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Intestinal transport of Monascus vinegar melanoidins and their amelioration of insulin resistance in HepG2 cells via IRS-1/PI3K/Akt and AMPK pathways

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ABSTRACT: The small intestinal digestion melanoidins (IDM), a product of Monascus vinegar melanoidins (LVM) digestion, were subjected to an analysis of their transport mode in intestinal epithelial cells, as well as their effect and mechanism of mitigating insulin resistance (IR) in HepG2 cells. The results show that IDM undergoes intestinal epithelial cell transport through a transcellular pathway, involving multiple endocytic mechanisms. IDM (200 - 400 µg/mL) increased glucose uptake and consumption, promoted glycogen synthesis, and inhibited gluconeogenesis in IR-HepG2 cells. After endocytosis into HepG2 cells, IDM initiates downstream reactions by promoting IRS-1 tyrosine phosphorylation. The PI3K/Akt pathway serves as the core pathway for improving IR in HepG2 cells. Activation of the PI3K/Akt pathway enhances GLUT4 translocation to promote glucose uptake and promotes glycogen synthesis by inhibiting p-GS expression. Additionally, IDM attenuates gluconeogenesis by activating the AMPK pathway, thereby regulating the nuclear protein HNF-4α and key enzymes. *In vivo*, oral administration of LVM effectively improved IR and liver metabolic disorders in T2DM mice. These findings indicate that Monascus vinegar melanoidins can serve as a potential functional factor for preventing and improving IR, providing a scientific basis for the development of new functional foods and dietary additives.

Keywords: Monascus vinegar melanoidins, insulin resistance, intestinal transport, HepG2 cells, PI3K/Akt signaling

1. Introduction

Type 2 diabetes mellitus (T2DM), a prevalent chronic metabolic disorder, poses a significant global health challenge, often leading to severe complications like cardiovascular disease, renal failure, and neuropathy ^[1]. With the global rise in obesity and unhealthy lifestyles, T2DM cases have dramatically increased, projected to reach 693 million by 2045 ^[2]. A hallmark of T2DM is insulin resistance (IR), which impairs the ability of insulin-sensitive organs, particularly the liver, to regulate glucose homeostasis ^[3]. This

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dysfunction disrupts crucial processes such as glucose uptake, glycogen synthesis, and lipolysis, making the liver a key target for therapeutic interventions [4]. Current clinical treatments for IR, including biguanides, thiazolidinediones, and α -glycosidase inhibitors, are often associated with long-term side effects like edema and liver injury, highlighting the need for safe, food-derived alternatives [1, 5].

Melanoidins, the end products of the Maillard reaction, are complex brown macromolecules found in various thermally processed and fermented foods, including vinegar [6]. These compounds exhibit diverse bioactivities, such as antioxidant, antihypertensive, and anti-inflammatory effects [6]. However, macromolecule compounds need to cross the intestinal barriers before entering the systemic circulation to exert functional activity [7]. This transport primarily occurs via the transcellular pathway, involving endocytosis and transport across epithelial cells, as opposed to the paracellular pathway, which is limited to smaller molecules [8, 9]. Our previous studies successfully extracted melanoidins (LVM) from aged *Monascus* vinegar and demonstrated that their small intestinal digestion products (IDM) effectively scavenged reactive oxygen species (ROS) in Caco-2 cells and mitochondrial superoxide [10, 11]. Given that IR is often associated with oxidative stress and disrupted glucose metabolism, the ROS-scavenging ability of IDM suggests its potential to ameliorate IR [11, 12].

While the bioactivities of melanoidins from various food sources are being investigated, their hypoglycemic effects and underlying molecular mechanisms remain largely unexplored [6]. As a key target organ for insulin, the liver plays a central role in maintaining glucose homeostasis and regulating IR-related signaling pathways [13]. IRS-1 is a direct substrate of the insulin receptor, and phosphorylation of its tyrosine site directly promotes insulin metabolism [14]. Emerging evidence suggests that the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) signaling pathway plays a key role in insulin receptor substrate-1 (IRS-1)-regulated glucose metabolism, glycogen synthesis, and gluconeogenesis [15]. Phosphorylation of the PI3K/Akt pathway recruits glucose transporter-4 (GLUT4) in the trans-Golgi network and vesicles for translocation to the plasma membrane, resulting in the uptake of blood glucose along a concentration gradient into the hepatocyte [1]. In addition, activation of PI3K/Akt regulates glycogen synthase kinase-3 β (GSK3 β) activity to inhibit glycogen synthase (GS) phosphorylation, which ultimately stimulates glycogen synthesis [13]. As a major regulator of lipid and glucose metabolism, AMPK inhibits gluconeogenesis by inhibiting the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) [16]. Therefore, these pathways represent potential targets for IR management.

In this study, we aimed to elucidate the transport mechanism of IDM across intestinal epithelial cells and investigate its hypoglycemic effects in IR-induced HepG2 cells. We utilized a Caco-2 cell monolayer model to simulate intestinal transport and employed transcriptomics, Western blotting, and RT-qPCR to analyze gene expression and signaling pathway activation in HepG2 cells. In addition, we also verified the effective effects of oral administration of LVM on improving IR and liver metabolic disorders in a T2DM mouse model. This research will provide valuable insights into the absorption and hypoglycemic activity of

Monascus vinegar melanoidins, contributing to the development of novel functional foods and dietary supplements for IR management.

2. Materials and methods

2.1 Materials

Monascus Vinegar was supplied by Taoxi Yongchun Monascus Vinegar Co., Ltd. (Quanzhou, China). 3-(4,5-Cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), DMEM (high-glucose), phosphate buffered solution (PBS, 0.01M), hanks balanced salt solution (HBSS solution), 0.25% trypsin-EDTA solution, SDS-PAGE loading buffer (5×), D-Glucose, DMSO, palmitic acid (PA), insulin, metformin, chlorpromazine, cytochalasin D, 5-(N-ethyl-N-isopropyl) amiloride (EIPA), Citric acid, streptozotocin (STZ), paraformaldehyde (4%), Glucogen Content Assay Kit, and BCA Protein Assay Kit were obtained from Solarbio Life Science (Beijing, China). AZD5363 (Akt inhibitor), Dorsomorphin (AMPK inhibitor), phenylmethanesulfonyl fluoride (PMSF, a protease inhibitor), cell lysis buffer, nystatin, 2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose (2-NBDG), Periodic Acid-Schiff Staining (PAS) Kit, Glucose Assay Kit, Total Lactate Dehydrogenase (LDH) Assay Kit, Nuclear and Cytoplasmic Protein Extraction Kit, and Enhanced Chemiluminescence Kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Sodium azide, fetal bovine serum (FBS), bile salt, all the digestive enzymes, and all the chemicals and solvents (analytical grade) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Antibodies

Akt, PI3K, AMPK α , GLUT4, GS, IRS-1, GSK-3 β , HNF-4 α , Lamin B1, β -actin, p-Akt (S473), p-AMPK α (T172), p-GS (S641), p-GSK-3 β (S9) and p-IRS-1 (S307) primary antibodies and goat anti-rabbit IgG (H + L) and goat anti-mouse IgG (H + L) secondary antibody (1:2000 dilution) were purchased from Cell Signaling Technology (Danvers, MA, USA). p-PI3K (Y467/Y199) was purchased from Abcam (Cambridge, UK). All primary antibodies were diluted by 1:1000.

2.3 Preparation of IDM from *in vitro* simulated digestion

The extraction and *in vitro* simulated digestion of LVM was carried out exactly as in our previous method [11]. Briefly, LVM was extracted from Monascus Vinegar by sedimentation with Sevage reagent (1-butanol: chloroform = 1:4, v/v) and lyophilized. The LVM sample of 100 mg was dissolved in 20 mL of simulated saliva (containing 0.3 M CaCl₂ and 75 U/mL α -amylase, pH=7.0) and stirred at 37°C for 2 min. Subsequently, the pH was modified to 2.0 using 2 M HCl, and the mixture was incubated with 500 U/mL pepsin at 37°C for 2 h. Then, the pH of the mixture was adjusted to 7.0 using 2 M NaOH, and 500 U/mL trypsin and 10 mM bile salt were added and incubated at 37°C for 2 h. Digestion was terminated in a boiling water bath, and the simulated digestive fluid were dialyzed and lyophilized to obtain IDM.

2.4 Cell culture and treatment

Caco-2 cells and HepG2 cells were acquired from the National Infrastructure of Cell Line Resource (Beijing, China). All cells were cultured at 37°C in a 5% CO₂ incubator in DMEM (high-glucose) supplemented with 1% penicillin-streptomycin and 10% FBS. The insulin resistance model of HepG2 cells (IR-HepG2) was established by co-incubation with 50 mM glucose and 0.1 mM PA for 24 h. In addition, all inhibitor experiments involved in this study were performed by incubating the corresponding inhibitor 1 h prior to the addition of IDM, followed by co-incubation of HepG2 cells with IDM and inhibitor.

2.5 Cell viability assay

Cells were cultured on a 96-well plate at a density of 1×10^4 cells per well for 24 h. Then, the medium of Caco-2 cells and HepG2 cells were exchanged for DMEM containing IDM at concentrations of 25-400 µg/mL and 50-800 µg/mL, respectively. After 24 h of incubation, 10 µL MTT solution (5 mg/mL in PBS) was added to each well and treated for 2 h. Finally, 150 µL of DMSO was used to replace the medium per well. Absorbance values were measured at A570 nm using a microplate reader (Synergy HT, BioTek Instruments, USA) after 10 min incubation.

2.6 Transport of the IDM across Caco-2 cells monolayer

2.6.1 Lactate dehydrogenase (LDH) release assay

Caco-2 cells were cultured on a 6-well plate at a density of 7.5×10^5 cells per well for 24 h. Then, the IDM group was replaced with serum-free DMEM containing 100 µg/mL IDM, and the other groups were replaced with serum-free DMEM. After the cells were incubated for 4 h, the LDH concentration in the culture medium of each group was detected using the Total LDH Assay Kit. In the positive control group, DMEM was replaced with 600 µL of cell lysis buffer one hour earlier. The absorbance at 450 nm was measured using a microplate reader and again after 20 min of incubation at 37°C. LDH activity was calculated using the difference between the two absorbances.

2.6.2 IDM transport through Caco-2 cells monolayer

Caco-2 cells were cultured on the upper compartment of Transwell plate (Corning, NY, USA) at a density of 1.25×10^5 cells per well. The cells were cultured continuously for 14 days, during which the medium was changed every 2 days. When the transepithelial electrical resistance (TEER) value of the Caco-2 cells monolayer exceeded 600 Ω/cm², the cells monolayer was considered complete and ready for subsequent experiments. The TEER values were determined by a Millicell-ERS-2 volt-ohmmeter (Millipore Company, Bedford, MA). In the transport study, Caco-2 cells were first equilibrated in HBSS solution for 30 min. Then, discard the HBSS solution and add fresh HBSS containing 100 µg/mL IDM to the apical chamber and 1.5 mL of HBSS solution to the basal chamber. At different time points within 4 h, 1.5 mL of HBSS solution was removed from the basal chamber of each well and the absorption value at 420 nm was detected using a UV-vis spectrometer (UV-2550, Shimadzu, Tokyo, Japan). According to our previous method (Wang et al., 2024) the standard curve of melanoidin content was established and the IDM transport amount at different time points

was calculated. The apparent permeability coefficient (P_{app}) was calculated to evaluate the transportation ability of IDM.

The P_{app} was calculated as below ^[17]

$$P_{app} = (dQ/dt)/(A \times C_0) \quad (1)$$

Where, “ dQ/dt ” is the change of the IDM transport volume in the basal chamber over time ($\mu\text{g/s}$), “ A ” represents the membrane surface area (cm^2), and “ C_0 ” is the initial concentration in the apical chamber ($\mu\text{g}/\text{cm}^3$).

2.6.3 Investigation of endocytosis pathways in the Caco-2 cells monolayer

When the TEER value of the Caco-2 cells monolayer exceeded $600 \Omega/\text{cm}^2$, different transport inhibitors were added to pretreat them for 1 h: (I) sodium azide (1 mg/mL), (II) nystatin ($30 \mu\text{M}$), (III) chlorpromazine ($30 \mu\text{M}$), (IV) EIPA ($100 \mu\text{M}$), (V) Cytochalasin D ($5 \mu\text{M}$). Then, a fresh HBSS solution containing $100 \mu\text{g/mL}$ IDM and different transport inhibitors were replaced in the apical chamber. After 2 h of co-incubation, the absorption value at 420 nm was measured by a UV-vis spectrometer and the transport amount of IDM was calculated.

2.7 Glucose consumption and production assay

HepG2 cells were seeded in 6-well plates at a density of 3×10^5 cells per well for 24 h. After the IR-HepG2 cell model was established, the cells were incubated with DMEM containing IDM ($0\text{--}400 \mu\text{g/mL}$), insulin ($1 \mu\text{M}$), or metformin (2 mM) for 24 h. According to the Glucose Assay Kit, the culture medium of each group was collected and the glucose assay reagent was added. The mixed solution was heated at 95°C for 8 min, cooled and $A_{630 \text{ nm}}$ was determined using a microplate reader. In addition, the cells were lysed on ice, and the protein concentration of the cells in each group was determined using the BCA Protein Assay Kit. Glucose consumption was calculated by subtracting the glucose concentration of each group from the glucose concentration of the blank group (no cells, only medium). After IDM incubation of IR-HepG2 cells for 24 h, the cells were rinsed twice with PBS and then cultured in glucose-free DMEM for 3 h. The glucose content in the culture medium of each group was the gluconeogenesis. The final results of glucose consumption and gluconeogenesis were corrected by protein concentration.

2.8 Glucose uptake assay

HepG2 cells (3×10^5 cells per well) were seeded in 6-well plates for 24 h. After the IR-HepG2 cell model was established, the cells were incubated with different concentrations of IDM for 24 h. Then, 2-NBDG was added at $0.1 \mu\text{M}$ final concentration, and incubated at 37°C for 30 min. Cells were collected after being rinsed twice using pre-cooled PBS. Subsequently, the collected cells were assayed by flow cytometry (CytoFLEX, Beckman Coulter, CA, USA).

2.9 Glycogen synthesis assay and glycogen staining

HepG2 cells (3×10^5 cells per well) were seeded in 6-well plates for 24 h. After the IR-HepG2 cell model was established, the cells were incubated with DMEM containing $0\text{--}400 \mu\text{g/mL}$ IDM for 24 h. Subsequently,

the glycogen content of cells in each group was measured using the Glucogen Content Assay Kit. In brief, cells were collected after lysis, sonicated for 30 s and boiled in a boiling water bath for 20 min. The volume was fixed to 5 mL with distilled water and centrifuged at 8000 g for 10 min. The collected supernatant was mixed with the working solution at a ratio of 1:4 (v/v) and then boiled in a boiling water bath for 10 min. Finally, absorbance values were measured at A570 nm by a microplate reader. The final results were calibrated by protein concentration.

HepG2 cells were incubated with different concentrations of IDM for 24 h after establishing IR models in 6-well plates. Cells were fixed using 70% ethanol for 10 min. Then, the cells were stained with periodic acid solution, Schiff reagent and hematoxylin staining solution. Stained cells were rinsed twice using distilled water. Subsequently, the images were obtained using fluorescence microscopy (Leica DMI8, Wetzlar, Germany).

2.10 RNA-Seq and Data analysis

HepG2 cells were placed in 6-well plates at a density of 3×10^5 per well for 24 h. Then, the vehicle group was replaced with DMEM containing 10% FBS, and the HepG2 cells in the Glu_PA group (IR group) and the IDM group were established IR models. After 24 h of incubation, the control and IR groups were replaced with DMEM containing 10% FBS, and the IDM group was replaced with DMEM containing 400 $\mu\text{g/mL}$ IDM for 24 h. Total RNA was extracted from the three groups of cells using Trizol reagent (SPARK jade, Jinan, China), and RNA integrity was measured using Agilent 2100 bioanalyzer (Thermo Fisher Scientific, MA, USA). The RNA libraries were prepared by application of Fast RNA-seq Lib Prep Kit V2 (RK20306) from ABclonal Biotechnology (Wuhan, China). After enrichment and quantification, the high-throughput sequencing was performed using the Novaseq X plus from Illumina (Carlsbad, CA, USA). After a quality screening of the raw data, HISAT2 (v2.0.4) was used to compare the sequencing data with the reference genes. Gene expression levels were calculated using RSEM (v1.2.12) and differential expression analysis was performed using DESeq2 (v1.4.5) with p -values < 0.05 and Log (Fold-Change) > 1 .

2.11 Western blot assay

HepG2 cells (3×10^5 cells per well) were seeded in 6-well plates and established IR-HepG2 cell model and exposed to IDM (0–400 $\mu\text{g/mL}$) for 0–24 h. After the cells were rinsed twice with pre-cooled PBS, 70 μL of cell lysis buffer containing PMSF was added to each well. Then, the collected cells were sonicated (ultrasound 3 s, interval 3 s, 10 times) using a cell ultrasonicator (JY96-IIN, Xinyi Ultrasonic Equipment Co., Ningbo, China). After the supernatants were collected by centrifugation, the protein concentration of lysates from each group was measured and quantified using the BCA Protein Assay kit. After that, SDS-PAGE loading buffer was added to each group of lysates and heated in a boiling water bath for 10 min. Denatured proteins were stored at -20°C . The Western blot method was followed by our previous study [18]. HNF-4 α nuclear proteins were extracted using the Nuclear and Cytoplasmic Protein Extraction Kit. Protein bands were identified using an Enhanced Chemiluminescence Kit and an automated chemiluminescence image analysis

system (Tanon, Shanghai, China). Finally, the protein band intensity was measured using ImageJ software (National Institutes of Health, Bethesda, MD).

2.12 Analysis of RT-qPCR

HepG2 cells (3×10^5 cells per well) were seeded in 6-well plates for 24 h. IR-HepG2 cell model was established and the cells were treated with different concentrations of IDM as previously described. After that, the total RNA was extracted using Trizol reagent. The ratio of A260/280 > 1.8 was considered to be qualified for RNA purity. The cDNA was constructed using a SPARK script II RT Plus Kit (SPARK jade, Jinan, China). The primer sequences of the targeted genes referred to the design of Xia et al. (Table S1) ^[13]. mRNA expression levels of each group were normalized relative to GAPDH levels. The expression levels of corresponding mRNAs were analyzed using the $2^{-\Delta\Delta C_q}$ method.

2.13 Animals experiment

2.13.1 Animal and experimental design

Animal experiments were executed according to the guidelines of the institutional animal ethics committee, and were supported by the institutional animal committee of Tianjin University of Science and Technology University (2025025, permission date: March 6, 2025). The male C57BL/6J mice (SPF grade, 6 weeks old) were purchased from Jinan Pengyue Experimental Animal Breeding Co. Ltd. (Jinan, China). All animals were housed under SPF conditions with a standard 12 h light/dark cycle, free access to food and water, and a controlled temperature (25 ± 2 °C).

C57BL/6J mice were randomly divided into basal diet group (Control group, n=8) and high fat diet group (HFD group, 60% fat, D12492, n=24). After 4 weeks of feeding, HFD mice were intraperitoneally injected with STZ 5 times (50 mg/kg b.w., once a day). After 3 days, the HFD/STZ mice with fasting blood glucose levels ≥ 11.1 mM were considered T2DM mice. Studies have shown that the effective dose of vinegar is 1.5-6 mL/kg b.w., and the content of melanoidins in Monascus vinegar is 130.87 mg/mL ^[10, 19]. Therefore, LVM at 250 mg/mL and 750 mg/mL were selected for subsequent animal experiments. The T2DM mice were randomly divided into three groups: HFD/STZ group (n=8), low-dose LVM group (L-LVM, 250 mg/kg b.w., n=8) and high-dose LVM group (H-LVM, 750 mg/kg b.w., n=8). During the next 6 weeks, LVM dissolved in sterile water was administered to the L-LVM and H-LVM mice by gavage once a day, and the control and HFD/STZ mice were given the same amount of sterile water by gavage. During this period, the control group was fed with basal diet, and the remaining mice were fed with high-fat diet. At the end of the experiment, mice were sacrificed by cervical dislocation after intraperitoneal injection of sodium pentobarbital (50 mg/kg b.w.). Blood samples and liver were collected for subsequent experiments.

2.13.2 Metabolic tolerance tests

Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were performed before the mice were sacrificed. Mice participating in the OGTT were fasted overnight and then gavaged with D-glucose (0.5 g/kg b.w.), and mice participating in the ITT were fasted for 4 h and then injected intraperitoneally with insulin (0.5

U/kg b.w.). Blood from the tail tip was collected at 15, 30, 60, 90, and 120 min for glucose measurement. The data were visualized by the area under the curve (AUC).

2.13.3 Biochemical analysis

The concentrations of triglyceride (TG) and total cholesterol (TC) were measured using a microplate reader (Molecular Devices, San Jose, CA) according to the manufacturer's instructions (Nanjing Jiancheng; TG, A110-1-1; TC, A111-1-1).

2.13.4 Histopathological analysis

After mice were sacrificed, livers were rapidly fixed in 4% paraformaldehyde for 48 hours. Subsequently, paraffin-embedded tissue slides were stained with hematoxylin and eosin (H&E) and section images were obtained using an optical microscope (Leica DMI8, Wetzlar, Germany). Liver sections were scored under blinded conditions according to the NAFLD activity score (NAS).

2.14 Statistical Analysis.

All the data are presented as means $\pm$ SD of three to four independent experiments. Significant differences were evaluated by one-way ANOVA and Tukey's test in GraphPad Prism 10.0 (GraphPad Software, San Diego, CA, USA). P value < 0.05 were considered significant.

3. Results and disscussion

3.1 Physical and chemical properties of IDM

In our previous study, *Monascus* vinegar melanoidins (LVM) extracted from 5-year-old *Monascus* vinegar exhibited high small intestinal bioaccessibility (80.05%)¹¹. The small intestinal digestion products (IDM), while partially degraded during simulated digestion, retained their characteristic melanoidin structure (Fig. S1). IDM had a molecular weight (Mw) of 46,645 Da and comprised primarily carbohydrates (632.6 mg GE/g), proteins (45.9 mg BSA/g), and phenols (31.0 mg GAE/g) (Table S2). The monosaccharide, amino acid, and phenolic compositions were further characterized by HPLC-MS (Fig. S2).

3.2 Transport of IDM across Caco-2 cells monolayer

Given the high bioaccessibility and stability of IDM during digestion, we investigated its transport across Caco-2 cell monolayer, a well-established model for intestinal epithelial permeability. Initially, we determined that IDM at 100 $\mu\text{g/mL}$ did not significantly affect Caco-2 cell viability ($p > 0.05$), while concentrations above 200 $\mu\text{g/mL}$ were cytotoxic (Fig. 1A). Therefore, 100 $\mu\text{g/mL}$ IDM was used for subsequent transport studies.

Because the confluent monolayer of Caco-2 cells has functional properties of the intestinal epithelium, they have been widely used in studies to assess the permeability of nutrients in the gut^[20]. To verify whether IDM could be transported through intestinal epithelial cells, we monitored the amount of IDM in the basal chamber over a 4 h period. IDM transport across the Caco-2 monolayer increased linearly over 4 hours, reaching 8.75 $\mu\text{g/cm}^2$ (Fig. 1B), with an apparent permeability (Papp) of 1.19×10^{-6} cm/s. Due to the

heterogeneity and structural complexity of melanoidins, they are generally considered to play beneficial roles in colonic fermentation, while absorption in the gastrointestinal tract is neglected [6, 21]. However, our results support the notion of intestinal uptake of melanoidins. To determine the transport mechanism, we assessed TEER values and LDH release. IDM did not significantly alter TEER values (Fig. 1C) or LDH levels (Fig. 1D), indicating that transport was not due to paracellular diffusion or cell membrane disruption ($p > 0.05$). Therefore, IDM likely undergoes transcellular transport.

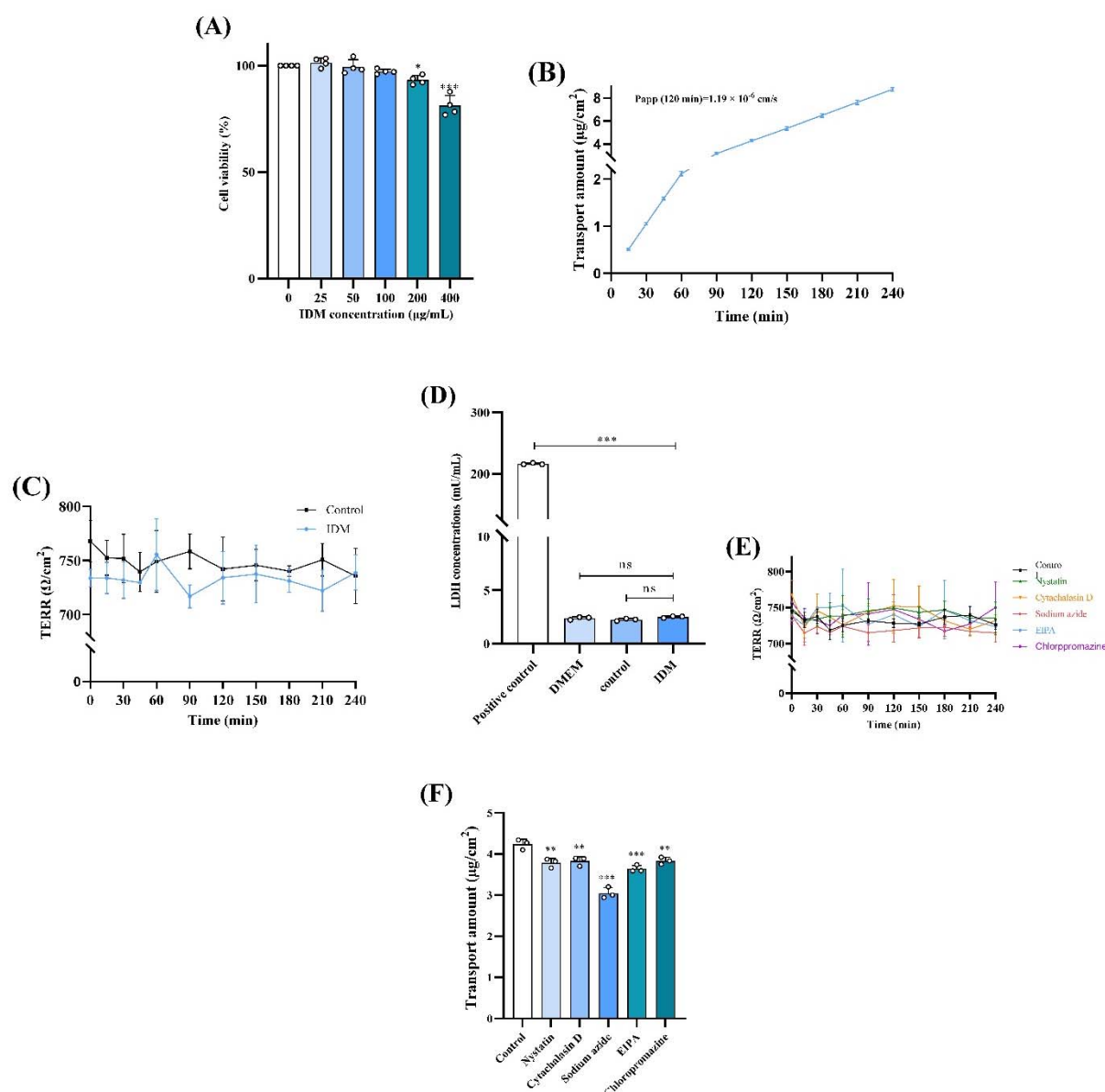


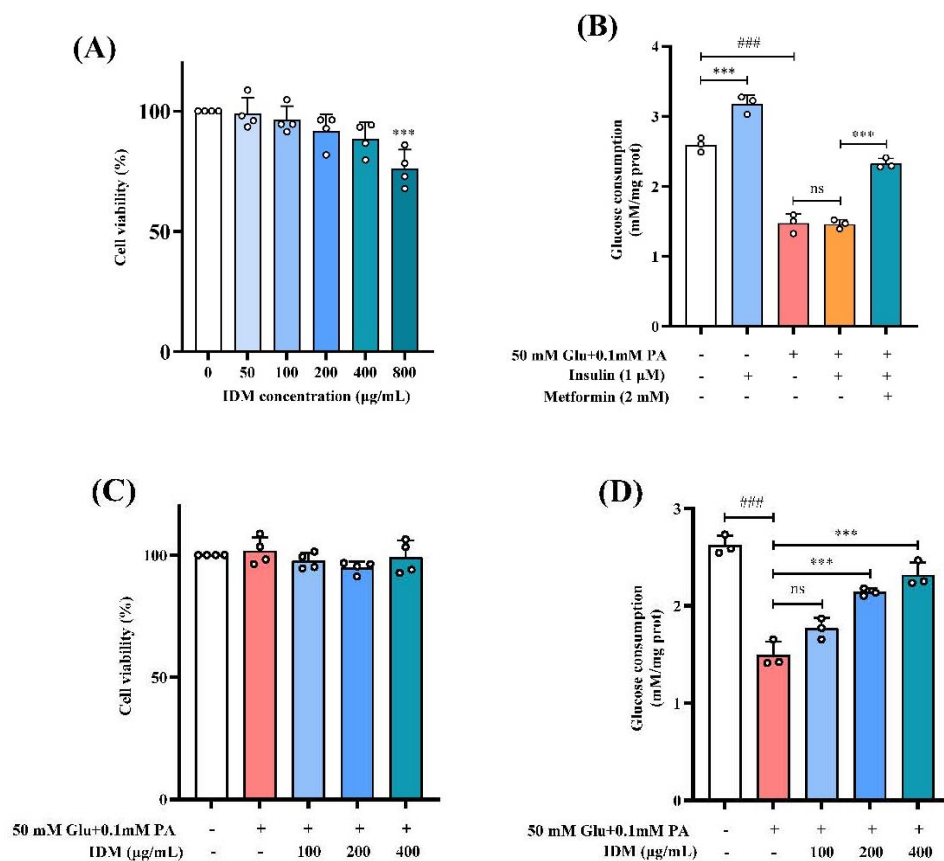
Fig. 1. Evaluation of the transport of IDM across Caco-2 cells monolayer. (A) Effect of different concentrations (25–400 μg/mL) of IDM on the viability of Caco-2 cells incubated for 24 h. (B) Changes in TEER during incubation of Caco-2 cells monolayer with IDM and fresh HBSS solutions. (C) The cumulative amount of IDM in the basolateral chamber as the function of time. (D) Effect of 100 μg/mL IDM on the integrity of cell membrane in Caco-2 cells. (E) Changes in TEER during incubation of Caco-2 cells monolayer with different endocytosis inhibitors. (F) The effects of different endocytosis inhibitors on the transport of IDM across Caco-2 cells monolayer. The values are presented as mean $\pm$ SD, $n=3-4$, the statistical significance was determined with * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ when compared to control group.

To further verify the mechanism of IDM transport through intestinal epithelial cells, we selected a variety of inhibitors to treat Caco-2 cells monolayer. Fig. 1E showed that these inhibitors had almost no effect on

TEER values, indicating that they did not open the tight junctions between cells [8]. As shown in Fig. 1F, sodium azide inhibited IDM transport most significantly by 28.1% ($P < 0.05$), indicating that IDM transport was heavily dependent on energy. Furthermore, macropinocytosis (EIPA), actin filament dynamics (cytochalasin D), clathrin-mediated endocytosis (chlorpromazine), and caveolae/lipid raft-mediated endocytosis (nystatin) also contributed to IDM uptake, albeit to a lesser extent (9.6–14.1% reduction, $P < 0.05$) (Fig. 1F) [22–24]. Since the formation and development of vinegar melanoidins during aging are dynamically aggregated, the IDM showed strong heterogeneity (the molecular weight distribution showed a typical multi-peak structure) [11]. This determined its involvement in multiple endocytic pathways during intestinal epithelial cell transport. These endocytic pathways have both synergistic and competitive relationships. On the one hand, macropinocytosis, clathrin-mediated endocytosis and caveolae/lipid raft-mediated endocytosis are responsible for the co-transport of IDM with different particle sizes, respectively [24]. On the other hand, macropinocytosis and clathrin-mediated endocytosis mediated IDM transport are both dependent on actin, which could lead to competitive inhibition [25].

3.3 Effect of IDM on glucose uptake and consumption in IR-HepG2 cells

To assess the impact of IDM on HepG2 cell viability, we first conducted an MTT assay. IDM concentrations up to 400 $\mu\text{g/mL}$ did not significantly affect cell viability ($p > 0.05$), while 800 $\mu\text{g/mL}$ exhibited significant cytotoxicity (Fig. 2A, $P < 0.001$). Consequently, we proceeded with IDM concentrations ranging from 0 to 400 $\mu\text{g/mL}$ for subsequent experiments.



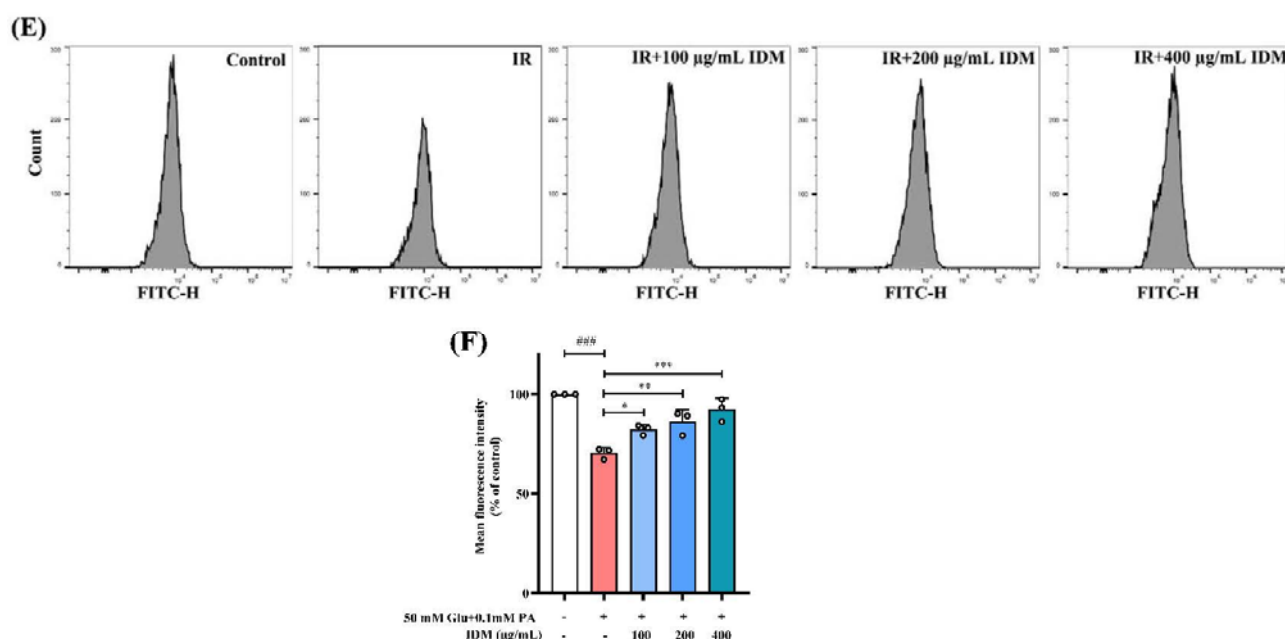


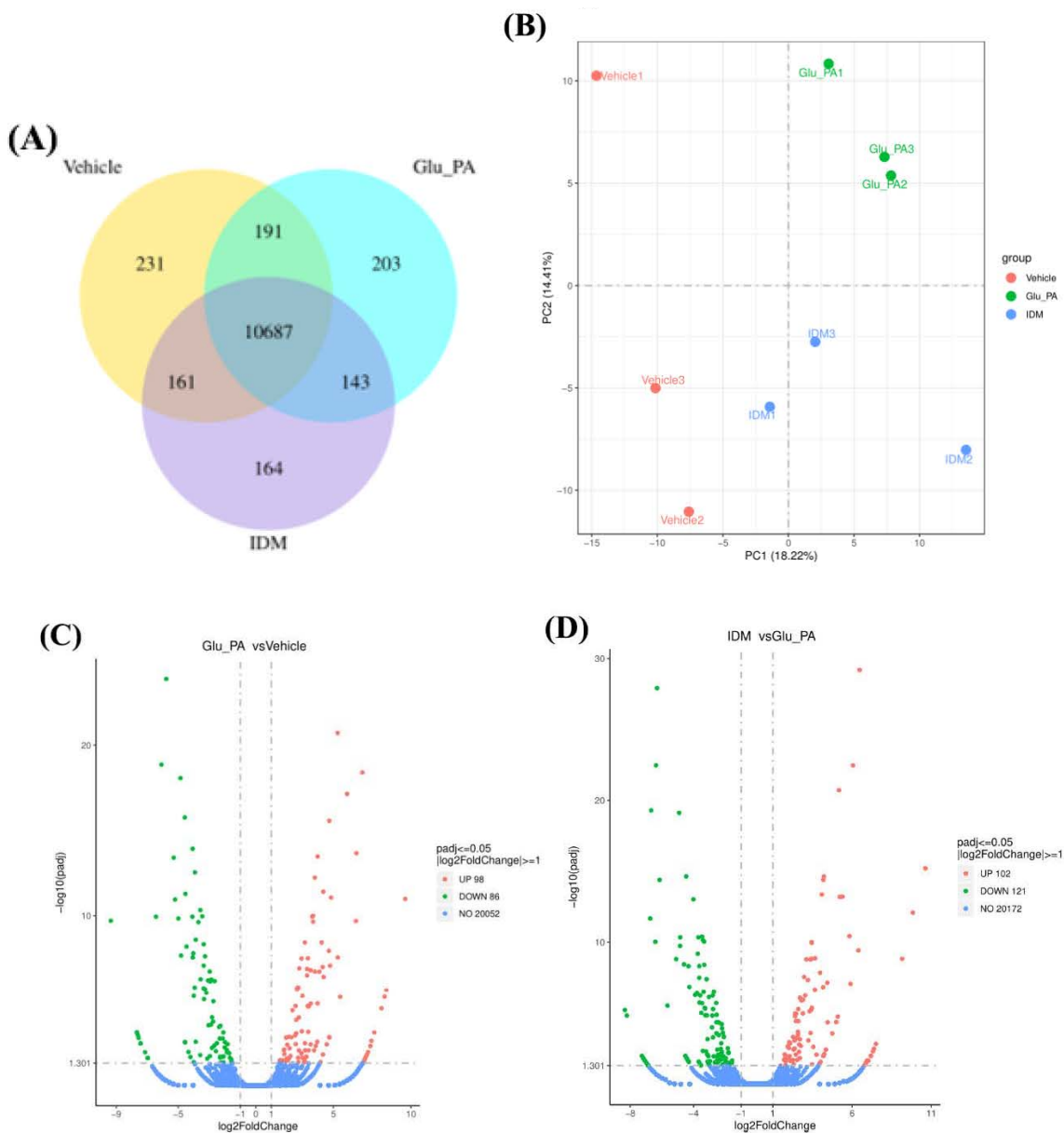
Fig. 2. Effect of IDM on cell viability, glucose uptake and glucose consumption in IR-HepG2 cells. (A) Effect of different concentrations IDM (50–800 µg/mL) on the viability of HepG2 cells incubated for 24 h. (B) Induction of insulin resistance in HepG-2 cells with DMEM containing 0.1 mM PA and 50 mM glucose. (C) Effect of IDM (100–400 µg/mL) on the viability of IR-HepG2 cells incubated for 24 h. (D) Effect of different concentrations of IDM on glucose consumption in IR-HepG2 cells after 24 h of treatment. (E) The mean fluorescence intensity of 2-NBDG was measured by flow cytometry to represent intracellular glucose uptake. (F) Quantification of the corresponding 2-NBDG fluorescence. All data are expressed as mean $\pm$ SD ($n = 3$ –4) for each group. P value significance represented by ***, <0.001 ; ###, <0.001 , ns, not significant.

Given that chronic exposure to high glucose and free fatty acids induces IR, we established an IR-HepG2 cell model using 50 mM glucose and 0.1 mM PA [13, 26]. This model displayed a significant reduction in glucose consumption ($P < 0.001$), which was not restored by 1 µM insulin (Fig. 2B, $p > 0.05$). In contrast, 2 mM metformin, a known insulin sensitizer, effectively increased glucose consumption in the IR-HepG2 cells (Fig. 2B, $P < 0.001$) [27]. Importantly, IDM (0–400 µg/mL) did not affect the viability of the IR-HepG2 cells (Fig. 2C, $p > 0.05$). These results confirm the successful establishment of the IR-HepG2 model.

Since IR is characterized by impaired glucose uptake and consumption in the liver, we investigated the effects of IDM on these parameters. IDM significantly increased glucose consumption in IR-HepG2 cells in a dose-dependent manner. Specifically, 200 and 400 µg/mL IDM increased glucose consumption by 0.64 mM/mg and 0.82 mM/mg, respectively (Fig. 2D, $P < 0.001$). Furthermore, IDM enhanced glucose uptake in IR-HepG2 cells in a dose-dependent manner (Fig. 2E–F, $P < 0.05$). These findings suggest that IDM effectively ameliorates IR by improving glucose utilization in HepG2 cells. The observed insulin-sensitizing effects of IDM may be attributed to its phenolic components. Previous studies have reported that daily vinegar consumption improves insulin sensitivity [28, 29]. Our previous research demonstrated that LVM in *Monascus* vinegar act as "reservoirs" for phenolic compounds, contributing significantly to its antioxidant capacity (Wang et al., 2024). Notably, IDM shares 12 phenolic compounds with Zhenjiang aromatic vinegar (ZAVE), which has been shown to promote glucose uptake and consumption by mitigating oxidative stress in HepG2 cells (Fig. S2) [13]. Therefore, it is likely that the hypoglycemic effect of IDM is mediated by phenolic compounds that bind to carbohydrate backbone.

3.4 Transcriptomic Analysis of IDM's Effects on IR-HepG2 Cells

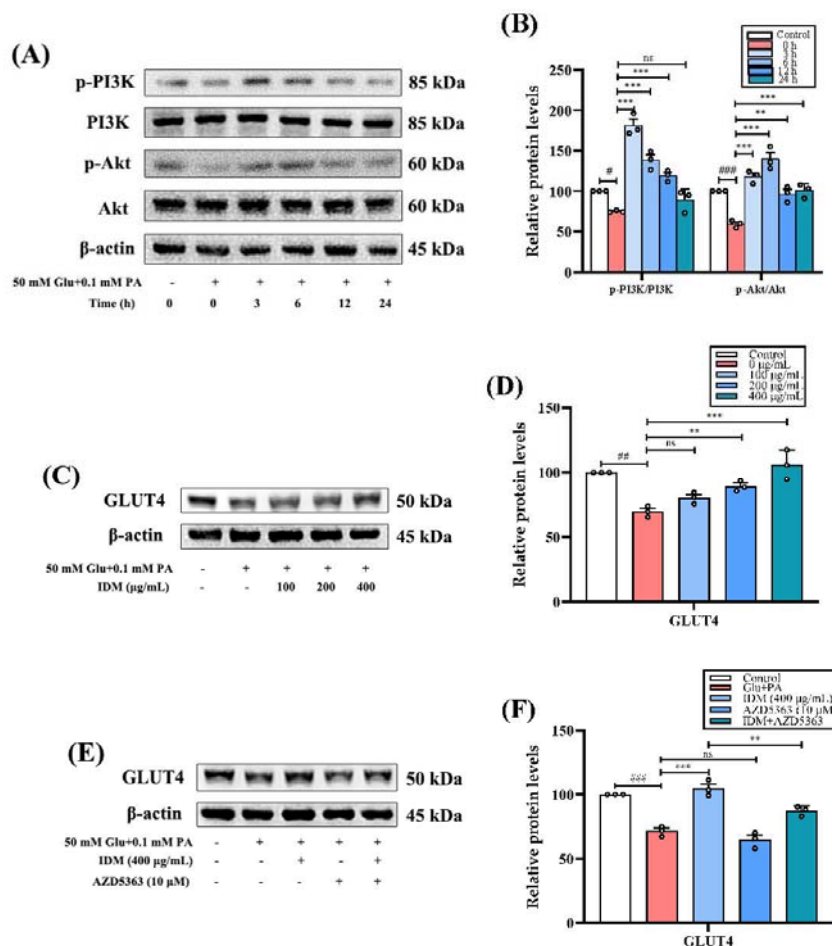
To elucidate the mechanisms underlying IDM's anti-IR effects, we performed RNA-seq analysis. Venn diagram showed that 11,270, 11,1224, and 11,155 genes were detected in the vehicle, IR, and IDM groups, respectively, most of which were commonly expressed in the three groups (Fig. 3A). The results of principal component analysis (PCA) showed clear clustering of transcriptome profiles in the vehicle, IR and IDM groups (Fig. 3B). There were 98 up-regulated DEGs (Differentially Expressed Genes) and 86 down-regulated DEGs in the IR group compared with the control group (Fig. 3C). Compared with the IR group, the IDM group had 102 up-regulated DEGs and 121 down-regulated DEGs (Fig. 3D). IDM treatment reversed the expression of 80 DEGs (Fig. 3E, F), with the gene expression profile of the IDM group clustering closer to the vehicle group (Fig. 3G), indicating a partial restoration of gene expression.



Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed to elucidate the possible functional changes caused by DEGs. GO enrichment analysis revealed that IDM significantly affected genes related to liver development, bone resorption, and endocytic vesicles (Fig. 3H). These findings suggest that IDM may improve glucose homeostasis, potentially mitigate diabetic bone disease by inhibiting bone resorption, and modulate endocytic pathways [30, 31]. KEGG pathway analysis indicated that IDM may modulate T2DM and glucagon signaling pathways (Fig. 3I), which are critical in IR development.

3.5 IDM promotes glucose uptake by activating GLUT4 through the PI3K/Akt pathway in IR-HepG2 cells

Consistent with the KEGG pathway analysis, which suggested IDM's influence on T2DM and glucagon signaling (Fig. 3I), we investigated the role of the PI3K/Akt pathway, a key regulator of growth and metabolism, in IDM's hypoglycemic activity [32]. We examined the activation of PI3K/Akt by IDM in IR-HepG2 cells. Exposure to 50 mM glucose and 0.1 mM PA for 24 h significantly suppressed the phosphorylation of PI3K (p-PI3K) and Akt (p-Akt) compared to the control group (Fig. 4A, B, $P < 0.05$). IDM treatment (400 $\mu\text{g/mL}$) significantly increased p-PI3K and p-Akt levels, albeit with different temporal profiles. p-PI3K peaked at 3 h (1.81-fold of control, $P < 0.001$) and returned to baseline by 24 h, indicating a transient activation. In contrast, p-Akt peaked at 6 h (1.40-fold of control) and remained activated at 24 h (1.70-fold of IR group, $P < 0.001$), suggesting sustained activation. This lag in Akt activation is consistent with the sequential nature of signal transduction pathways [33, 34].



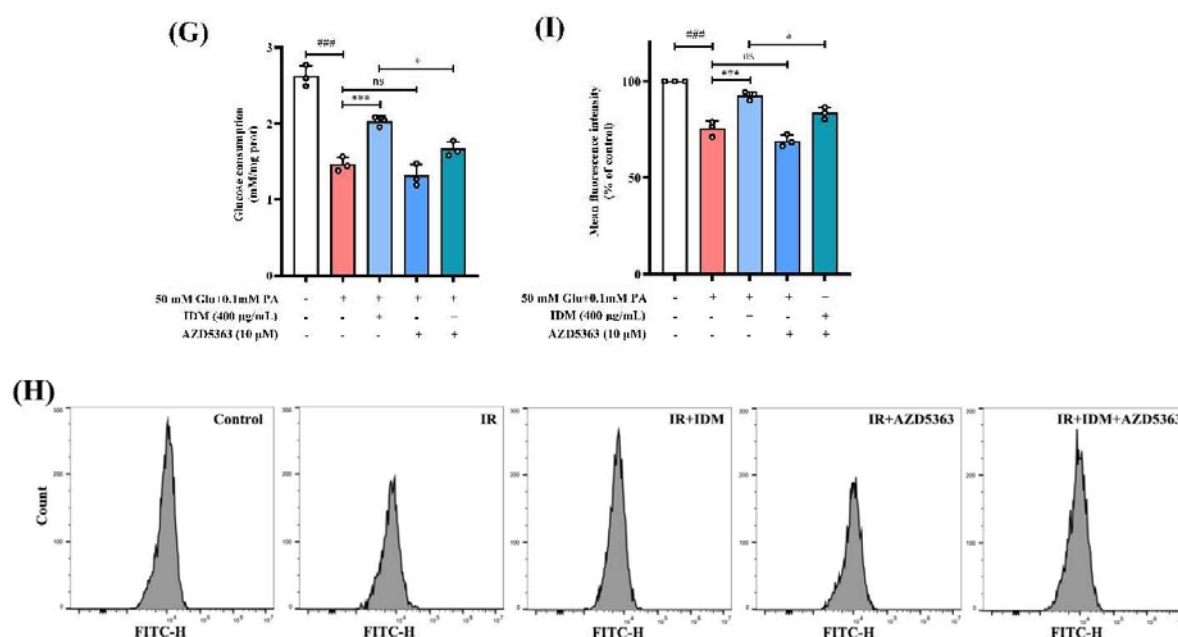


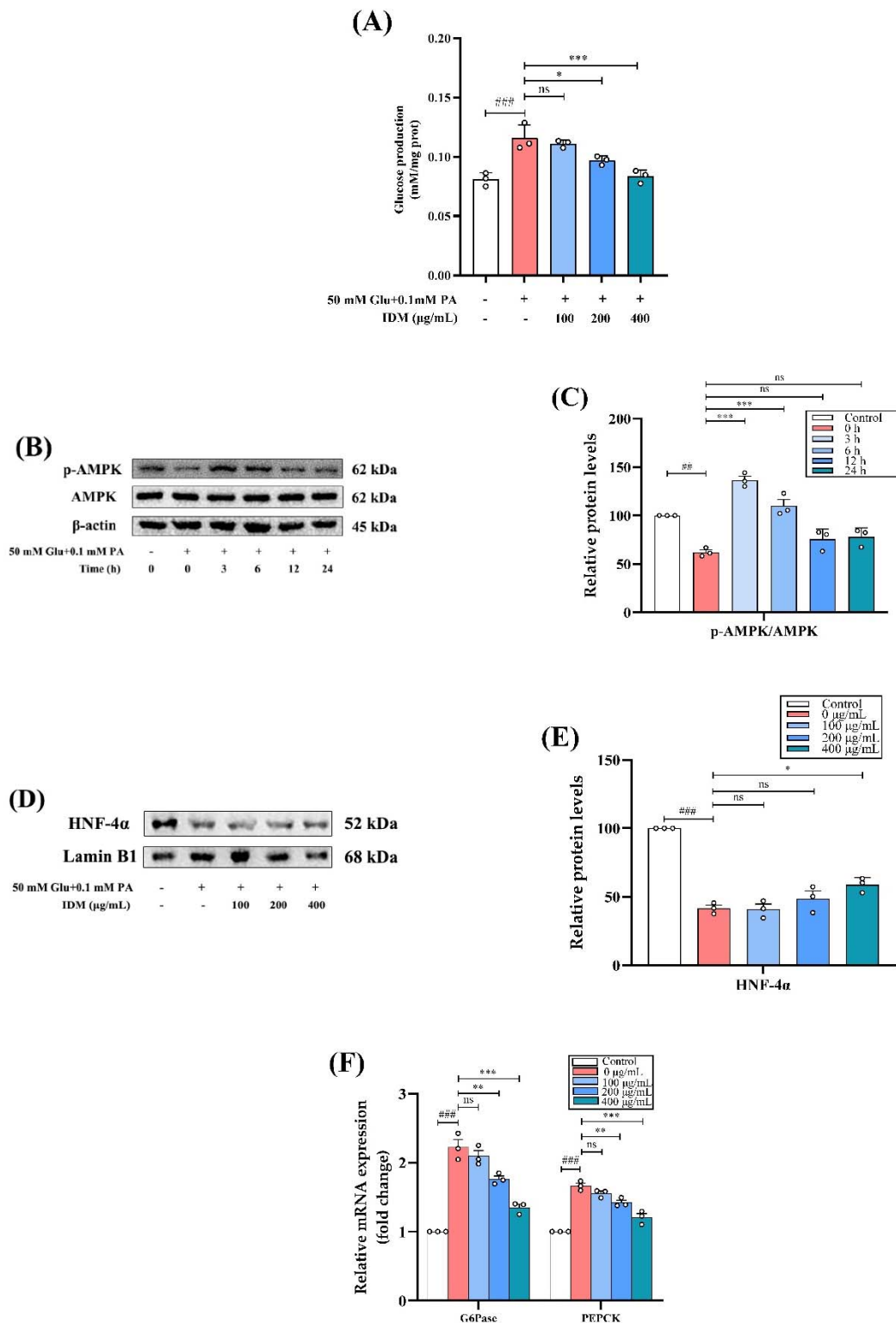
Fig. 4. IDM increases glucose consumption and uptake in IR-HepG2 cells by promoting GLUT4 expression through PI3K/Akt pathway. (A, B) Effect of 400 µg/mL IDM on PI3K/Akt pathway phosphorylation levels in HepG2 cells within 24 h. (C, D) Effect of different concentrations IDM (100–400 µg/mL) on GLUT4 protein expression in HepG2 cells. (E, F) Effect of 10 µM AZD5363 (Akt inhibitor) on 400 µg/mL IDM to increase the GLUT4 expression levels in HepG2 cells. Effect of AZD5363 on IDM to stimulate glucose consumption (G) and glucose uptake (H, I) in IR-HepG2 cells. All data are expressed as mean $\pm$ SD ($n = 3$) for each group. P value significance represented by #, <0.05; ##, <0.01; ###, <0.001; *, <0.05; **, <0.01; ***, <0.001, ns, not significant.

Then we evaluated the effect of IDM on GLUT4 expression, a protein critical for glucose transport [35]. IR-HepG2 cells exhibited significantly reduced GLUT4 expression ($P < 0.01$), which was restored by IDM (200 and 400 µg/mL) (Fig. 4C, D, $P < 0.01$). Notably, 400 µg/mL IDM restored GLUT4 expression to near-control levels (1.06-fold). To confirm the involvement of the PI3K/Akt pathway in IDM-mediated GLUT4 activation, we used AZD5363, an Akt inhibitor. AZD5363 alone did not affect GLUT4 expression in IR-HepG2 cells (Fig. 4E, F, $p > 0.05$). However, it abolished the IDM-induced increase in GLUT4 expression ($P < 0.01$), demonstrating that IDM's effect on GLUT4 is dependent on Akt activation (Fig. 4E, F). Furthermore, inhibiting Akt also significantly reduced IDM's ability to promote glucose uptake and consumption (Fig. 4G–I, $P < 0.05$). These findings collectively suggest that IDM promotes GLUT4 translocation to the cell membrane via the PI3K/Akt pathway, thereby facilitating glucose uptake [36]. This study further elucidates the relationship between signal transduction and the hypoglycemic activity of IDM, expanding on previous research [27, 37].

3.6 IDM inhibits gluconeogenesis by modulating gene expression through the AMPK pathway in IR-HepG2 cells

Enhanced hepatic gluconeogenesis is a hallmark of T2DM, disrupting the balance with glycogen synthesis [26]. Therefore, inhibiting hepatic glucose production from non-carbohydrate sources is crucial for glycemic control [38]. Consistent with this, IDM (200–400 µg/mL) significantly reduced glucose production in IR-HepG2 cells compared to the IR group (Fig. 5A, $P < 0.05$). As an energy sensor that controls glucose and lipid metabolism, AMPK is one of the kinases known to inhibit hepatic gluconeogenesis [5, 39]. To

investigate whether AMPK mediates IDM's inhibitory effect on gluconeogenesis, we examined AMPK phosphorylation. The IR group showed significantly reduced AMPK phosphorylation compared to the control group (0.62-fold) (Fig. 5B, C, $P < 0.01$). IDM treatment induced a rapid and transient increase in AMPK phosphorylation, peaking at 3 h (1.37-fold of control, $P < 0.01$) and declining thereafter. By 12 h, AMPK phosphorylation returned to levels comparable to the IR group ($p > 0.05$), indicating that the activation of AMPK pathway by IDM was rapid and transient, and the action time may be concentrated in 0–6 h.



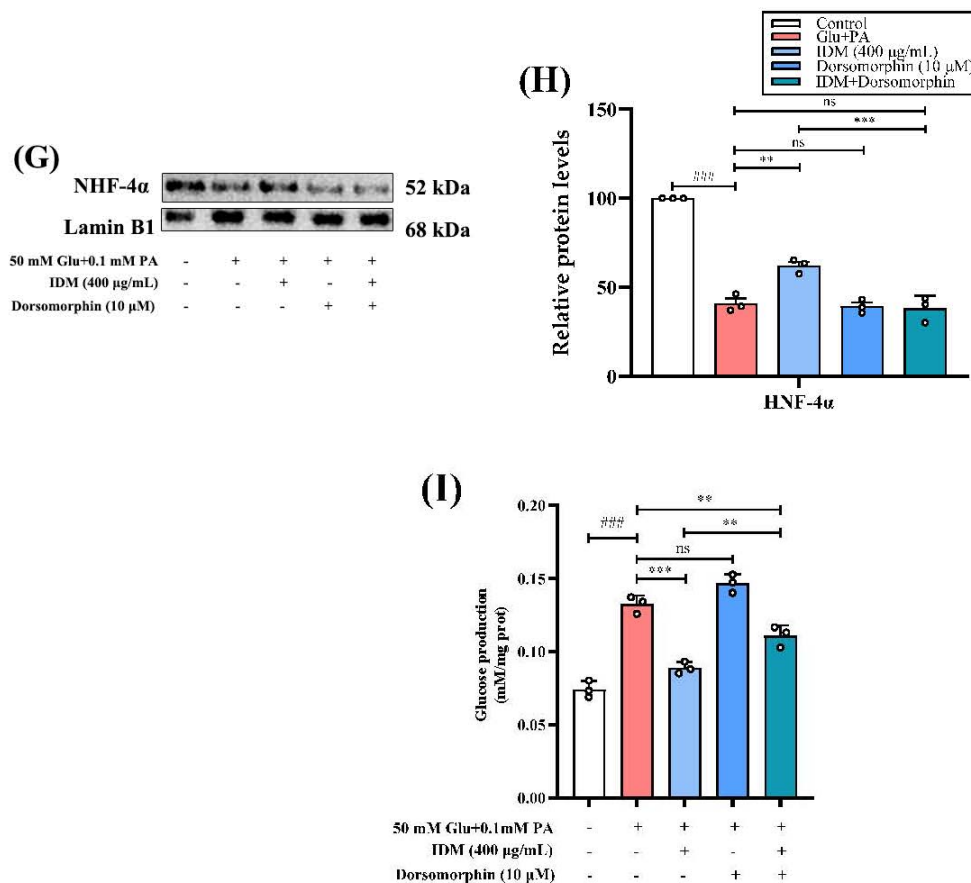


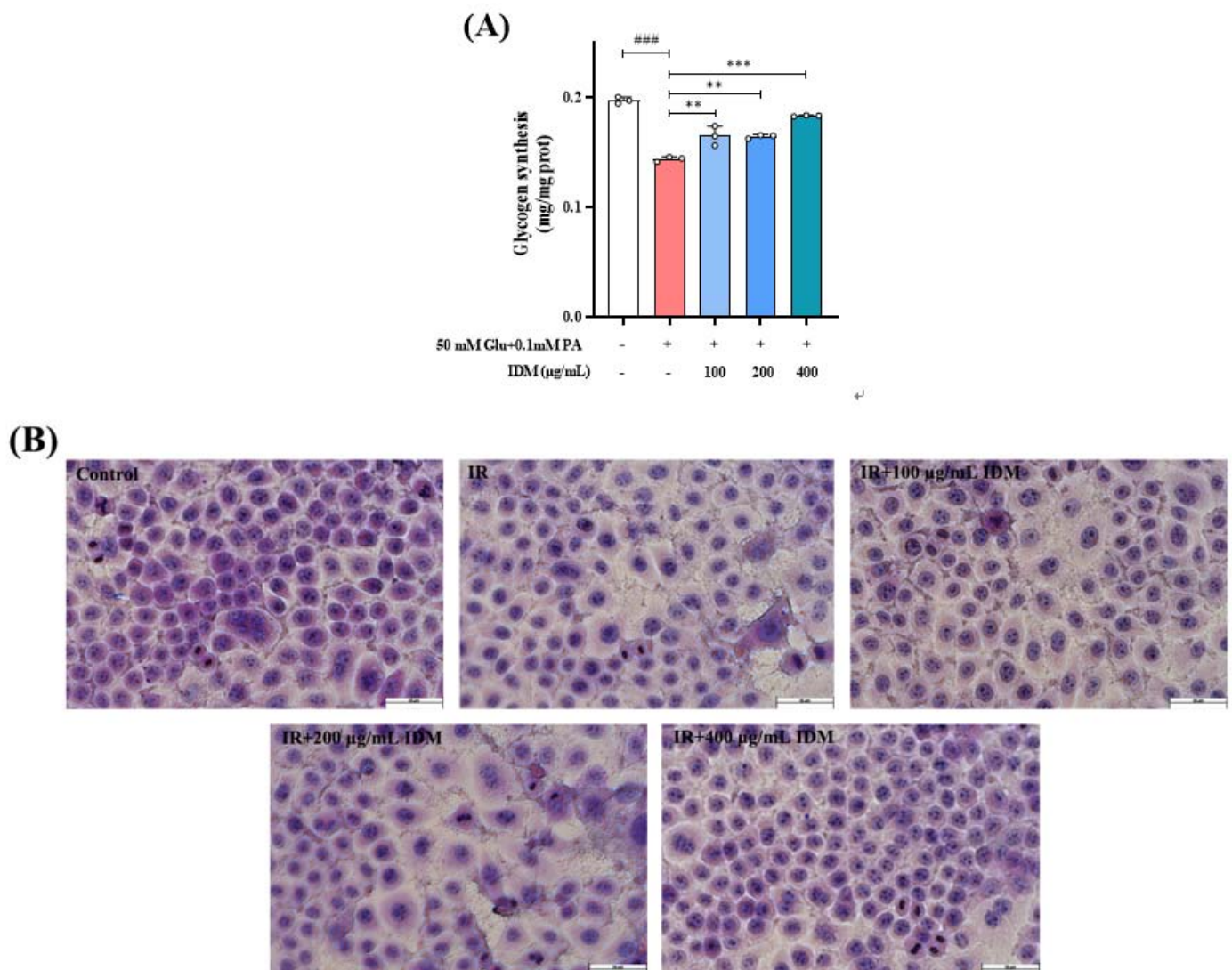
Fig. 5. IDM attenuates gluconeogenesis in IR-HepG2 cells by inhibiting related genes expression through AMPK pathway. (A) Effect of of IDM (100–400 μg/mL) on gluconeogenesis in IR-HepG2 cells after 24 h of treatment. (B, C) Effect of 400 μg/mL IDM on AMPK pathway phosphorylation levels in HepG2 cells within 24 h. (D, E) Effect of IDM (100–400 μg/mL) on HNF-4α expression (nuclear protein) in HepG2 cells. (F) The mRNA expression levels of G6Pase and PEPCK was determined by RT-qPCR. (G, H) Effect of 10 μM dorsomorphin (AMPK inhibitor) on IDM (400 μg/mL) to increase the HNF-4α expression levels in HepG2 cells. (I) Effect of dorsomorphin on IDM to inhibit gluconeogenesis in IR-HepG2 cells. All data are expressed as mean ± SD (n = 3) for each group. P value significance represented by ##, <0.01; ###, <0.001; *, <0.05; **, <0.01; ***, <0.001, ns, not significant.

HNF-4α, a nuclear factor regulated by AMPK, activates gluconeogenic genes in the fasting liver by interacting with PGC-1α [30, 40]. G6Pase and PEPCK are two rate-limiting enzymes that promote gluconeogenesis, and their abnormal expression is closely related to IR [41]. Compared with IR group, IDM (400 μg/mL) significantly restored HNF-4α expression ($P < 0.05$), but only to 0.59-fold of the control group (Fig. 5D, E). In addition, IDM (200–400 μg/mL) significantly suppressed the IR-induced overexpression of G6Pase and PEPCK (Fig. 5F, $P < 0.01$). At present, it is no longer controversial that G6Pase and PEPCK are regulated by nuclear transcription factor HNF-4α in IR related studies [40, 42]. Interestingly, while 200 μg/mL IDM did not significantly affect HNF-4α expression ($p > 0.05$), it effectively inhibited G6Pase and PEPCK expression (Fig. 5D–F, $P < 0.01$). This suggests that G6Pase and PEPCK may be regulated by other transcription factors in addition to HNF-4α, indicating multiple pathways mediating IDM's effect [30, 43]. Blocking AMPK with dorsomorphin completely abolished the IDM-induced increase in HNF-4α expression ($p > 0.05$ vs. IR group), confirming that HNF-4α activation is AMPK-dependent (Fig. 5G, H). Dorsomorphin also attenuated IDM's inhibitory effect on gluconeogenesis ($P < 0.01$ vs. IDM alone), but gluconeogenesis remained significantly lower than in the IR group (Fig. 5I, $P < 0.01$). This supports the

involvement of additional pathways in IDM's suppression of gluconeogenesis, beyond the AMPK/HNF-4 α axis.

3.7 IDM enhanced glycogen synthesis by inhibiting *p*-GS through the PI3K/Akt/GSK3 β pathway in IR-HepG2 cells

The liver plays a crucial role in maintaining glucose homeostasis by regulating glucose uptake and storage through glycogen synthesis [44]. To investigate IDM's effect on glycogen synthesis in IR-HepG2 cells, we analyzed glycogen levels and PAS-stained glycogen. Glycogen synthesis was significantly suppressed in the IR group compared to the control group ($P < 0.001$). IDM (100–400 $\mu\text{g/mL}$) dose-dependently restored glycogen synthesis in IR-HepG2 cells (Fig. 6A, $P < 0.01$). PAS staining corroborated these findings, visually demonstrating the differences in glycogen levels among the groups (Fig. 6B). These results indicate that IDM effectively enhances glycogen synthesis in IR-HepG2 cells, likely contributing to the restoration of metabolic balance by increasing glucose uptake and inhibiting gluconeogenesis (Fig. 2E, F, Fig. 5A).



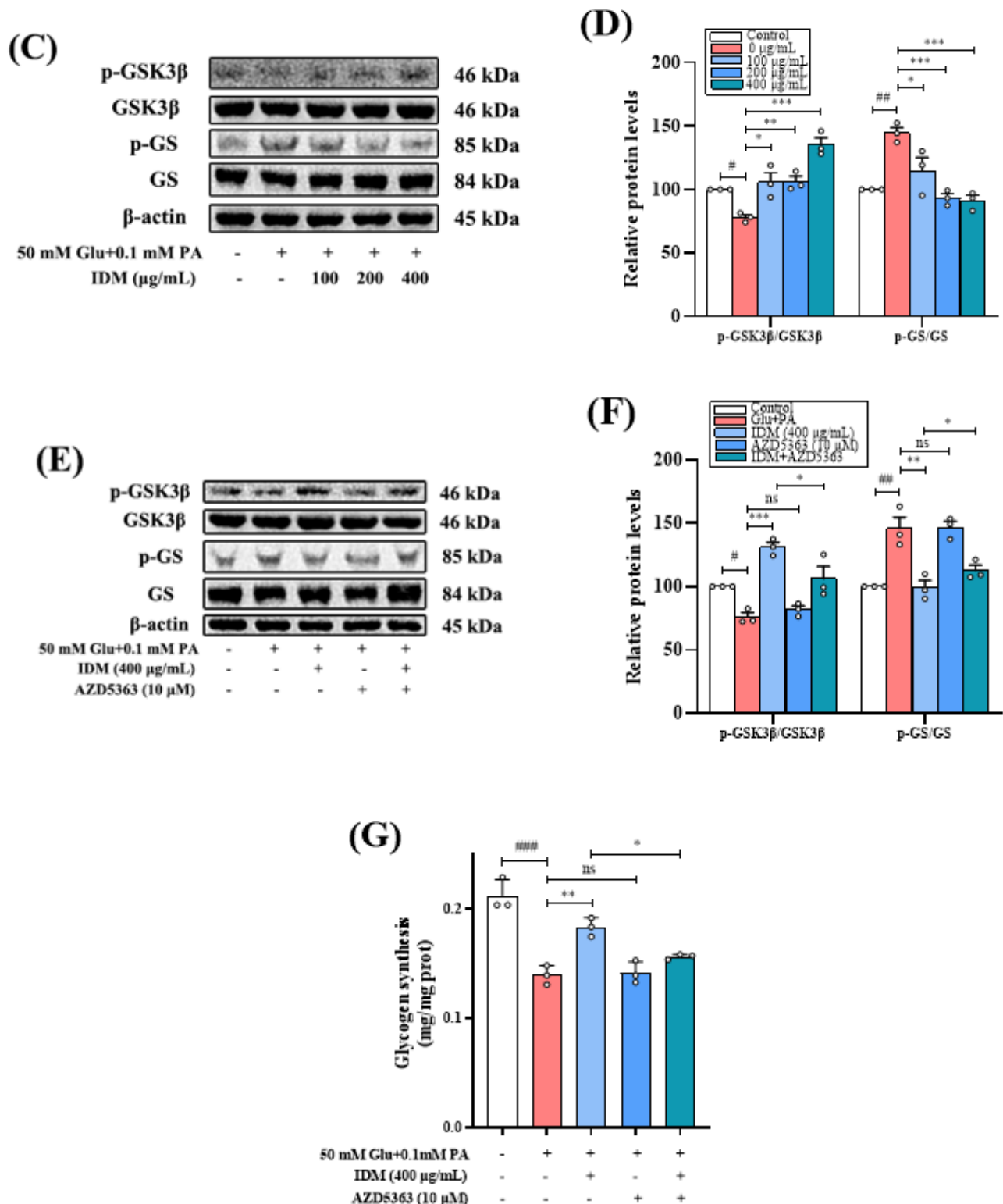


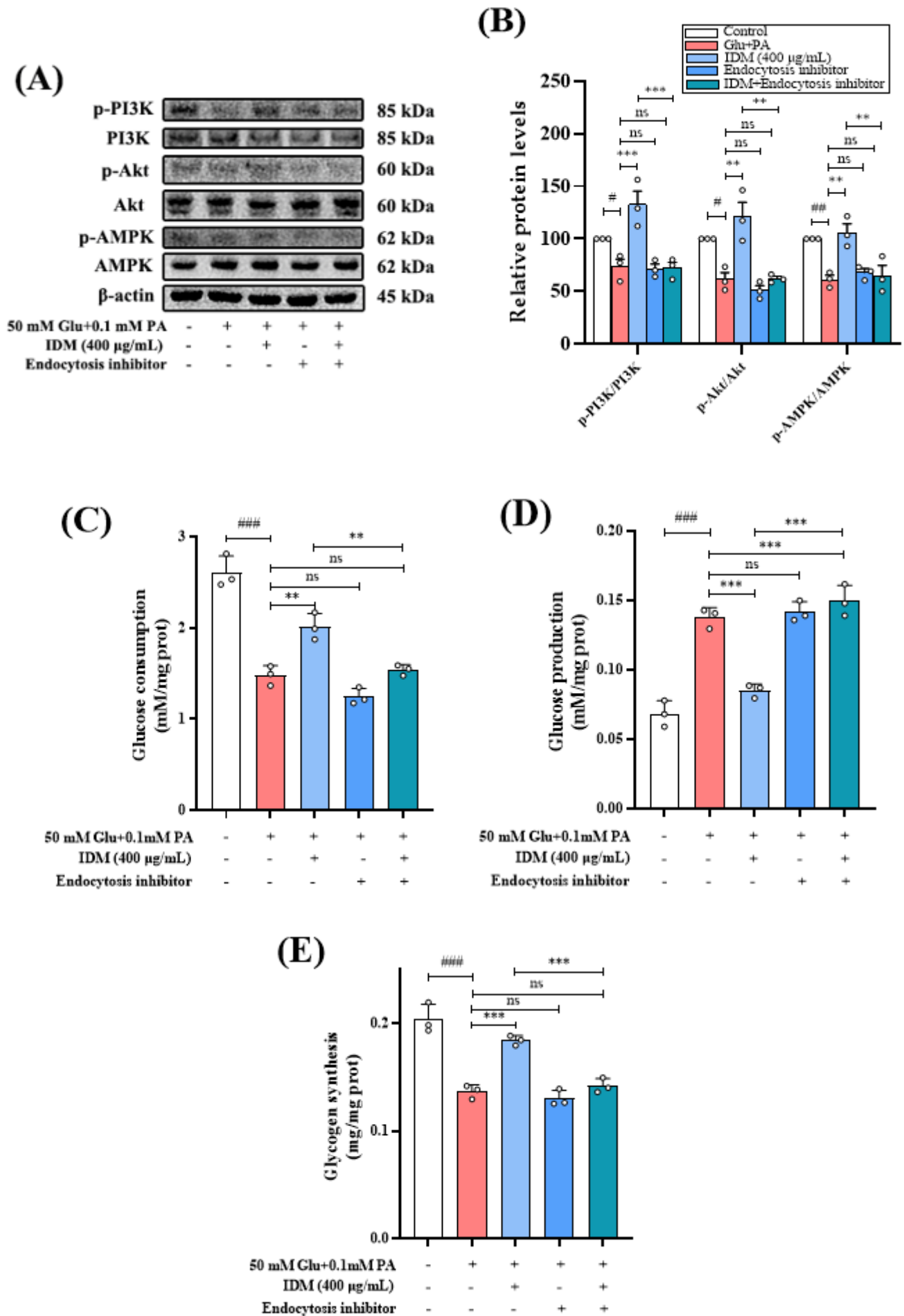
Fig. 6. IDM increases glycogen synthesis in IR-HepG2 cells by inhibiting p-GS expression through PI3K/Akt/GSK3 β pathway. (A) Effect of of IDM (100–400 μ g/mL) on glycogen synthesis in IR-HepG2 cells after 24 h of treatment. (B) glycogen staining (the microscope magnification is 40 $\times$). (C, D) Effect of different concentrations IDM (100–400 μ g/mL) on GSK3 β , p-GSK3 β , GS and p-GS expression levels in HepG2 cells. (E, F) Effect of 10 μ M AZD5363 on the regulation of GSK3 β /GS expression levels by IDM (400 μ g/mL) in HepG2 cells. (G) Effect of AZD5363 on IDM to promote glycogen synthesis in IR-HepG2 cells. All data are expressed as mean $\pm$ SD ($n = 3$) for each group. P value significance represented by #, <0.05; ##, <0.01; ###, <0.001; *, <0.05; **, <0.01; ***, <0.001, ns, not significant.

GSK3 β and GS, key enzymes in hepatic glycogen synthesis, are regulated by the upstream Akt pathway [45]. In IR hepatocytes, GS primarily exists in its inactive phosphorylated form (p-GS) [13].

Conversely, GSK3 β phosphorylation (p-GSK3 β) promotes GS dephosphorylation and glycogen synthesis [46]. Compared to the control group, the IR group showed significantly reduced p-GSK3 β expression (0.78-fold, $P < 0.05$) and increased p-GS expression (1.45-fold, $P < 0.01$) (Fig. 6C, D). IDM (100–400 $\mu\text{g/mL}$) significantly restored p-GSK3 β expression and suppressed p-GS expression ($P < 0.05$), indicating that IDM promotes glycogen synthesis by modulating these phosphorylation states. To further explore the molecular mechanism of IDM promoting glycogen synthesis in IR-HepG2 cells, the Akt pathway was blocked using the inhibitor AZD5363. AZD5363 alone did not significantly affect p-GSK3 β or p-GS expression in IR-HepG2 cells (Fig. 6E, F, $p > 0.05$). However, co-treatment with IDM and AZD5363 significantly suppressed p-GSK3 β expression (1.06-fold of control, $P < 0.05$) and increased p-GS expression (1.13-fold of control, $P < 0.05$) compared to IDM alone. Additionally, inhibiting Akt signaling significantly reduced IDM's ability to promote glycogen synthesis (Fig. 6G, $P < 0.05$). These results confirm that IDM promotes glycogen synthesis in IR-HepG2 cells by inhibiting p-GS expression through the PI3K/Akt/GSK3 β pathway, further defining the signal transduction mechanisms involved.

3.8 Endocytosis-dependent activation of IRS-1/PI3K/Akt and AMPK by IDM mediates hypoglycemic effects

In general, extracellular active substances usually stimulate cells in two ways, one is to bind to specific receptors on the cell surface and trigger the conduction of intracellular signals, thus allowing the cell to respond to exogenous stimuli [47]. The other way is that extracellular active substances enter the cell interior through the endocytic pathway and directly bind to intracellular effector targets to cause downstream reactions [48]. Given that IDM altered endocytic vesicle-related gene expression in IR-HepG2 cells (Fig. 3H) and has a large molecular weight (46,645 Da), we hypothesized that IDM exerts its hypoglycemic effects through endocytosis. To test this, we used nystatin, EIPA, and chlorpromazine to inhibit endocytosis. These inhibitors did not significantly alter p-PI3K, p-Akt, or p-AMPK levels in the IR group (Fig. 7A, B, $p > 0.05$). However, co-treatment with IDM and these inhibitors significantly reduced IDM's ability to activate these pathways ($P < 0.01$), effectively abolishing the activation ($p > 0.05$ vs. IR group). Furthermore, inhibiting endocytosis prevented IDM from ameliorating IR-related effects, including promoting glucose consumption and glycogen synthesis, and inhibiting gluconeogenesis (Fig. 7C–E, $p > 0.05$ vs. IR group). These results indicate that IDM's hypoglycemic activity is entirely dependent on endocytosis, not cell surface receptor binding. While melanoidins have been shown to exhibit various bioactivities, the mechanism of their cellular interaction remains unclear [11, 49, 50]. This study demonstrates for the first time that melanoidins can enter cells via endocytosis to exert their effects. Given the heterogeneity of melanoidins due to factors like food source and processing conditions, their cellular interaction may vary [6].



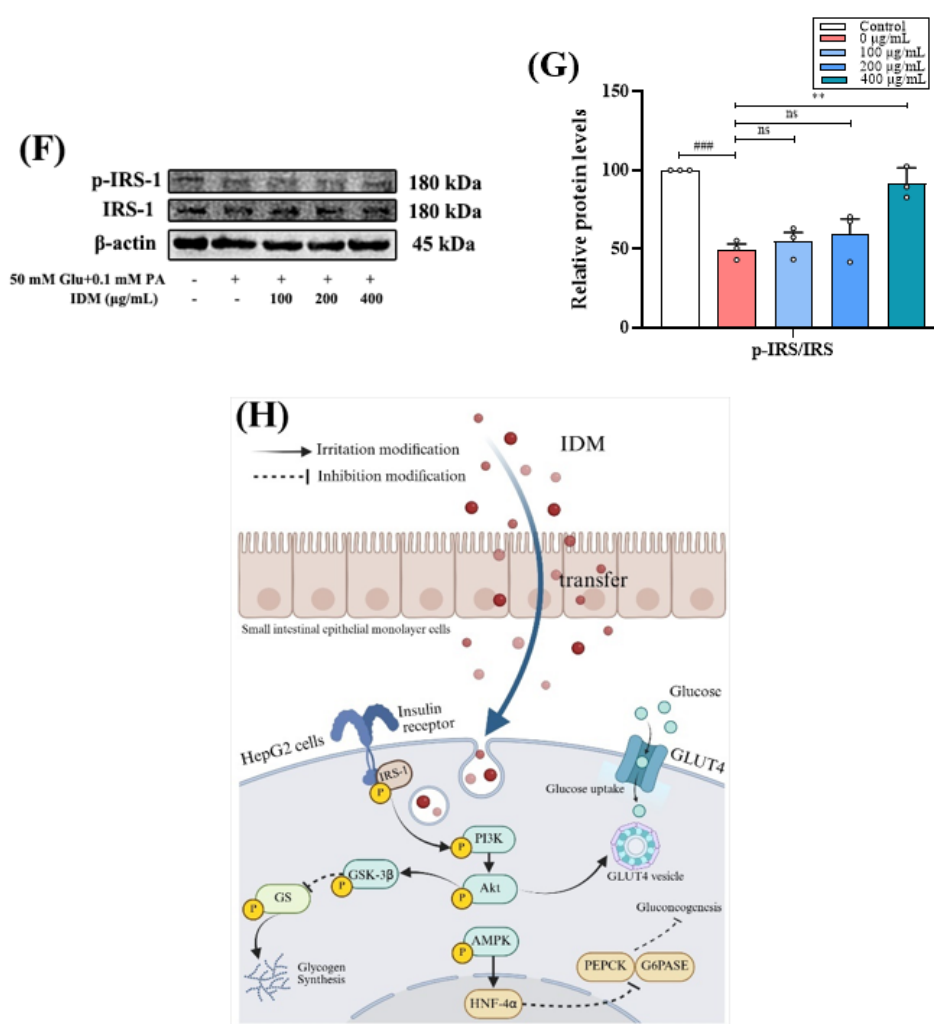


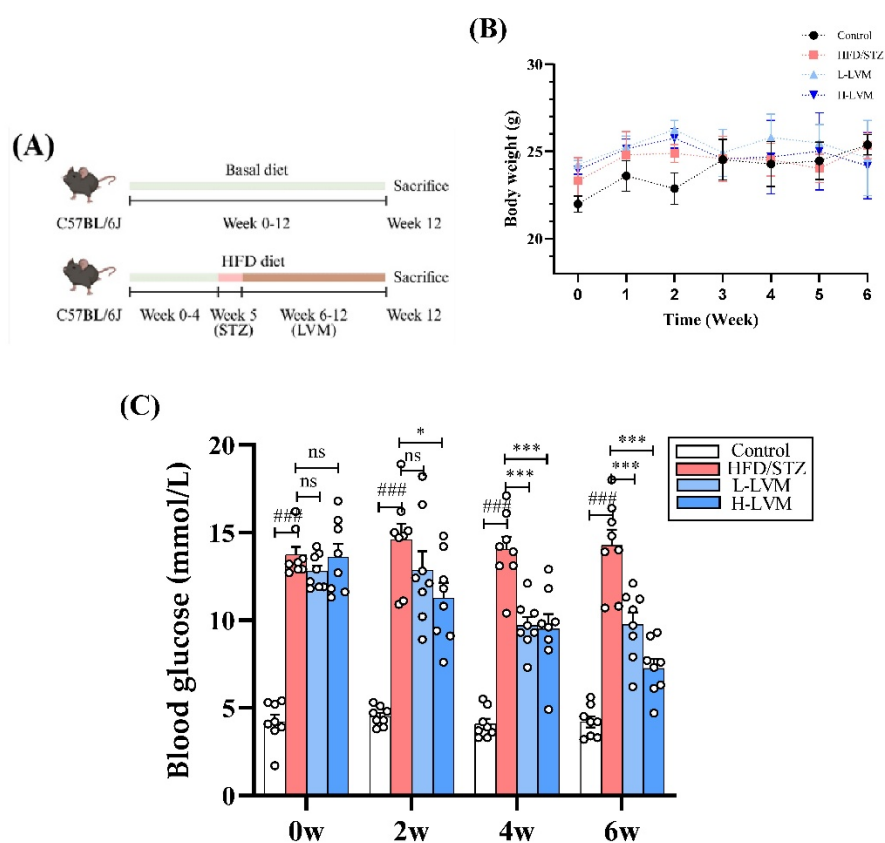
Fig. 7. IDM promotes IRS-1 tyrosine phosphorylation through the endocytic pathway to cause downstream responses. (A, B) Effect of endocytosis inhibitor (30 μM nystatin, 30 μM chlorpromazine, 100 μM EIPA) on IDM to activate the PI3K/Akt pathway and AMPK pathway in HepG2 cells. Effect of endocytosis inhibitor on IDM to stimulate glucose consumption (C), gluconeogenesis (D), and glycogen synthesis (E). (F, G) Effect of IDM (100–400 μg/mL) on IRS-1 tyrosine phosphorylation levels in IR-HepG2 cells. (H) Potential molecular mechanism of IDM alleviating insulin resistance in IR-HepG2 cells. All data are expressed as mean ± SD (n = 3) for each group. P value significance represented by #, <0.05; ##, <0.01; ###, <0.001; **, <0.01; ***, <0.001, ns, not significant.

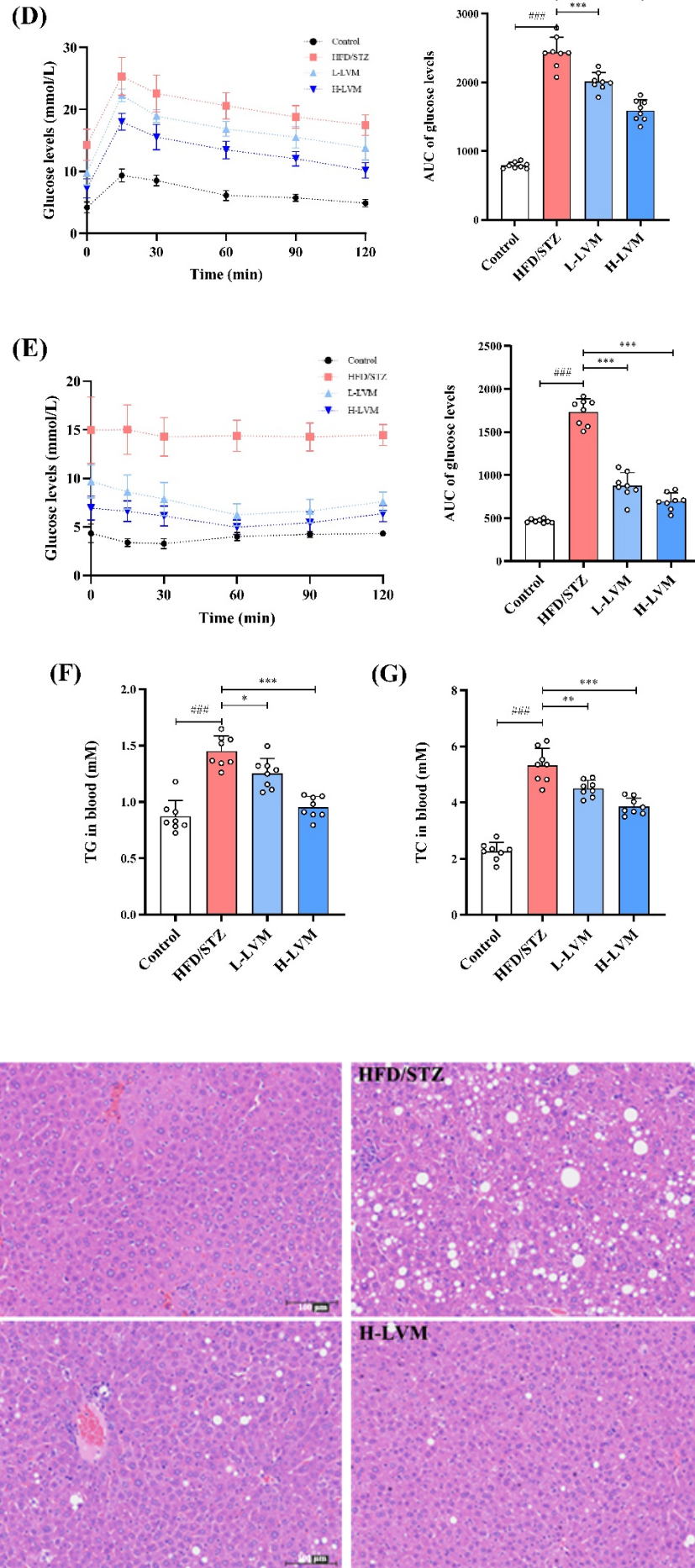
As a direct substrate of insulin receptor and a key upstream target of PI3K/Akt pathway, IRS-1 plays an important role in insulin metabolism [14, 15]. We investigated whether IDM improves insulin metabolism by promoting IRS-1 tyrosine phosphorylation (p-IRS-1). p-IRS-1 levels were significantly suppressed in the IR group (0.50-fold of control group) (Fig. 7F, G, $P < 0.001$). IDM (400 μg/mL) significantly restored p-IRS-1 levels (0.92-fold of control group, $P < 0.01$), suggesting that IDM promotes p-IRS-1 expression by binding to its SH-2 domain [51]. This indicates that IDM's anti-IR effects may be mediated by enhancing insulin metabolism. Consistent with our findings, chrysin and black quinoa polyphenols have been shown to exert hypoglycemic effects by restoring p-IRS-1 levels [1, 5]. Additionally, Xia et al. showed that Zhenjiang aromatic vinegar extract enhances insulin sensitivity by inhibiting IRS-1 serine phosphorylation [13].

3.9 Oral administration of LVM improved insulin resistance and liver metabolic disorders in T2DM mice

In previous studies, we identified the hypoglycemic effects and underlying molecular mechanisms of *Monascus* vinegar melanoidins in IR-HepG2 cells, but *in vivo* validation is still lacking. Therefore, the

hypoglycemic activity of LVM *in vivo* and its effect on hepatic metabolism were verified by daily gavage of LVM in T2DM mice (Fig. 8A). The results showed that LVM treatment had no significant effect on the body weight of mice within 6 weeks (Fig. 8B, $p > 0.05$ vs. HFD/STZ group). Compared with the control group, the fasting blood glucose of the HFD/STZ group was significantly increased to 13.74 mmol/L, indicating that the T2DM mouse model was successfully established (Fig. 8C, $P < 0.001$) [52]. From the two weeks of oral administration of LVM, high dose LVM can significantly reduce fasting blood glucose in T2DM mice ($P < 0.05$ vs. HFD/STZ group). Low dose LVM also demonstrated hypoglycemic activity after four weeks of oral administration ($P < 0.001$ vs. HFD/STZ group). These results suggest that oral administration of LVM can alleviate hyperglycemia induced by T2DM [4]. In addition, oral administration of LVM also significantly reduced the AUC of OGTT and ITT in T2DM mice ($P < 0.001$ vs. HFD/STZ group), indicating that LVM may improve glucose metabolism by increasing insulin sensitivity in T2DM mice (Fig. 8D, E). IR is often accompanied by liver metabolic disorders during the development of T2DM [3]. The intervention with LVM significantly reduced the contents of TG and TC in blood ($P < 0.05$ vs. HFD/STZ group), indicating that oral administration of LVM improved dyslipidemia (Fig. 8F, G). In addition, the results of H&E staining showed that T2DM mice developed significant liver steatosis and vacuolar degeneration (significantly increased NAS, $P < 0.001$ vs. control group), while oral administration of LVM significantly alleviated liver lipid deposition and injury (significantly decreased NAS, $P < 0.01$ vs. HFD/STZ group), indicating that the liver metabolic disorder of mice was effectively improved (Fig. 8H, I). In summary, these findings provide evidence that oral administration of LVM improves IR and liver metabolic disorders in T2DM mice.





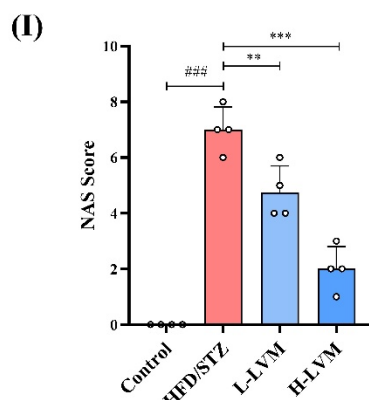


Fig. 8. Oral administration of LVM attenuates hyperglycemia and liver metabolic disorders in HFD/STZ mice. (A) A schematic diagram showing the procedure of HFD/STZ mice treated with LVM. (B) Body weight curve. (C) Fasting blood glucose levels at 2, 4 and 6 weeks. OGTT (D) and ITT (E) with AUC. (F, G) TG and TC levels in blood. (H) Representative photomicrographs of liver H&E staining (scale bar, 100 μ m). (I) Histological scores. The values are presented as mean $\pm$ SD, n=4-8. ###, $P < 0.001$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no significance.

4. Conclusion

In conclusion, the present study explored the transport mode of IDM in Caco-2 cells monolayer and revealed its hypoglycemic effect and underlying molecular mechanism in IR-HepG2 cells. Our study confirmed that IDM can transport intestinal epithelial cells through transcellular pathway, and multiple endocytic pathways are involved in this process. To the best of our knowledge, this is the first study to reveal the mechanism of melanoidin transport in Caco-2 cells monolayer. In addition, IDM significantly improved IR by increasing insulin metabolism, increasing glucose uptake and consumption, and promoting glycogen synthesis in IR-HepG2 cells. Mechanistically, after IDM enters cells through endocytosis, it first activates the PI3K/Akt pathway by promoting IRS-1 tyrosine phosphorylation, causing downstream reactions. On the one hand, IDM promotes GLUT4 translocation and increases glucose uptake through the PI3K/Akt pathway. On the other hand, IDM promoted glycogen synthesis by inhibiting the expression of p-GS through the PI3K/Akt/GSK3 β pathway. In addition, the inhibitory effect of IDM on gluconeogenesis may be mediated by multiple pathways, of which AMPK/HNF-4 α pathway is one of the confirmed pathways in this study. *In vivo*, we preliminarily verified the effect of oral administration of LVM on hyperglycemia and liver metabolic disorders in T2DM mice. However, the absorption metabolism of LVM *in vivo* and the potential role played by the gut microbiota are not well understood, and this is clearly the focus of our future studies. The findings of this study will provide important insights into the hypoglycemic activity of Monascus vinegar melanoidins, which will contribute to the development of novel functional foods and dietary supplements.

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.

Data availability

Data will be made available on request.

Acknowledgments

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