug and Chemical Toxicology, DOI: 10.1080/01480545.2025.2609950

To link to this article: https://doi.org/10.1080/01480545.2025.2609950

DRUG AND CHEMICAL TOXICOLOGY
https://doi.org/10.1080/01480545.2025.2609950

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RESEARCH ARTICLE

N-nitrosodimethylamine increased glucose production by promoting hyperglycemia in hepatocyte via AMPK signaling pathway in vivo and in vitro

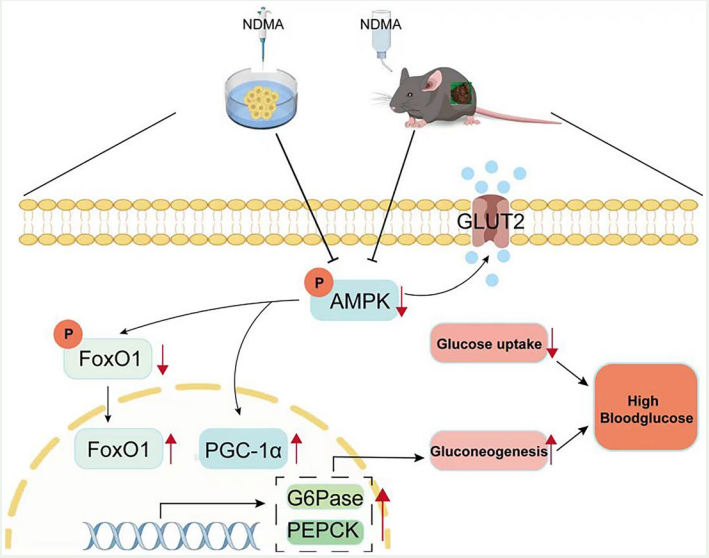
Wenxin Wang[b,c], Shuhua Xi[b,c] and Yue Wang[a,b]

[a]School of Public Health, Shenyang Medical College, Shenyang, China; [b]Key Laboratory of Environmental Stress and Chronic Disease Control and Prevention, Ministry of Education, China Medical University, Shenyang, China; [c]Department of Environmental Health, School of Public Health, China Medical University, Shenyang, China

ABSTRACT

N-nitrosodimethylamine (NDMA), as a disinfection by-product of drinking water, has been detected in various water. Epidemiological investigations have found that exposure to NDMA can exacerbate the development of diseases related to insulin resistance. In this study, male C57BL/6 mice were exposed to 0.5 and 12.5 mg/L NDMA by drinking water for 12 weeks. The mice in 12.5 mg/L NDMA group appeared fasting blood glucose elevation, and glucose tolerance and insulin sensitivity decreased. NDMA exposure enhanced hepatic gluconeogenesis and suppressed glycolysis of mice, but not affecting glycogen synthesis. In addition, NDMA exposure inhibited the phosphorylation of AMPK and FoxO1 as well as GLUT2 protein expression, and increased PGC-1α protein levels. GLUT2 protein decreasing could reduce glucose uptake of hepatocyte and enhance blood glucose concentration. Dephosphorylated FoxO1 might translocate to the nucleus, binding promoters of gluconeogenesis key enzymes PEPCK and G6Pase to upregulate their expression and promote gluconeogenesis. PGC-1α could also stimulate the expression of PEPCK and G6Pase. Furthermore, human normal liver cells (MIHA) were treated with NDMA for 24 hours. AMPK as a central energy sensor, regulates hepatic glucose production, uptake, and the process of glycolmetabolism. Therefore, AMPK agonist AICAR was used to explore the mechanism of NDMA causing hyperglycemia. AICAR reversed p-FoxO1, PGC-1α, GLUT2, PEPCK and G6Pase proteins expression and improved the glycolysis and glucose uptake capacity in NDMA treated MIHA cells. In conclusion, NDMA in drinking water led to fasting blood glucose enhancing, impairment of glucose tolerance and insulin sensitivity, increasing hepatic gluconeogenesis via AMPK signaling.

GRAPHICAL ABSTRACT



ARTICLE HISTORY

Received 11 September 2025
Revised 15 November 2025
Accepted 20 December 2025

KEYWORDS

N-nitrosodimethylamine; blood glucose; AMPK; gluconeogenesis; PGC-1α

CONTACT Yue Wang yuewang@cmu.edu.cn School of Public Health, Shenyang Medical College, No. 146 Huanghe North Street, Shenyang 110034, China; Key Laboratory of Environmental Stress and Chronic Disease Control and Prevention, Ministry of Education, China Medical University, Shenyang, Liaoning, 110122, China.

1. Introduction

N-Nitrosamines (NAs) are widely present in aquatic environments surrounding humans (Fahrer & Christmann, 2023). People can come into contact with NAs substances through various channels, such as drinking water and food (Fan & Lin, 2018; Li et al., 2021; Niklas et al., 2022). There are nine common N-nitrosamines in water, including N-nitrosodimethylamine (NDMA), N-nitrosodiamine, N-nitrosodibutylamine, N-nitrosodipentylamine, N-nitrosomorpholine, N-nitrosodiphenamine, N-nitrosomethylethylamine, N-nitrosopiperidine, and N-nitrosopyrrolidine (Wang et al., 2011). Among them, the first NAs discovered in the environment is NDMA (1), which is the simplest in structure but the most carcinogenic among NAs (Krasner et al., 2013). In recent years, as a new disinfection by-product of drinking water, NDMA has been detected in various water in different regions, including source water, treated water and tap water (Chen et al., 2019; Fan & Lin, 2018; Yin et al., 2019). NDMA has high water solubility and relatively stable chemical properties, making it difficult to remove from water. It is mainly metabolized by the cytochrome P450 superfamily enzymes in the liver microsomal oxidation enzyme system, such as P4502E1 (Cytochrome P450 2E1, CYP2E1) (Dicker & Cederbaum, 1991), and has been classified as a 2A category carcinogen by the International Agency for Research on Cancer (Seo et al., 2023). Epidemiological investigations and experimental studies have found that exposure to NAs can exacerbate the development of diseases related to insulin resistance such as diabetes mellitus (DM), accompanied by changes in insulin signal transduction mechanisms (Tong et al., 2010).

Type 2 Diabetes Mellitus (T2DM), the most prevalent form accounting for approximately 90% of diabetes cases, is an important factor threatening human health and quality of life (Sharma & Tripathi, 2019), which characteristics include elevated fasting blood glucose (FBG), impaired glucose tolerance (IGT), and insulin resistance (IR). The liver, as an important metabolic organ, regulates the production and transformation of glucose (Glu) to maintain the homeostasis of metabolism. The production of hepatic Glu consists of glycogenolysis and gluconeogenesis, both of which are strictly regulated to sustain normoglycemia (Morgan et al., 2024). Under normal circumstances, the liver converts excess Glu into glycogen stored in the body to balance blood glucose levels. Glycogen synthase 2 (GYS2) is a key rate-limiting enzyme catalyzing the synthesis process of glycogen, and its expression is inhibited during IGT, resulting in reduced hepatic glycogen synthesis and subsequently elevated blood glucose. Gluogenesis refers to the process of converting lactate, pyruvate, and other non-sugar substances into Glu. Hepatic gluconeogenesis accounts for approximately 90% of endogenous Glu production, and excessive gluconeogenesis is considered an early driving factor for fasting hyperglycemia in T2DM patients (Liu et al., 2024a). Gluconeogenesis is mainly regulated by phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), and both levels reflect the gluconeogenic capacity of the liver (Wang & Dong, 2019). Adenosine 5′-monophosphate (AMP) -activated protein kinase (AMPK) plays a pivotal role in regulating hepatic glucose metabolism (Zhang et al., 2024). Studies demonstrated that the activation of AMPK significantly inhibited the expression of key enzymes in gluconeogenesis, thereby reducing hepatic gluconeogenesis (Fan et al., 2023; Foretz et al., 2005; Min et al., 2024). Forkhead transcription factor O1 (FoxO1) and peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α), as downstream signaling molecules of AMPK are important transcription factors regulating gluconeogenesis. Inhibition of FoxO1 and PGC-1α ameliorated hyperglycemia (Liu et al., 2019; Saline et al., 2019), with synergistic effects observed between these two factors (Deng et al., 2023; Duan et al., 2024). In addition, hepatocytes can also maintain systemic homeostasis through glycolysis to consume the synthesized glucose. Glycolysis is a process of breaking down glucose into small molecules and releasing energy, which can be regarded as the reverse reaction of gluconeogenesis (Guo et al., 2024). The glycolytic metabolic rate is mainly influenced by hexokinase 2 (HK2), phosphofructokinase (PFK), and pyruvate kinase M2 (PKM2) (Yang et al., 2024a). HK2 is the rate-limiting enzyme of the first step of glycolysis and can affect the glucose uptake capacity of tissue cells. The decrease in HK2 expression may be one of the reasons for IR in diabetic patients. The increase of PFK can promote the absorption and utilization of glucose. PKM2 regulates the final stage of glycolysis, converting phosphenolpyruvate into pyruvate. Three enzymes together regulate glycolysis, which is essential for normal cellular activities (Wu et al., 2024b). Inhibiting the phosphorylation activity of AMPK reduced the level of glycolysis by regulating the expression of key enzymes in the glycolytic process (Zhu et al., 2023).

Glucose transporter (GLUT) is a protein family that transports Glu (Klip et al., 2024). Glucose transporter 2 (GLUT2), first discovered in the liver tissues, is the most abundant GLUT subtype in hepatocyte of rodent and human, and responsible for most of the glucose uptake (Morrice et al., 2023; Yang et al., 2024b). Genome-wide association studies showed that variations in GLUT2 elevated the risk of fasting hyperglycemia and diabetes. AMPK activation enhanced GLUT2 protein expression in the liver (Kang et al., 2017), promoted extracellular glucose translocation into cells, and improves cellular glucose uptake, thereby ameliorating blood glucose levels and insulin resistance.

Currently, the association between NAs and diabetes is mainly based on epidemiological data analysis, and the study on the specific role of NDMA in diabetes remains limited. In this study, we demonstrated that NDMA exposure disrupted hepatic glucose metabolism by suppressing AMPK signaling and its downstream effectors, leading to increased glucose production and elevated fasting blood glucose. These findings provide foundational experimental data for developing preventive strategies against NDMA-induced metabolic dysregulation.

2. Materials and methods

2.1. Animal and treatments

Twenty-four SPF-grade male C57BL/6 mice (4 weeks old), with an initial body weight ranging from 13 to 15 g, were purchased from Beijing Vital River Laboratory Animal Technology Company (China). The mice were randomly divided into three groups

based on their body weight, with the control group drinking distilled water, low NDMA (Aladdin, China, purity 99%) concentration group drinking 0.5 mg/L NDMA water, and high NDMA concentration group drinking 12.5 mg/L NDMA water. All experimental mice were raised in clean animal rooms with suitable temperature and humidity, and had free access to water and food. At the end of the experiment, mice were anesthetized via intraperitoneal injection of 20% urethane solution (1 mL/100 g body weight), and eyeballs were removed to obtain blood for subsequent relevant index detection. The liver of the mice was weighed to calculate the organ coefficient, and a portion of the liver was fixed for subsequent experiments, while the rest was stored at −80 °C for subsequent experiments. All animal raising and disposal processes were in accordance with the experimental animal welfare and ethical review standards of China Medical University (IACUC numbers: CMU 2023941).

2.2. Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT)

The mice were fasted but not dehydrated for 12 hours, and their fasting blood glucose levels were measured in the morning of the next day. IPGTT was determined as follows. After the mice were fasted for 12 hours, their fasting blood glucose levels were measured and recorded as the 0 min blood glucose level. Then, 2 g/kg of 20% glucose was injected intraperitoneally, and the blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 minutes, respectively. The curves were plotted and the area under the curves were calculated. After the mice were fasted for 4 hours, fasting blood glucose levels were measured and recorded as the 0 min blood glucose level. Then, 0.75 U/kg of insulin was injected intraperitoneally, and the blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 min respectively. The curves were plotted and the area under the curves were calculated for IPITT (Lv et al., 2024a). Fasting blood glucose levels were measured using a blood glucose meter (Contour Plus, Bayer Healthcare Pharmaceuticals Inc., China) and expressed in mmol/L.

2.3. Serum insulin level detection

Blood was collected via eye-dropper and diluted 40 times with sample diluent according to the results of the pre-experiment. Then, the reagents such as sample, detection antibody, SABC complex, TMB chromogenic solution were added in accordance with the instructions of the insulin ELISA detection kit (Biotech Well Technology Co, Ltd, Shanghai, China), and the absorbance was measured at 450 nm. The standard curve of insulin content was plotted and the content was calculated.

2.4. Cell culture and viability assays

Human normal liver cell (MIHA) was purchased from Fenghui Biotechnology Co., Ltd. (Hunan, China). The cells were cultured with RPMI-1640 basic medium adding 10% FBS, 1% penicillin and streptomycin in a 37 °C constant temperature incubator with 5% CO2. MIHA cells were separately exposed to 0, 0.135, 1.35, and 13.5 mM NDMA for 24 hours for subsequent experiments.

2.5. Cell viability assays

The cell viability was determined by CCK-8 kit (Beyotime, Shanghai, China). The cells were inoculated in 96-well plates and continued culture for 24 hours. Then, CCK-8 detection reagents were added into the cells, incubating in the dark to observe the color of the cell culture plate. The absorbance at 450 nm wavelength was detected using an enzyme detector to calculate the cell survival status.

2.6. Western blot

Mice liver tissues or MIHA cells were added into the lysis buffer for high-speed grinding, and the supernatant was performed for protein quantification using the BCA Protein Quantification Kit (Dingguo, Beijing, China). Western blotting was carried out according to the usual methods in our laboratory (Liu et al., 2022). Containing the antibodies as follows: AMPK Rabbit pAb (Cell Signaling Technology, USA)、p-AMPK Rabbit pAb (Cell Signaling Technology, USA)、FoxO1 Rabbit pAb (Abclonal, China)、p-FoxO1 Rabbit pAb (Sangon Biotech, China)、PGC-1α Rabbit pAb (Abclonal, China)、GLUT2 Rabbit pAb (Abclonal, China)、GYS2 Rabbit pAb (ImmunoWay Biotechnology, USA)、PEPCK Rabbit pAb (Sangon Biotech, China)、G6Pase Rabbit pAb (Sangon Biotech, China)、HK2 Rabbit pAb (Proteintech Group, China)、PFKM Rabbit pAb (Proteintech Group, China)、PKM2 Rabbit pAb (UpingBio, China).

2.7. Glycogen content measurement

100 mg of liver tissue was put into 1.5 mL EP tube, adding lysis buffer and boiling for 20 min. Then, it was transferred to a centrifuge tube and diluted to 5 mL. The mix was centrifuge to obtain the supernatant for measurement of glycogen content according to the instructions of the glycogen quantitative kit (Hengyuan Biological Technology Co., Ltd, Shanghai, China). Cultivate MIHA cells were treated with glycogen extraction solution. After sonication of cells, glycogen content is determined by the same method as the detection of animal liver glycogen.

2.8. Glucose production test in cells

Sodium pyruvate, sodium lactate and FBS were respectively added into the basic medium (without glucose and phenol red), which was filtered as carbohydrate medium. MIHA cells were inoculated in 6-well plates and added carbohydrate medium for further culture for 6 h. The concentration of Glu in the supernatant was detected using the Glucose Assay Kit (Jiancheng, Nanjing, China).

2.9. Glucose uptake experiment

The cells were inoculated into 6-well plates and incubated with the NDMA for 24 hours. After that, the supernatant was discarded and the cells were washed twice with PBS. Then, the cells were transferred to Ep tubes and starved for 4 hours in the absence of glucose. After the starvation was completed,

the cells were washed three times with PBS and transferred to the tubes. Each tube was added with 300 µL of 2-NBDG (Selleck, USA) at a concentration of 200 µM **(**Lv et al., 2024b**).** The tubes were placed in the incubator for 45 minutes and then centrifuged. The cells were washed twice with PBS and resuspended in 1 mL of PBS before being transferred to the flow cytometry tubes for fluorescence intensity detection of each group of cells.

2.10. RNA extraction and RT-qPCR

Total RNA was extracted from cells using Trizol reagent (TaKaRa, Dalian, China). The concentration of the extracted RNA was detected by Nano Drop 2000 and quantified. The mRNA levels of PGC-1α, PEPCK and G6Pase were detected by RT-qPCR. The GAPDH gene was selected as the internal reference. The relative expression levels of the target genes were calculated using the 2-ΔΔCT method. The primer sequences for the target genes showed in Table 1.

Table 1. Primers sequence(5′-3′).

Name	Forward	Reverse
PGC-1α	CCAAAGGATGCGCTCTCGTTCA	CGGTGTCTGTAGTGGCTTGACT
PEPCK	ATCCCCAAAACAGGCCTCAG	ACGTACATGGTGCGACCTTT
G6Pase	GACTGGCTCAACCTCGTCTT	CGTAGTATACACCTGCTGTGC
GAPDH	GGACCTGACCTGCCGTCTA	AGTGGGTGTCGCTGTTGA

2.11. Statistical analysis

Data were shown as mean ± standard deviation (SD), and one-way ANOVA and LSD test were conducted using SPSS 23.0 and Graphpad Prism 8.0 software. $p < 0.05$ indicated statistically significant differences

3. Results

3.1. NDMA induced elevation of fasting blood glucose and impairment of glucose tolerance in mice

During the exposure process, the mice were in good mental state with smooth fur, and all survived until the end of the experiment. Body weight increased with age across all groups, but the 12.5 mg/L NDMA group showed significant decrease in body weight from 7 to 12 weeks compared to the control group (Figure 1(A)). There were no significant differences in food consumption and water consumption among the groups (Figure 1(B,C)). Compared with the control group, the liver organ coefficient of mice in the 12.5 mg/L NDMA group decreased significantly (Figure 1(D)). The FBG levels of mice fluctuated during 12 weeks, and the FBG of mice in the 12.5 mg/L NDMA group was higher than the control group at 12 weeks ($p < 0.05$) (Figure 1(E)). The content of insulin in mice serum was shown in Figure 1(F), and there was no significant difference in the insulin secreted among groups ($p > 0.05$).

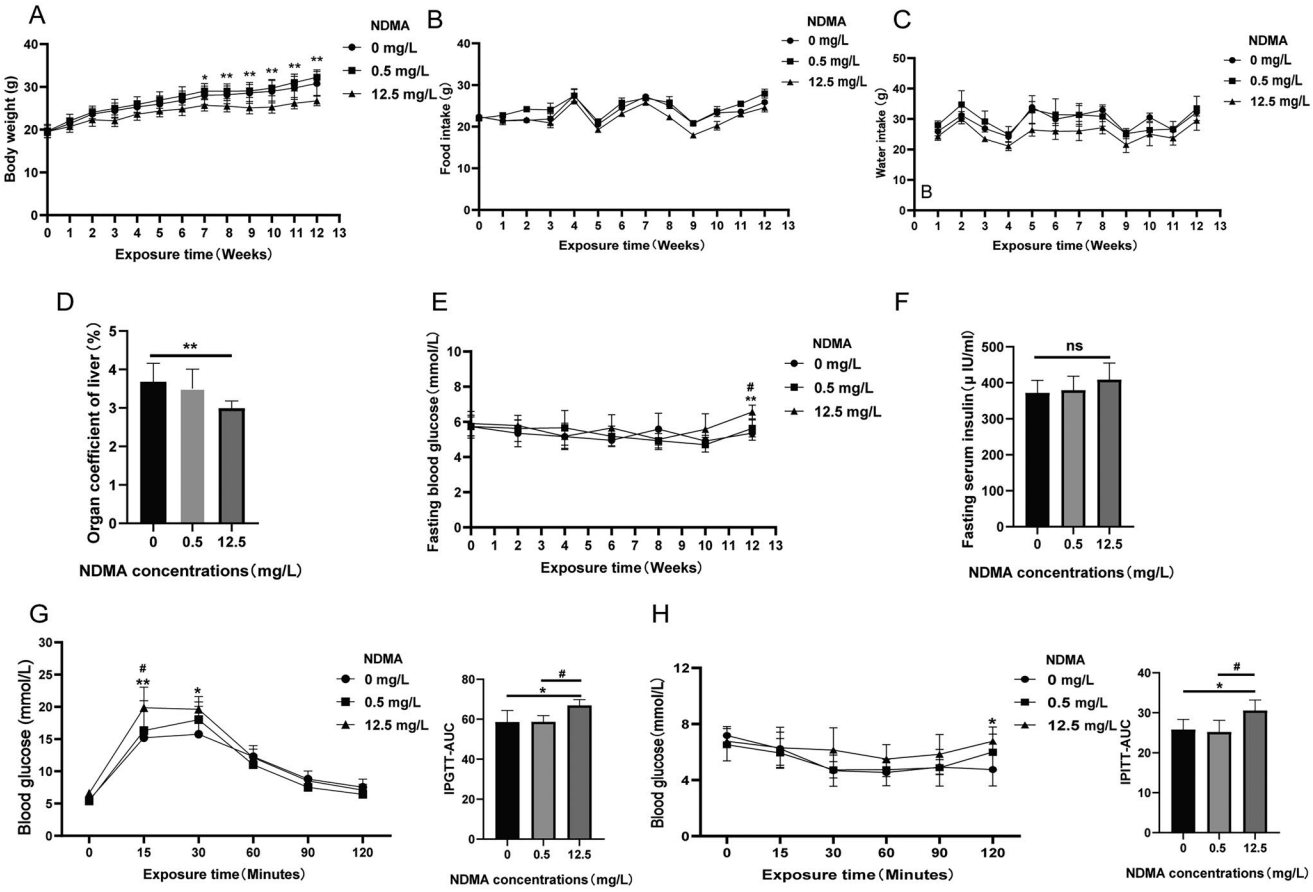


Figure 1. NDMA exposure elevated fasting blood glucose and decreased glucose tolerance in mice. (A) Body weight of mice ($n = 8$). (B) Food consumption of mice ($n = 8$). (C) Water consumption of mice ($n = 8$). (D) Liver organ coefficient ($n = 8$). (E) Fasting blood glucose of mice ($n = 8$). (F) Fasting serum insulin content of mice ($n = 5$). (G) Glucose tolerance test ($n = 5$). (H) Insulin tolerance test ($n = 5$). *$p < 0.05$ and **$p < 0.01$ compared with the control group; #$p < 0.05$ compared with the 0.5 mg/L NDMA group; nsno significant.

However, IPGTT test showed that the levels of blood glucose in 12.5 mg/L NDMA group rapidly increased after intraperitoneal injection of glucose, and the area under the curve increased significantly compare to the control group ($p < 0.05$), suggesting a decreased glucose tolerance in 12.5 mg/L NDMA exposure mice (Figure 1(G)). The results of insulin sensitivity detection were shown in Figure 1(H). Compared with the control group, the levels of blood glucose in the 12.5 mg/L NDMA group decreased slowly, the gluconeogenesis speed was faster, and the area under the curve increased after insulin injection ($p < 0.05$), which indicated that the insulin sensitivity decreased after exposed to NDMA in mice.

3.2. NDMA exposure increased gluconeogenesis and changed AMPK pathway-related proteins expression in mice

The production of hepatic glucose is strictly regulated by glycogenesis and gluconeogenesis to maintain normal blood glucose levels. GYS2 is a key enzyme in the glycogen synthesis of liver tissue. In this study, there were no significant differences in the liver glycogen content and GYS2 protein expression in livers of mice among groups ($p > 0.05$) (Figure 2(A,B)). Excessive gluconeogenesis is considered to be the main early driving factor of fasting hyperglycemia in T2DM

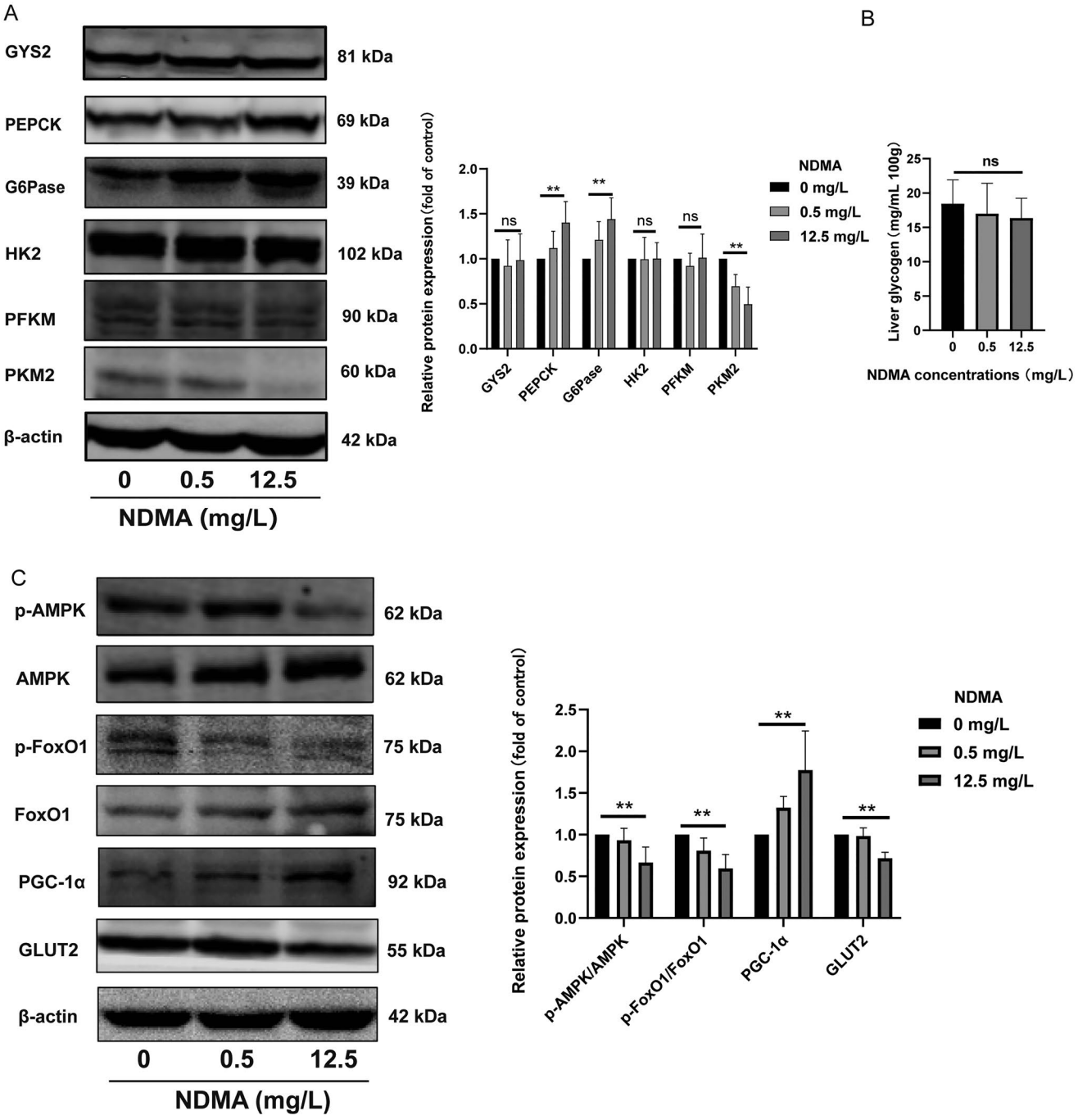


Figure 2. Effects of NDMA exposure on glycogen content and proteins expression of gluconeogenesis and AMPK pathway-related in mouse liver. (A) and (C) Protein expression in mice liver by Western blot ($n = 5$). (B) Glycogen content of the liver ($n = 5$). $^{**}p < 0.01$ compared with the control group; ^{ns}no significant.

patients. G6Pase and PEPCK are two key rate-limiting enzymes in the gluconeogenesis process. The protein expression levels of G6Pase and PEPCK in the 12.5 mg/L NDMA group increased significantly as compared with the control group ($p < 0.01$) (Figure 2(A)).

Glycolysis is a process of breaking down glucose into small molecules and releasing energy. To some extent, Glycolysis can be as the reverse process of gluconeogenesis. The protein expression levels of the rate-limiting enzymes HK2, PFKM, and PKM2 in glycolysis were determined in the livers of mice. There was no significant difference in the protein expression of PFKM and HK2 among groups, but the expression of PKM2 decreased significantly compared to control group (Figure 2(A)). These results indicate that NDMA exposure enhances hepatic gluconeogenesis, suppresses glycolysis, and ultimately promotes glucose production in mice.

GLUT2 is the most important glucose transporter in the liver. Compared with the control group, the 12.5 mg/L NDMA group exhibited significantly reduced GLUT2 protein expression ($p < 0.01$). AMPK and related proteins FoxO1 and PGC-1α play the critical roles in regulating glucose and lipid metabolism. The proteins expression of p-AMPK and p-FoxO1 decreased and PGC-1α increased in 12.5 mg/L NDMA group, and there were significant differences for the protein levels of p-AMPK/AMPK ratio, p-FoxO1/FoxO1 ratio and PGC-1α between the 12.5 mg/L NDMA group and the control group ($p < 0.01$), see Figure 2(C).

3.3. NDMA exposure disturbed glucose metabolism in normal human liver cells (MIHA)

The above research showed that NDMA exposure induced an increase in fasting blood glucose and changes in glucose metabolism in mice. An *in vitro* experiment was conducted in NDMA treated human normal liver cells (MIHA). Firstly, the cell viabilities were detected by CCK-8 assay after treated with NDMA. MIHA cell viabilities decreased dose-dependently after NDMA treatment for 24h, and 1.35 and 13.5 mM NDMA treatment MIHA cells reduced significantly cell viabilities as compared with the control cells ($p < 0.05$ or $p < 0.01$) (Figure 3(A)). The glucose levels in the cell culture supernatant increased in dose-dependent after NDMA treatment, and there was significant difference between 13.5 mM NDMA group and the control cells ($p < 0.01$) (Figure 3(B)). The mRNA and protein expression levels of the key enzymes of gluconeogenesis PEPCK and G6Pase in the 13.5 mM NDMA treatment cells were significantly increased, while the protein expression level of PKM2 was significantly decreased as compared with the control cells ($p < 0.01$) (Figure 3(C,D)). No significant differences were observed in HK2, PFKM, GYS2 protein levels and glycogen content among NDMA treatment cells (Figure 3(D,E)). Similar to the results in mice, NDMA treatment enhances gluconeogenesis, suppresses glycolysis, and thereby promotes glucose overproduction in MIHA cells.

Furthermore, the glucose uptake of NDMA treatment MIHA cells was measured with 2-NBDG, a deoxyglucose analog, by flow cytometry analysis. 2-NBDG uptake reduced in 13.5 mM NDMA cells compared to the control cells ($p < 0.05$) (Figure 3(F)). The expression of GLUT2, a protein of glucose uptake, also decreased significantly in 13.5 mM NDMA treated cells compared with the control cells (Figure 3(G)). The protein expression levels of p-AMPK/AMPK and p-FoxO1/FoxO1 in the 13.5 mM NDMA treated cells were the same as the results in vivo, and decreased significantly (Figure 3(G)). The protein and mRNA expression levels of PGC-1α were significantly increased in 13.5 mM NDMA treated cells compared to the control cells (Figure 3(G,H)). These results indicate that NDMA treatment suppresses AMPK activation and disturb glucose metabolism in MIHA cells.

3.4. Activating AMPK improves NDMA-induced glucose metabolic disorders in MIHA cells

Some studies reported that AMPK plays a pivotal role in regulating hepatic glucose metabolism, and previous results show phosphorylated AMPK decreasing in NDMA-treated MIHA cells. Therefore, a pharmacological AMPK activator, 5-aminoimidazole-4-carboxyamide ribonucleoside (AICAR) (Med Chem Express, USA) was employed to verify the roles of AMPK signaling pathway on glucose metabolism. Firstly, we detected the cytotoxicity of AICAR to MIHA cells. MIHA cells were treated with 0, 0.25, 0.5, and 1.0 mM AICAR for 24 hours, and the results showed that 0.5 mM AICAR could maintain the cell viability at around 90%, while 1.0 mM AICAR significantly decreased the cell viability (Figure 4(A)). Therefore, 0.5 mM AICAR was used for subsequent studies. AICAR treatment inhibited the increase of NDMA-induced glucose content, and mRNA and protein expression of PEPCK and G6Pase, and reversed PKM2 expression (Figure 4(B–D)). Furthermore, AICAR restored p-FoxO1/FoxO1 ratio and GLUT2 protein expression, as well as glucose uptake in NDMA treated cells (Figure 4(E–G)). These findings collectively highlight the pivotal role of AMPK signaling in mediating NDMA-induced glucose metabolic disorders.

4. Discussion

The incidence of diabetes is increasing exponentially, with approximately 90% of patients attributed to environmental factors (Galicia-Garcia et al., 2020; Zhou et al., 2025). The liver, as an important target organ of insulin plays an important role in regulating glucose metabolism and insulin sensitivity. Hepatic glucose production accounts for approximately 90% of endogenous production. Excessive hepatic gluconeogenesis is an early major driving factor for hyperglycemia in T2DM. NDMA exposure had been thought associating with the risk of diabetes.

In this study, the fasting blood glucose increased in mice exposed 12.5 mg/L NDMA in drinking water, accompanying by impaired glucose regulation ability and decreased insulin sensitivity. Multiple studies have shown that in T2DM and IR model groups of animals, there are impaired glucose tolerance and decreased insulin sensitivity (Liu et al., 2017; Zou et al., 2024). The liver maintains the dynamic balance of blood glucose through regulation of both the generation and utilization of glucose. Glycogen is formed by the polymerization of many glucose molecules. Insulin normally promotes hepatic glycogen

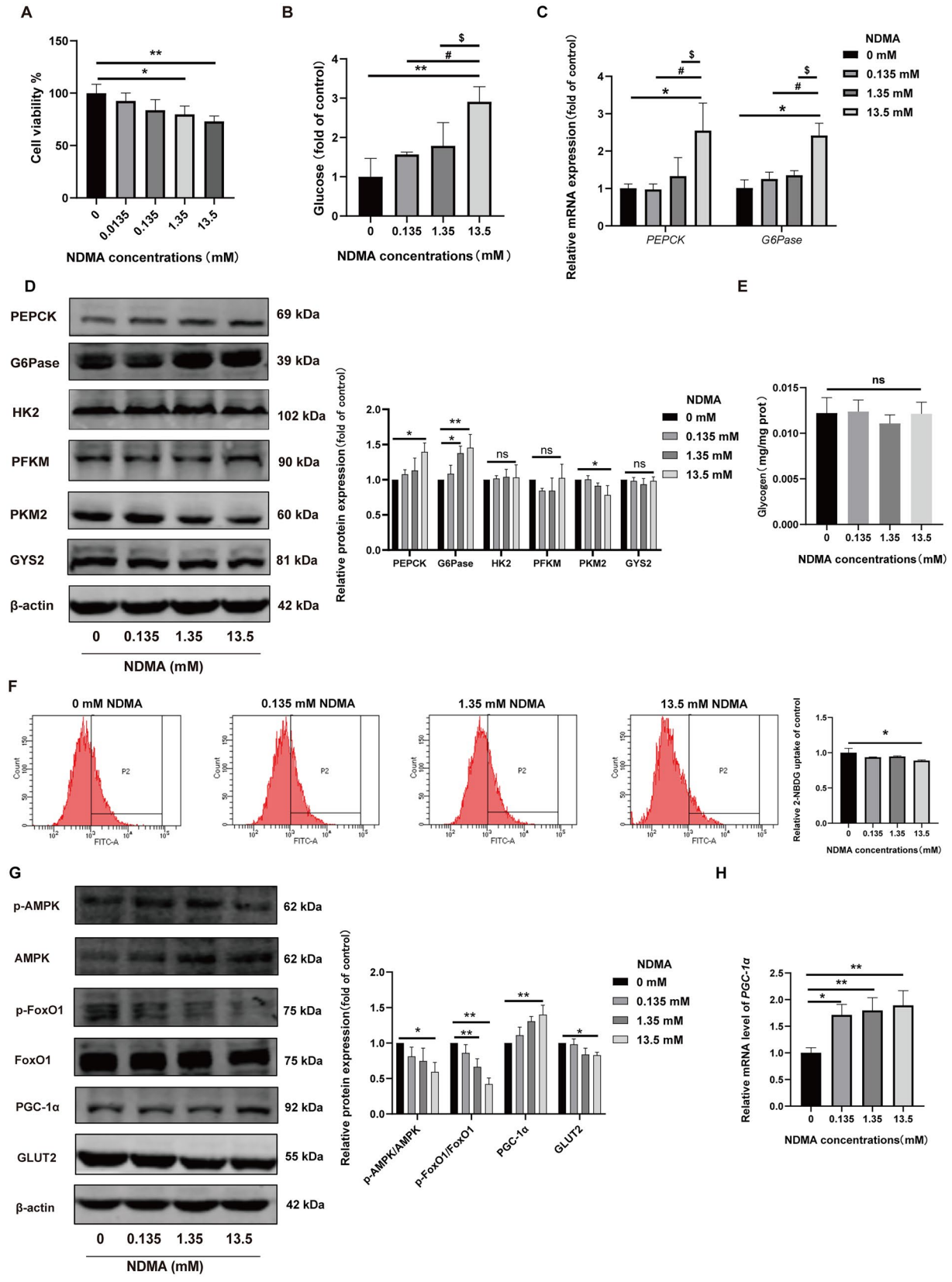


Figure 3. NDMA disturbed the glucose metabolism in MIHA cells ($n = 3$). (A) CCK-8 assay to test the viability. (B) Glucose production. (C) and (H) mRNA levels by RT-qPCR. (D) and (G) Protein expression by Western blot. (E) Glycogen content. (F) The glucose uptake by flow cytometry detection of the proportion of 2-NBDG positive cells. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the control group; $^{\#}p < 0.05$ compared with the 0.135 mM NDMA group; $^{\$}p < 0.05$ compared with the 1.35 mM NDMA group; ^{ns}no significant.

synthesis to lower blood glucose, while there is often a decrease in glycogen synthesis in the liver of T2DM due to insulin resistance. In our study, NDMA exposure did not alter hepatic glycogen content or GYS2 expression, suggesting that NDMA induced the increase in mouse FBG was not through affecting hepatic glycogen synthesis. Gluconeogenesis is the

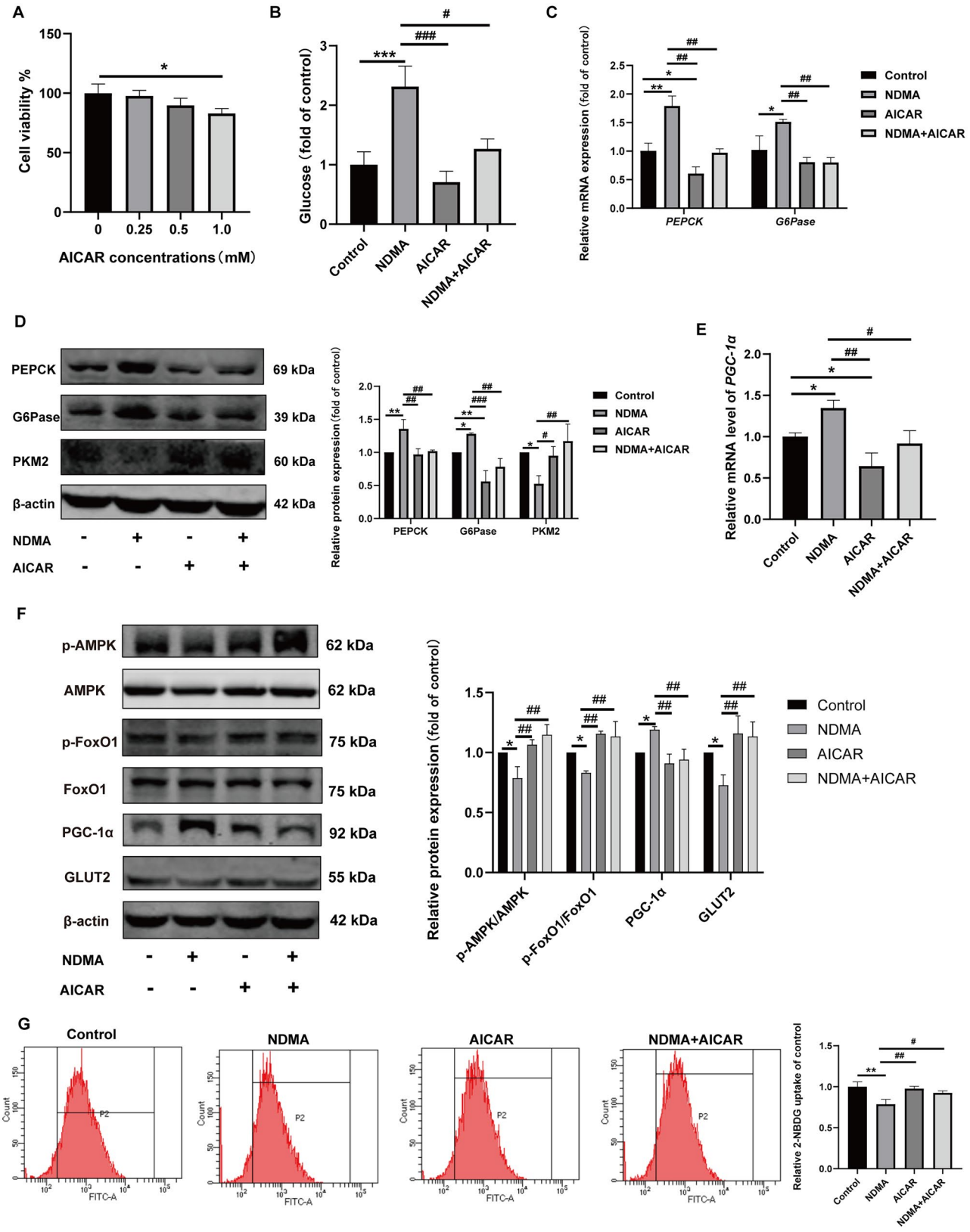


Figure 4. AMPK activator AICAR attenuated the glucose metabolism disorder induced by NDMA in MIHA cells ($n = 3$). (A) the viability of MIHA cells by CCK-8 assay. (B) Glucose content of MIHA cells. (C) and (E) mRNA levels by RT-qPCR. (D) and (F) Protein expression by Western blot assay. (G) The glucose uptake by flow cytometry detection of the proportion of 2-NBDG positive cells. AICAR, 5-aminoimidazole-4-carboxyamide ribonucleoside. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ compared with the NDMA group.

synthesis of glucose from non-carbohydrate precursors and is regulated by multiple factors (Fang et al., 2025). A large number of studies have shown that the elevated fasting blood glucose in T2DM patients in the early stage is closely related to the elevated level of hepatic gluconeogenesis (Barroso et al., 2024). There have been corresponding reports that the fasting

blood glucose increases accompanied by the expression increasing of PEPCK and G6Pase under the induction of dexamethasone and high-fat diet mice. Knocking down these two enzymes in mice led to a decrease in blood glucose (Bielczyk-Maczynska et al., 2022; Cui et al., 2019). Similar results were also obtained in our experimental study, and NDMA exposure enhanced the ability of hepatic gluconeogenesis in mice. Glycolysis is the process of consuming glucose to produce energy. Of the three key enzymes HK2, PFKM, and PKM2 in the glycolytic process, only PKM2 was down-regulated in the mice liver exposed to NDMA. PKM2 is a key rate-limiting enzyme in the final step of the glycolytic process, and its reduced expression indicates suppressed glycolytic activity and markedly slowed glucose catabolism. In addition, the protein and mRNA expression of GLUT2 decreased in the liver tissue of NDMA exposed mice. GLUT2 is essential for the transport and uptake of glucose in the liver. In response to insulin stimulation, hepatocytes GLUT2 promotes the uptake of glucose from the blood to maintain blood glucose stability. Studies showed that the expression of GLUT2 in liver tissues of mice was significantly decreased with fasting hyperglycemia and insulin resistance. The protein and mRNA expression levels of GLUT2 in the liver tissue of mice and rats with T2DM induced by high-fat diet and streptozotocin were significantly decreased (Liu et al., 2024b; Luo et al., 2022). Active substances such as protocatechuic acid, astragalus polysaccharides, and pandanus fruit or regulation by other factorscould improve the expression of GLUT2 and reduced FBG, enhancing insulin sensitivity (González-Rodriguez et al., 2008; Shi et al., 2022). These findings indicate that NDMA-induced FBG elevation may be the combined action of decreasing glucose uptake of hepatocyte, increasing gluconeogenesis, and suppressing glycolysis.

AMPK, a central energy sensor, senses changes in the AMP/ATP ratio within the cell. It regulates hepatic glucose production and uptake, participates in the process of glycolmetabolism through different pathways. It was reported that AMPK played a major role in the therapeutic benefit of metformin for type 2 diabetes and related metabolic disorders. The activation of AMPK promotes ATP breakdown by triggering catabolic pathways and inhibits anabolic pathways to maintain cellular energy stores (Cantó et al., 2009). FoxO1 is a transcription factor involved in a variety of cellular processes, including glucose metabolism (Saline et al., 2019). AMPK could activate SIRT1 that deacetylated FoxO1 and enhanced FoxO1 activity, impart the biological effect of AMPK on energy metabolism (Cantó et al., 2009). Dephosphorylated FoxO1 could translocate to the nucleus, binding promoters of PEPCK and G6Pase to upregulate their expression (Yoo et al., 2023). AMPK phosphorylated directly and indirectly FoxO1 and inhibited FoxO1 into the nucleus (Saline et al., 2019). Oxyberberine inhibitory effect on gluconeogenesis was by activating AMPK that phosphorylated and promoted nuclear exclusion of FoxO1 (Wu et al., 2024a). PGC-1α is a main regulator of fatty acid β-oxidation and gluconeogenesis, and plays a key role in hepatic gluconeogenesis. AMPK/SIRT1/PGC-1α network involved in the beneficial effects of resveratrol on the glucose and lipid metabolism (Shi et al., 2018). The anti-insulin resistance effect of tomatine was by activating AMPK/PGC1α pathway (Lee & Kim, 2025). Sodium butyrate improved insulin tolerance and kidney injury in mice with diabetic kidney disease, and the mechanism was increased protein expression of PGC-1α and p-AMPK (Yu et al., 2023). PGC-1α strongly stimulated the expression of PEPCK and G6Pase to promote glucose production in primary hepatocytes (Chang et al., 2016). FoxO1 and PGC-1α interacted in insulin-regulated gluconeogenesis, and the coordinated inactivation of FoxO1 and PGC-1α was associated with suppression of gluconeogenic enzyme expression induced by insulin (Puigserver et al., 2003; Sajan et al., 2018). In addition, the activating of AMPK could enhance glucose uptake by GLUT2 in a non-energy-dependent mechanism (Walker et al., 2005). AMPK inhibition leads to the decreased expression of glucose transport proteins and the reduced ability of glucose uptake (Hu et al., 2024). Similar results were presented in this study. AMPK and FoxO1 phosphorylation and GLUT2 expression decreased in NDMA exposed cells and liver of mice, while PGC-1α protein expression increased. AMPK agonist AICAR reversed these proteins expression and improved the glycolysis and glucose uptake capacity in NDMA treated MIHA cells. AICAR also inhibited the glucose over-production induced by NDMA. Enhanced AMPK signaling improved insulin sensitivity and mitigated hyperglycemia. In our study, the underlying mechanisms of NDMA affecting glucose production and hyperglycemia were influencing the expression of FoxO1, PGC-1α and PKM2 key enzyme of glycolysis by inhibiting AMPK signaling pathway in liver cells.

The findings of this study highlight the importance of monitoring and controlling human exposure to NDMA, particularly through drinking water. Given that NDMA is a disinfection by-product detected in various water sources and has been shown in this study to promote hyperglycemia and impair glucose metabolism, it is advisable to implement stricter regulatory limits on NDMA levels in drinking water. Water treatment strategies such as advanced oxidation processes, ultraviolet treatment, and activated carbon filtration could be employed to reduce NDMA formation and accumulation (Astuti et al., 2023; Minakata & Coscarelli, 2018; Pu & Zeng, 2023). Furthermore, public awareness should be raised regarding potential dietary sources of NDMA, including certain processed foods and cured meat products. Long-term biomonitoring of NDMA and its metabolites in high-risk populations may also aid in the early identification of metabolic dysfunction.

5. Conclusion

In summary, NDMA exposure in drinking water led to fasting blood glucose enhancing, impairment of glucose tolerance and insulin sensitivity decreasing of mice, due to enhanced hepatic gluconeogenesis. These glucose metabolic disorders were induced by NDMA suppressing AMPK activation that further regulated FoxO1, PGC-1α, and GLUT2 expression. The results provide a foundation for further revealing the mechanism of NDMA disrupting glucose metabolism.

Authors contributions

Wenxin Wang: Writing—original draft, Validation, Visualization, Conceptualization. Shuhua Xi: Conceptualization. Yue Wang: Conceptualization, Supervision, Writing—review & editing.

Disclosure interest

No potential conflict of interest was reported by the author(s).

Funding

This study was not funded.

Data availability statement

Data are available on request to the authors.

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