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Research Article

FER1L6 ameliorates insulin resistance by regulating GLUT4 expression

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Abstract: Insulin resistance, a prominent characteristic of type 2 diabetes, has been extensively studied. In our investigation, we examined the effect of Eudesmin on insulin resistance in 3T3-L1 adipocytes and HepG2 cells. Additionally, we analyzed the alterations in mRNA expression in insulin-resistant HepG2 cells through transcriptomic techniques. Subsequently, we assessed the changes in mRNA expression in insulin-resistant HepG2 cells following Eudesmin treatment. Our analysis revealed 13 differentially expressed genes that were commonly observed. Notably, FER1L6 exhibited the most significant changes, with a marked upregulation in mRNA expression in insulin-resistant HepG2 cells. Consequently, we conducted functional experiments to validate the role of fer-1 like family member 6 (FER1L6). Specifically, through gene knockdown experiments, we observed a significant enhancement in glucose transporter type 4 (GLUT4) expression and an improvement in insulin resistance status in cell models. Therefore, FER1L6 emerges as a promising therapeutic target for insulin resistance.

Keywords: insulin resistance; 3T3-L1 adipocytes; HepG2 cells; Eudesmin

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1 Introduction

Diabetes mellitus has emerged as one of the most prevalent metabolic disorder in contemporary society, often accompanied by a multitude of complications including diabetic nephropathy, diabetic foot, and cardiovascular disease. These complications pose a significant threat to individuals' health and overall quality of life[1-3]. Type 2 diabetes mellitus (T2DM) represents over 90% of all cases and is characterized by a combination of insulin resistance (IR) and progressive impairment of pancreatic β-cell function. These physiological changes give rise to both microvascular and macrovascular complications, leading to substantial psychological and physical distress for both patients and caregivers, as well as imposing a substantial burden on healthcare systems[4]. The discovery of insulin in 1,921 greatly facilitated research efforts in the field of diabetes mellitus and related metabolic disorders. One of the main effects of insulin is to increase glucose uptake by fat, liver and muscle cells[5]. This process is enabled by promoting locational changes in the glucose transporter glucose transporter type 4 (GLUT4) from the cytoplasm to the surface of the cell membrane. However, the response is defective in the disease states of IR and type 2 diabetes[6]. Thus, the characteristics of GLUT4 transport may identify potential therapeutic targets for its pathophysiology. The central role of GLUT4 in systemic metabolism is strongly supported by various genetically engineered mouse models, with mice with muscle-specific GLUT4 deficiencies showing reduced insulin responsiveness in adipose tissue and liver[7], while patients with fat-specific GLUT4 depletion show muscle and liver IR[8]. Notably, overexpression of GLUT4 in adipose tissue of muscle-specific GLUT4 deficient mice overcame glucose intolerance and diabetes[9].

However, to date, a definitive cure for diabetes remains elusive[10]. IR stands as the principal hallmark of T2DM, manifesting as a reduction in insulin sensitivity within target tissues and resulting in a dysregulation of the body's metabolic processes[11]. Adipose tissue and the liver are the primary target organs for insulin action[12, 13]. When energy intake surpasses energy expenditure, adipose tissue expands and stores excess nutrients as inert triacylglycerols (TAGs) within lipid droplets, thereby maintaining metabolic homeostasis[14]. However, prolonged overnutrition overwhelms the capacity of adipose tissue to store fat, resulting in adipocyte hypertrophy and the release of TAGs and other lipid metabolites into non-adipose tissues like muscle, liver, and pancreas. This phenomenon, known as "ectopic storage of fat", may ultimately contribute to the development of

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diabetes mellitus[15]. In regulating carbohydrate and fat metabolism, the liver plays a crucial role in meeting the body's energy needs[16]. Elevated blood glucose levels prompt the liver to store glucose as glycogen, which can be broken down into glucose again as necessary to meet energy requirements[17].

Conventional classes of drugs commonly utilized in the management of type 2 diabetes encompass sulfonylureas, biguanides, peroxisome proliferator-activated receptor γ (PPARγ) agonists, and α-glucosidase inhibitors[18]. These medications often elicit a range of adverse effects, including severe hypoglycemia, weight gain, and gastrointestinal discomfort. Consequently, the exploration of novel targets for diabetes treatment and the development of alternative drugs with reduced side effects and enhanced efficacy have emerged as prominent areas for both domestic and international research[19]. To identify potential lead compounds for improving insulin resistance, we conducted a screening of compounds derived from *Patrinia scabiosaefolia* Fisch. And assayed their activities to improve cellular insulin resistance, found that Eudesmin (Eud) was able to significantly improve IR in 3T3-L1 adipocytes and HepG2 cells. Furthermore, in our quest for candidate target genes in the treatment of type 2 diabetes, transcriptomic analysis led us to identify fer-1 like family member 6 (FER1L6), a gene that exhibited elevated expression levels in IR-HepG2 cells. By conducting knockdown experiments in IR-HepG2 cells, we substantiated that FER1L6 represents a previously unidentified novel target for regulating GLUT4 expression.

2 Materials and methods

2.1 Culture and treatment of 3T3-L1 adipocytes

The 3T3-L1 adipocytes was obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). The 3T3-L1 adipocytes were cultured in DMEM medium supplemented with 10% neonatal bovine serum and 1% penicillin-streptomycin mixture. The cells were incubated at 37 °C and 5% CO_2. Once the cell fusion reached approximately 80%, the cells were passaged. The *in vitro* adipogenic differentiation of 3T3-L1 cells was performed as previously described[20]. To induce adipocyte differentiation, cells were treated with a medium containing 0.5 mmol/L isobutylmethylxanthine (IBMX), 1 μmol/L dexamethasone (Dex), and 10 μg/mL insulin for 3 days. Afterward, the supernatant was replaced with a medium containing 10 μg/mL insulin and cultured for an additional 2 days. Finally, the cells were cultured in normal medium until they differentiated into mature adipocytes. The IR model was established by treating mature adipocytes with 3 μmol/L Dex.

2.2 Culture and treatment of HepG2 cells

The HepG2 cells was obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). HepG2 cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin mixture. The cells were incubated at 37 °C in a CO_2 incubator with 5% CO_2. Once the cell fusion reached approximately 80%, the cells were passaged. According to previous methods, IR development was induced by cell stimulation with glucosamine (GlcN) dissolved in phosphate buffer at a concentration of 8 μmol/L[21].

2.3 Cell viability assay

3T3-L1 adipocytes were inoculated into 96-well plates with a density of 1×10^4 cells/well, HepG2 cells were inoculated into 96-well plates with a density of 2×10^4 cells/well. After 24 h, the blank group was replaced with a new complete medium, experimental group was exposed to various concentrations of Eud (3.125, 6.25, 12.5, 25, 50, 100, and 200 μmol/L) for a duration of 48 h. Following this, 10 μL of 5 mg/mL MTT solution was added to each well and the cells were incubated for an additional 4 h. After discarding the medium, 200 μL of DMSO was added. The absorbance was subsequently measured at 490 nm using an enzyme marker.

2.4 Determination of cellular glucose consumption

Following the cellular conversion into insulin resistance, the supernatant of the cells in both the control and model groups was substituted with complete medium. In the Eud-treated group, the supernatant was replaced with complete medium containing varying concentrations of Eud. As for the positive group, the supernatant was substituted with complete medium containing 10 μmol/L of sodium orthovanadate (Van) or rosiglitazone (ROSI), and the amount of glucose in the supernatant of the cells was detected by using the glucose oxidase-peroxidase (GOD-POD) assay after incubation for 48 h[22]. Subsequently, the total amount of glucose consumed by the cells during this period was calculated.

2.5 Oil Red O staining of cells

The differentiated adipocytes slowly washed with PBS and fixed with paraformaldehyde for 40 min. Subsequently, the cells were slowly rinsed with PBS buffer and stained with a solution of Oil Red O for 30 min. After discarding the Oil Red O staining solution, the cells were washed with PBS buffer and ultrapure water, respectively. The entire experiment was conducted in a light-protected environment and finally photographed using an inverted microscope.

2.6 Transcriptomic analysis in HepG2 cells

Transcriptomic analysis was conducted to examine the changes in mRNA expression in insulin resistance-HepG2 cells following Eud treatment. HepG2 cells from the control, model, and Eud groups (12 μmol/L) were collected using TRIzol, and three biological replicates were performed for each sample. The transcriptome sequencing was carried out by The Beijing Genomics Institute (Shenzhen, China).

2.7 Transfection of small interfering RNA

Primer sequences for siRNA were designed as follows: FER1L6 (5′-CCGGAAGAUUGGAGAUAAATT-3′) (purchased from GenePharma, Shanghai). The transfection of siRNA was carried out using the Lipo2000 reagent (Thermo Fisher Scientific) in accordance with the instructions provided by the manufacturer. Following transfection, the cells were incubated for 48 h to allow for subsequent processing.

2.8 Real time-quantitative PCR (RT-qPCR)

RT-qPCR was performed as described previously[23]. Total RNA was isolated from cells using TRIzol, cDNA was synthesized using SweScript RT I First Strand cDNA Synthesis Kit. cDNA was used for RT-qPCR using SYBR Green qPCR Master Mix reagent. The primer sequences of 3T3-L1 adipocytes are listed in Table 1, the primer sequences of HepG2 cells are listed in Table 2.

Table 1 Primers sequence of 3T3-L1 adipocytes

Name	Primer	Sequence (5'→3')
IRS	Forward	TCGGCTCACCGTTTCCTTG
	Reverse	TCGCTCTCGTAGCACTCCA
AKT	Forward	CACCGTGTGACCATGAACGAGTT
	Reverse	TGGCGACGATGACCTCCTTCTT
PI-3K	Forward	TCCTGGAATGTGCCCTCTTTCGT
	Reverse	TGACTGGCTGACTGCTGTGACT
Leptin	Forward	ATGTGCCCTTCCGATATACAACC
	Reverse	CGTGTCATCCACTAATCTTCTGG
Adiponectin	Forward	GGAGTGTTCGTGGGCTTAGG
	Reverse	GCAGCTCCGGTGATATAGAGG
β-actin	Forward	AGCCTTGTAGGTACCCAACC
	Reverse	TCCACTCACCTGAGGTGCTGAA

Table 2 Premer sequence of HepG2 cells

Name	Primer	Sequence (5'→3')
KNG1	Forward	TGCTCCAGGCTGCTACTAAGT
	Reverse	GGCTTCAGTTATGCGGTACAA
HPX	Forward	CGTGACTGAACGCTGCTCA
	Reverse	CTCCCGGTCCCATTTGTGAC
CYP3A7-CYP3A51P	Forward	GACCCAGAAACTGCATTGGC
	Reverse	GCGGGATCTGATGGTAGGAC
ADH1A	Forward	AGTCATCCCACTCGCTATTCC
	Reverse	GTCCCCTGAGGATTGCTTACA
UGT2B	Forward	CCAACCAATGAAGCCCCTG
	Reverse	GTTGTGAGCTGCGACTCGAA
LYG2	Forward	GTTTTGGGGACTAATTGCCCT
	Reverse	CAGGCGTGGATGTAGGTGAG
GSK3β	Forward	TGGCAGCAAGGTAACCACAG
	Reverse	CGGTTCTTAAATCGCTTGTCCTG
PEPCK	Forward	CTGCATAACGGTCTGGACTTC
	Reverse	CAGCAACTGCCCGTACTCC
G6Pase	Forward	CGACTCGCTATCTCCAAGTGA
	Reverse	GTTGAACCAGTCTCCGACCA
Fer1l6	Forward	CCAGAGGAGTCACCAAGAAGA
	Reverse	GGTGAGGACCAATCCCTTCTC
Fer1l5	Forward	CAGTCGGCCAAGATTGACCC
	Reverse	CTTCCACCACACGAGTTCTTT
EHD2	Forward	TCCGCAAACTCAACCCTTTC
	Reverse	TCTCCAGGACCTGATTAGGGA
GLUT4	Forward	GTGACTGGAACACTGGTCCTA
	Reverse	CCAGCCACGTTGCATTGTAG
β-actin	Forward	CATGTACGTTGCTATCCAGGC
	Reverse	CTCCTTAATGTCACGCACGAT

2.9 Western blot

Western blot was performed as described previously[24]. Cell samples were lysed using RIPA lysate, and the protein concentration was determined using the BCA method. Subsequently, the proteins were separated by electrophoresis on a 10% SDS-PAGE gel and transferred onto polyvinylidene difluoride membranes. The membranes were then incubated in TBST buffer containing 20% skimmed milk for a duration of 2 h. Following this, the membranes were exposed to primary antibodies overnight at a temperature of 4 °C, and subsequently to the appropriate secondary antibodies for 2 h at room temperature. Western blot was carried out using standard techniques with antibodies outlined in Table 3.

2.10 Statistical analysis

All experimental data are presented as mean ± standard deviation (SD) and were analyzed using SPSS 25.0 (SPSS Inc.).

Table 3 Antibodies information

Antibodies	Source	Identifier
Anti-PI-3K (p110)	Proteintech	Cat No. 67071-1-Ig
Anti-AKT	Proteintech	Cat No. 60203-2-Ig
Anti-phospho-AKT	Proteintech	Cat No. 66444-1-Ig
Anti-GLUT4	Proteintech	Cat No. 66846-1-Ig
Anti-GSK3B Monoclonal	Proteintech	Cat No. 22104-1-AP
Anti-Phospho-GSK3B (Ser9)	Proteintech	Cat No. 67558-1-Ig
Anti-PEPCK	Proteintech	Cat No. 67676-1-Ig
Anti-G6Pase	Proteintech	Cat No. 66860-1-Ig
Anti-*β*-actin	Servicebio	GB 15003-100
Rabbit IgG	Servicebio	GB 111738-100
Mouse IgG	Servicebio	GB 111739-100

Unpaired Student's *t*-test was employed to compare between two groups, while multiple comparisons were assessed using one-way ANOVA with Tukey's tests. Statistical significance was defined as $P < 0.05$, $P < 0.01$, and $P < 0.001$.

3 Results

3.1 Eud ameliorates IR in 3T3-L1 adipocytes

The PI3K/AKT pathway is crucial in regulating the effects of insulin on cellular function and energy metabolism[25]. To assess the biological activity of Eud in improving insulin resistance, we initially examined the impact of Eud on insulin resistance-3T3-L1 adipocytes (Fig. 1A). The results from the MTT assay demonstrated that Eud significantly inhibited the viability of 3T3-L1 adipocytes at a concentration of 200 μmol/L, with no observed cytotoxicity within the range of 12.5–100 μmol/L (Fig. 1B). We established an IR model by treating 3T3-L1 adipocytes with varying concentrations of Dex (1–3 μmol/L), and after 72 h of Dex treatment, insulin (3 μmol/L) was added to assess the success of the IR model in 3T3-L1 adipocytes. Compared to the control group, the insulin-added group significantly enhanced glucose uptake by 3T3-L1 adipocytes. However, the addition of insulin to the Dex group did not lead to a significant increase in glucose uptake by the cells compared to the insulin-added group, indicating the development of IR after Dex treatment (Fig. 1C). Consequently, for subsequent experiments, we selected a 3 μmol/L Dex treatment for 72 h to induce IR in 3T3-L1 adipocytes. Eud treatment notably enhanced the glucose uptake capacity of IR-3T3-L1 adipocytes (Fig. 1D). Additionally, Eud treatment also significantly enhanced the triglyceride metabolizing capacity of the cells (Fig. 1E). To elucidate the mechanism of action through which Eud improves IR in 3T3-L1 adipocytes, we observed that Eud significantly promoted the serine phosphorylation of Akt, the expression of the PI3K catalytic subunit p110, and the expression of GLUT4, but had no effect on total Akt levels (Fig. 1F). Furthermore, the PCR experiments revealed that Eud promoted the transcription of IRS, PI3K, Akt, leptin, and adiponectin (Fig. 1G).

3.2 Eud ameliorates IR in HepG2 cells

The results of MTT assay showed that Eud significantly inhibited HepG2 cells viability at concentrations ranging from 25 μmol/L to 200 μmol/L, with no cytotoxicity at concentration ranging from 3.125 μmol/L to 12.5 μmol/L (Fig. 2A); the IR model of HepG2 cells was established by varying concentrations of GlcN (1–20 mmol/L) for 24 h, insulin (3 μmol/L) was added to test whether the HepG2 cells IR model was successful or not.

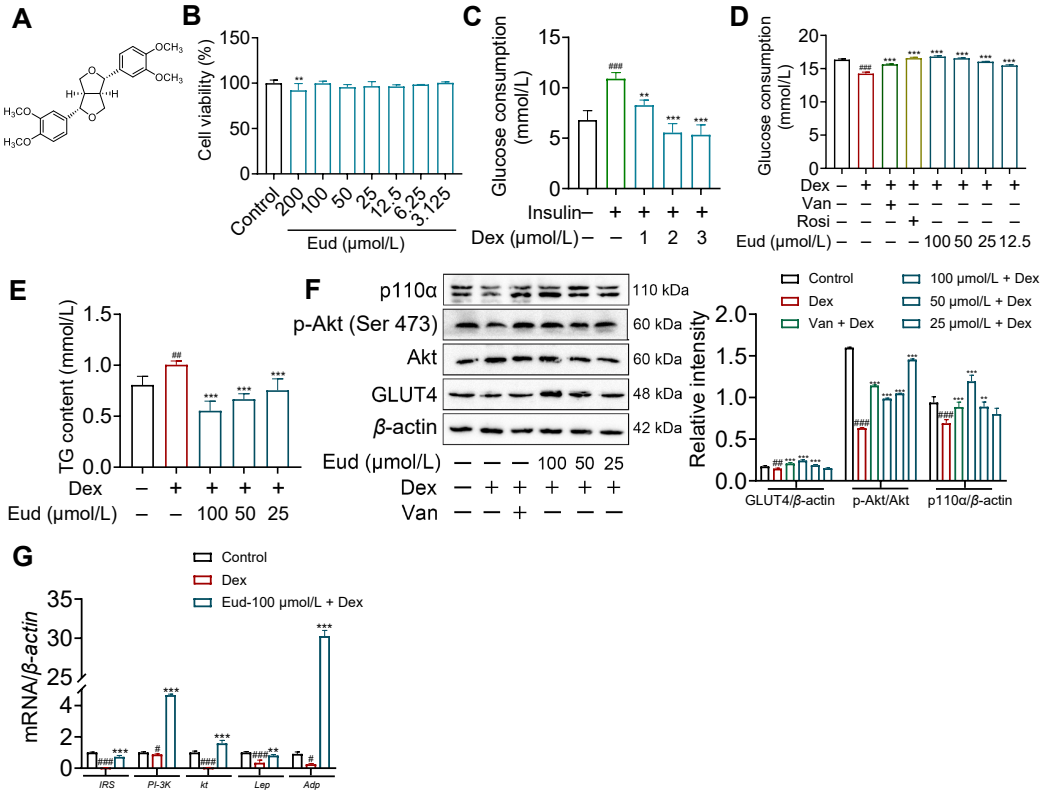

Figure 1 Eud ameliorates IR in 3T3-L1 adipocytes through the PI-3K/Akt signaling pathway. (A) Chemical structure of Eud. (B) Effect of Eud on 3T3-L1 adipocyte viability (n = 3). (C) Established a model of IR in 3T3-L1 adipocytes and validated model stability (n = 3). (D) Effect of Eud on glucose content in the supernatant of IR-3T3-L1 adipocytes (n = 3). (E) Effect of Eud on TG content in IR-3T3-L1 adipocytes (n = 3). (F) The effect of Eud on the expression of proteins related to the PI3K/Akt/GLUT4 signaling pathway (n = 3). (G) The effect of Eud on mRNA expression of *IRS*, *PI3K*, *Akt*, *leptin* (*Lep*) and *adiponectin* (*Adp*) (n = 3). Data expressed as mean ± SD. $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ *vs.* control group; $^{***}P < 0.001$, $^{**}P < 0.01$ *vs.* model group.

Compared with the control group, the insulin-added group was able to significantly promote glucose uptake by the cells, whereas the GlcN group (8–16 mmol/L) was still not able to significantly promote glucose uptake by the cells after the addition of insulin compared with the insulin-added group, which suggested that after GlcN treatment (8–16 mmol/L), the cells developed IR (Fig. 2B); the Eud treatment significantly promoted glucose uptake in IR-HepG2 cells (Fig. 2C). Also, the metabolizing ability of cells for TG was significantly promoted after Eud treatment of cells (Fig. 2D).

To investigate the regulatory targets of Eud on glucose metabolism in IR-HepG2 cells, we conducted RNA-Seq experiments on HepG2 cells, including a control group, a model group, and an Eud treatment group. In this study, we identified differentially expressed genes (DEGs) using a cutoff criterion of $Q < 0.001$. The DEGs volcano plot revealed 68 up-regulated DEGs and 199 down-regulated DEGs in the Control-*vs*-GlcN group. Similarly, the Eud-*vs*-GlcN group identified a total of 9 up-regulated DEGs and 16 down-regulated DEGs (Figs. 2E, 2F). We observed 13 shared DEGs between the two groups, and the cluster analysis plot displayed significant differences among these shared genes (Fig. 2G). To validate the reliability of the mRNA data obtained through RNA-seq technology, we performed RT-qPCR on six randomly selected DEGs including FER1L6. The results demonstrated a high level of agreement between the differential gene expression trends and the sequencing data (Fig. 2H).

3.3 Mechanisms of Eud improves IR in HepG2 cells

Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses were conducted on the DEGs obtained from mRNA sequencing. The KEGG analysis results revealed that the DEGs were predominantly enriched in the signaling pathways related to gluconeogenesis and glycolysis (Fig. 3A). On the other hand, the GO enrichment analysis demonstrated that, in terms of biological processes, the DEGs were primarily involved in glycogen synthesis, PI3K activation, and ATP-stimulated adenosine receptor activation (Fig. 3B). These findings suggest that Eud has the potential to improve IR in HepG2 cells by stimulating these biological processes. Further analysis through Western blot showed that the group treated with Eud exhibited a significant upregulation in the expression of PI3K, pAkt, and GLUT4 protein. Additionally, it promoted GSK3β phosphorylation and significantly reduced the expression of PEPCK and G6pase protein (Fig. 3C). Furthermore, the Eud treatment group exhibited a significant elevation in the mRNA expression of *PEPCK*, *G6pase*, and *GSK3β* (Fig. 3D).

3.4 Knockdown of *FER1L6* improves cellular IR mechanisms

To investigate the role of *FER1L6*, siRNA transfection experiments were carried out to knock down *FER1L6* expression in IR-HepG2 cells. The mRNA expression of *FER1L6* was significantly reduced in cells treated with siRNA at concentrations of 40, 60, and 80 nmol/L (Fig. 4A). Among these concentrations, 40 nmol/L siRNA had the most pronounced inhibitory effect on *FER1L6* mRNA expression. Additionally, when cells were treated with the 40 nmol/L siRNA concentration, the glucose content in the cell supernatant was significantly decreased compared to the control group (Fig. 4B). These findings suggest that silencing the *FER1L6* gene can improve sugar metabolism in IR-HepG2 cells.

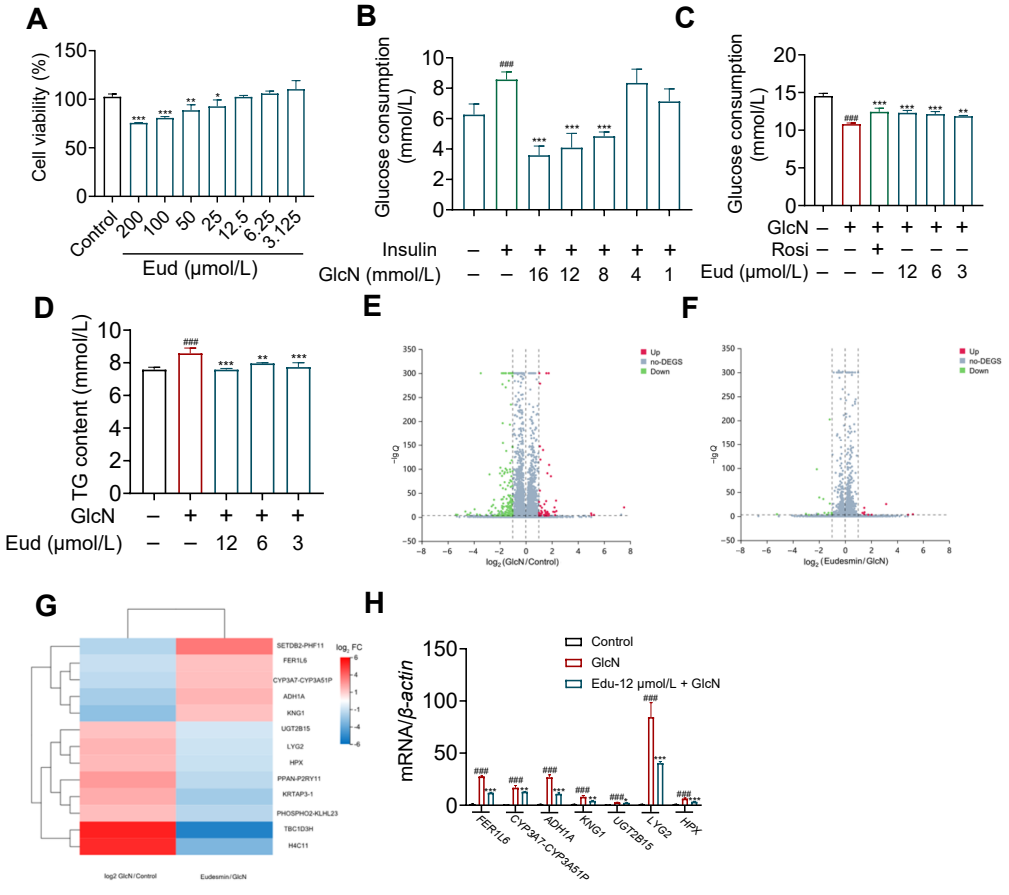

Figure 2 Transcriptomic analysis of the mechanism of Eud in improving IR in HepG2 cells. (A) Effect of Eud on HepG2 cells viability (n = 3). (B) Established a model of IR in HepG2 cells and validated model stability (n = 3). (C) Effect of Eud on glucose content in the supernatant of IR-HepG2 cells (n = 3). (D) Effect of Eud on TG content in IR-HepG2 cells (n = 3). The DEGs volcano plot of transcriptomics in Control-*vs*-GlcN (E) and Eud-*vs*-GlcN (F). (G) Clustering heatmap integrating the DEGs shared by the two groups of transcriptomics. (H) RT-qPCR validated part of the DEGs (n = 3). Data expressed as mean ± SD. $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ *vs*. control group; $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ *vs*. model group.

Previous studies have shown that Fer1L5 protein, a homolog of FER1L6, directly interacts with endocytosed cyclins EHD1 and EHD2[26]. EHD2 has been implicated in the insulin-induced translocation of GLUT4 to the cell membrane in rat adipocytes[27]. In our study, knockdown of *FER1L6* resulted in increased mRNA expression of *FER1L5*, *EHD2*, and *GLUT4* (Figs. 4C, 4D, 4E). This suggests that the deposition of *FER1L6* gene improves glucose metabolism in IR-HepG2 cells, possibly by promoting *EHD2* expression and subsequently facilitating the membrane transport of *GLUT4*.

4 Discussion

Adipose tissue is an important site of metabolism and one of the target tissues for the development of IR[28]. Eud is a bis-tetrahydrofuran-type lignan found in a variety of plants, such as Magnoliaceae, Umbelliferae, Chrysomelidae, and Rutaceae, and possesses a wide range of biological activities, such as anti-inflammatory[29], anti-tumour[30], and anti-convulsant activity[31]. In addition, it has been found that Eud is able to interfere with adipogenesis by inhibiting the S6K1 signaling pathway.[32] This study provides a theoretical basis for Eud to treat obesity and metabolic diseases. Insulin exerts its regulatory effects on the body's glucose and lipid metabolism mainly through the PI3K/AKT signaling pathway, and many literatures have reported that the mechanism of improving glucose metabolism of natural products is related to the activation of PI3K/AKT signaling[33-35]. However, there is still a lack of studies on the activity of Eud on improving IR. Here, we firstly chose 3T3-L1 cells as the *in vitro* study object, and we demonstrated that Eud could improve Dex-induced IR in 3T3-L1 cells, promote glucose uptake and metabolism of triglycerides in insulin-resistant cells, and promote and thus ameliorate IR by restoring the PI3K/AKT signaling pathway, and promoting the expression of GLUT4, leptin, and lipocalin.

It is well known that long-term unhealthy eating habits such as high sugar and oil and lack of exercise lead to adipose tissue expansion and increased lipid deposition in the body, which also induces hepatic IR[36,37]. The liver plays a central role in maintaining glucose homeostasis[38], and hepatic IR is an important cause of many metabolic diseases, such as obesity[39], T2DM[40], and non-alcoholic fatty liver disease[41]. HepG2 cells are one of the most commonly used cell lines to study hepatic IR. Here, we examined the effect of Eud on glucose metabolism in IR-HpeG2 cells and analysed the effect of Eud on glucose metabolism by transcriptomic. The effect of Eud on gene expression in IR-HpeG2 cells showed that Eud improved glucose metabolism in IR-HepG2 cells by activating the PI3K/AKT signaling pathway, promoting glycogen synthesis, and decreasing gluconeogenesis processes. Currently, the conventional classes of drugs used for the treatment of type 2 diabetes include sulfonylureas[42], biguanides[43], PPARγ agonists[44], and α-glucosidase inhibitors[45]. These drugs tend to produce a range of side effects, such as severe hypoglycaemia, weight gain,

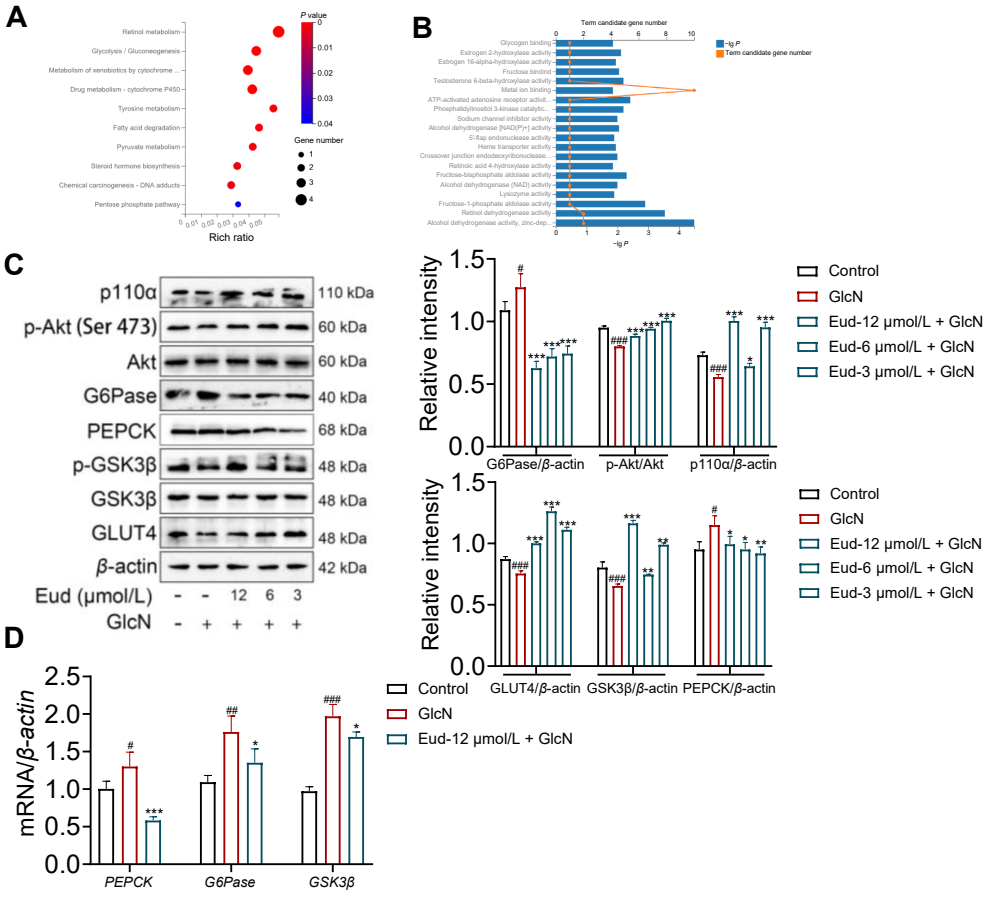

Figure 3 Transcriptomic analysis of the mechanism of Eud in ameliorating IR in HepG2 cells. (A) KEGG pathway analysis of DEGs in Eud-*vs*-GlcN group. Rich ratio denotes the number of DEGs located in the KEGG/the total number of genes located in the KEGG, and the greater the rich ratio value, the greater the KEGG enrichment degree. (B) The GO enrichment bar graph of DEGs in Eud-*vs*-GlcN group. (C) Effect of Eud on the protein expression of PI-3K/Akt/GLUT4, GSK3β, a key protein for glycogen synthesis, and PEPCK, a key protein for gluconeogenesis, and G6pase in IR-HepG2 cells (n = 3). (D) RT-qPCR detection of Eud on *PEPCK*, *G6pase*, *GSK3β* mRNA expression (n = 3). Data expressed as mean ± SD. $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ *vs.* control group; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ *vs.* model group.

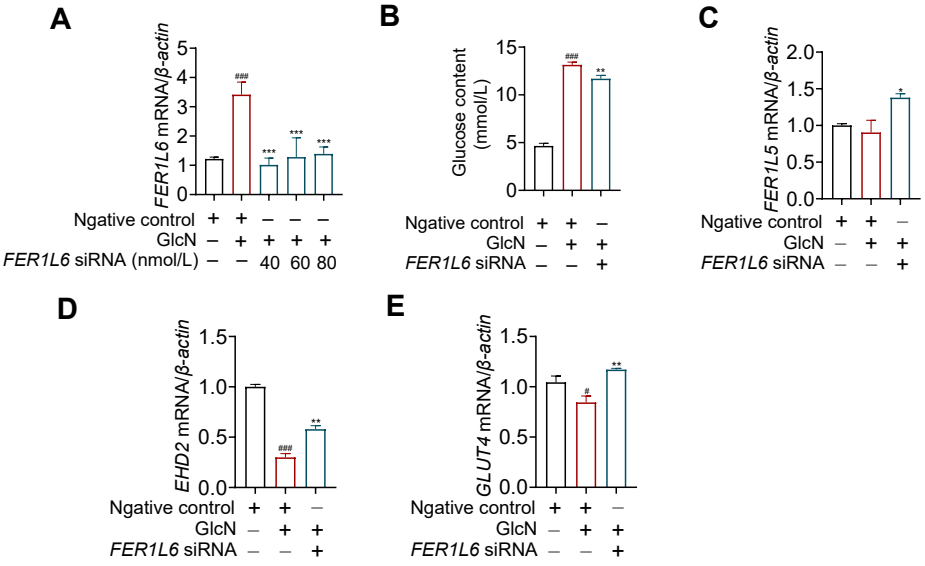

Figure 4 Knockdown of *FER1L6* in IR-HepG2 cell improves glucose metabolism. (A) Knock down *FER1L6* expression was achieved in IR-HepG2 cells (n = 3). (B) The impact of *FER1L6* knockdown on glucose content in the supernatant of IR-HepG2 cells was assessed (n = 3). (C) The influence of *FER1L6* knockdown on the mRNA expression level of *FER1L5* was examined (n = 3). (D) The effect of *FER1L6* knockdown on the mRNA expression level of *EHD2* was examined (n = 3). (E) The effect of *FER1L6* knockdown on the mRNA expression level of *GLUT4* was examined (n = 3). Data expressed as mean ± SD. $^{###}P < 0.001$, $^{#}P < 0.05$ *vs.* control group; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ *vs.* model group.

gastrointestinal discomfort. Therefore, research on new targets for diabetes treatment and the development of new drugs for diabetes treatment with fewer side effects and more significant efficacy have become hotspots for domestic and international research[19,46,47].

In this study, *FER1L6* is the gene with the most significant difference in the transcriptomics results, and the expression of this gene could be significantly upregulated in IR-HepG2 cells and diabetic mice. With the aim of finding new targets for diabetes treatment, we investigated the association of *FER1L6* with the pathogenesis of IR and the improvement of glucose metabolism in IR-HepG2 cells by Eud. Knockdown of *FER1L6* expression in IR-HepG2 cells significantly promoted cellular glucose consumption and *GLUT4* expression. It has been shown that FER1L3, a homologous protein of FER1L6, can mediate endocytosis cycle and participate in the transport of insulin-like growth factor receptor[48]; another homologous protein, FER1L5 protein, directly binds to endocytosis cycle proteins EHD1 and EHD2[26], which may be involved in insulin-induced GLUT4 transport to the cell membrane in rat adipocytes[27]. In this study, the mRNA expression of *FER1L5*, *EHD2*, *GLUT4* was examined in cells after *FER1L6* knockdown, and the results showed that the mRNA expression of *FER1L5* was compensatorily increased after knockdown of *FER1L6* expression, while the expression of the mRNA of *EHD2*, *GLUT4* was significantly elevated. Thus, we hypothesised that knockdown of *FER1L6* could improve IR by promoting *GLUT4* expression through *EHD2*. However, animal models are lacking in this study, and we will continue to investigate the function of *FER1L6* in animal models in subsequent studies.

5 Conclusion

Our study elucidated the mechanism by which Eud alleviates IR and thereby improves glucolipid metabolism. We found that Eud ameliorated IR in 3T3-L1 adipocytes, HepG2 cells by restoring the PI3K/AKT pathway. Meanwhile, we also found that inhibition of FER1L6 expression promoted GLUT4 expression to improve IR. Although the mechanism of action of FER1L6 in regulating IR remains to be further elucidated, our results provide some basis for the therapeutic application of Eud in metabolic diseases and give new targets for the diagnosis and treatment of IR occurrence.

Conflict of interest

The authors declare that there are no conflicts of interest in this work.

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References

[1] Zhu, X., Xu, X., Du, C., et al. An examination of the protective effects and molecular mechanisms of curcumin, a polyphenol curcuminoid in diabetic nephropathy. *Biomedicine & Pharmacotherapy*, **2022**, 153: 113438. https://doi.org/10.1016/j.biopha.2022.113438

[2] Aalaa, M., Amini, M. R., Delavari, S., et al. Diabetic foot workshop: a strategy for improving the knowledge of diabetic foot care providers. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, **2022**, 16: 102543. https://doi.org/10.1016/j.dsx.2022.102543

[3] Kim, H., Lee, K. N., Shin, D. W., et al. Association of comorbid mental disorders with cardiovascular disease risk in patients with type 2 diabetes: a nationwide cohort study. *General Hospital Psychiatry*, **2022**, 79: 33–41. https://doi.org/10.1016/j.genhosppsych.2022.10.005

[4] Chatterjee, S., Khunti, K., Davies, M. J. Type 2 diabetes. *The Lancet*, **2017**, 389: 2239–2251. https://doi.org/10.1016/S0140-6736(17)30058-2

[5] Nortion, L., Shannon, C., Gastaldelli, A., et al. Insulin: the master regulator of glucose metabolism. *Metabolism-Clinical and Experimental*, **2022**, 129: 155142. https://doi.org/10.1016/j.metabol.2022.155142

[6] Van, G. J., Shun-shion, A. S., Fazakerley, D. J. Insulin signalling and GLUT4 trafficking in insulin resistance. *Biochemical Society Transactions*, **2023**, 51: 1057–1069. https://doi.org/10.1042/BST20221066

[7] Zisman, A., Peroni, O. D., Abel, E. D., et al. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nature Medicine*, **2000**, 6: 924–928. https://doi.org/10.1038/78693

[8] Abel, E. D., Peroni, O., Kim, J. K., et al. Adipose-selective targeting of the *GLUT4* gene impairs insulin action in muscle and liver. *Nature*, **2001**, 409: 729–733. https://doi.org/10.1038/35055575

[9] Carvasho, E., Kotani, K., Peroni, O. D., et al. Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle. *American Journal of Physiology-Endocrinology and Metabolism*, **2005**, 289: E551–E561. https://doi.org/10.1152/ajpendo.00116.2005

[10] Falcetta, P., Aragona, M., Bertolotto, A., et al. Insulin discovery: a pivotal point in medical history. *Metabolism-Clinical and Experimental*, **2022**, 127: 154941. https://doi.org/10.1016/j.metabol.2021.154941

[11] Mastrototaro, L., Roden, M. Insulin resistance and insulin sensitizing agents. *Metabolism-Clinical and Experimental*, **2021**, 125: 154892. https://doi.org/10.1016/j.metabol.2021.154892

[12] Edgerton, D. S., Kraft, G., Smith, M., et al. Insulin's direct hepatic effect explains the inhibition of glucose production caused by insulin secretion. *JCI Insight*, **2017**, 2: e91863. https://doi.org/10.1172/jci.insight.91863

[13] Kim, J. Y., Bacha, F., Tfayli, H., et al. Adipose tissue insulin resistance in youth on the spectrum from normal weight to obese and from normal glucose tolerance to impaired glucose tolerance to type 2 diabetes. *Diabetes Care*, **2019**, 42: 265–272. https://doi.org/10.2337/dc18-1178

[14] Girousse, A., Virtue, S., Hart, D., et al. Surplus fat rapidly increases fat oxidation and insulin resistance in lipodystrophic mice. *Molecular Metabolism*, **2018**, 13: 24–29. https://doi.org/10.1016/j.molmet.2018.05.006

[15] Neeland, I. J., Ross, R., Dseprés, J. P., et al. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. *The Lancet Diabetes & Endocrinology*, **2019**, 7: 715–725. https://doi.org/10.1016/S2213-8587(19)30084-1

[16] Bechmann, L. P., Hannivoort, R. A., Gerken, G., et al. The interaction of hepatic lipid and glucose metabolism in liver diseases. *Journal of Hepatology*, **2012**, 56: 952–964. https://doi.org/10.1016/j.jhep.2011.08.025

[17] Tappy, L. Metabolism of sugars: a window to the regulation of glucose and lipid homeostasis by splanchnic organs. *Clinical Nutrition*, **2021**, 40: 1691–1698. https://doi.org/10.1016/j.clnu.2020.12.022

[18] Demarsilis, A., Reddy, N., Boutari, C., et al. Pharmacotherapy of type 2 diabetes: an update and future directions. *Metabolism-Clinical and Experimental*, **2022**, 137: 155332. https://doi.org/10.1016/j.metabol.2022.155332

[19] Stadler, L. K., Farooqi, I. S. A new drug target for type 2 diabetes. *Cell*, **2017**, 170: 12–14. https://doi.org/10.1016/j.cell.2017.06.024

[20] Liu, Z., Xu, L., Xu, X., et al. Effects and mechanisms of iridoid

glycosides from *Patrinia scabiosaefolia* on improving insulin resistance in 3T3-L1 adipocytes. *Food and Chemical Toxicology*, **2019**, 134: 110806. https://doi.org/10.1016/j.fct.2019.110806

[21] Zhu, D., Wang, Y., Du, Q., et al. Cichoric acid reverses insulin resistance and suppresses inflammatory responses in the glucosamine-induced HepG2 cells. *Journal of Agricultural and Food Chemistry*, **2015**, 63: 10903–10913. https://doi.org/10.1021/acs.jafc.5b04533

[22] Cui, L., Wang, J., Wang, M., et al. Chemical composition and glucose uptake effect on 3T3-L1 adipocytes of *Ligustrum lucidum* Ait. flowers. *Food Science and Human Wellness*, **2020**, 9: 124–129. https://doi.org/10.1016/j.fshw.2020.02.002

[23] Tang, Q., Chen, S., Rizvi, S. A. H., et al. Two alkaloids from *Delphinium brunonianum* Royle, their anti-inflammatory and anti-oxidative stress activity via NF-κB signaling pathway. *Frontiers in Nutrition*, **2021**, 8: 826957. https://doi.org/10.3389/fnut.2021.826957

[24] Zhang, Y., Lv, P., Ma, J., et al. Antrodia cinnamomea exerts an anti-hepatoma effect by targeting PI3K/AKT-mediated cell cycle progression *in vitro* and *in vivo*. *Acta Pharmaceutica Sinica B*, **2022**, 12: 890–906. https://doi.org/10.1016/j.apsb.2021.07.010

[25] Huang, X., Liu, G., Guo, J., et al. The PI3K/AKT pathway in obesity and type 2 diabetes. *International Journal of Biological Sciences*, **2018**, 14: 1483–1496. https://doi.org/10.7150/ijbs.27173

[26] Posey, A. D., Pytel, P., Gardikiotes, K., et al. Endocytic recycling proteins EHD1 and EHD2 interact with fer-1-like-5 (Fer1L5) and mediate myoblast fusion. *Journal of Biological Chemistry*, **2011**, 286: 7379–7388. https://doi.org/10.1074/jbc.M110.157222

[27] Park, S. Y., Ha, B. G., Choi, G. H., et al. EHD2 interacts with the insulin-responsive glucose transporter (GLUT4) in rat adipocytes and may participate in insulin-induced GLUT4 recruitment. *Biochemistry*, **2004**, 43: 7552–7562. https://doi.org/10.1021/bi049970f

[28] Fazakerley, D. J., Krycer, J. R., Kearney, A. L., et al. Muscle and adipose tissue insulin resistance: malady without mechanism? *Journal of Lipid Research*, **2019**, 60: 1720–1732. https://doi.org/10.1194/jlr.R087510

[29] Kim, J. Y., Lim, H. J., Lee, D. Y., et al. *In vitro* anti-inflammatory activity of lignans isolated from *Magnolia fargesii*. *Bioorganic & Medicinal Chemistry Letters*, **2009**, 19: 937–940. https://doi.org/10.1016/j.bmcl.2008.11.103

[30] Yu, M., Li, Y., Li, M., et al. Eudesmin exerts antitumor effects by down-regulating EZH2 expression in nasopharyngeal carcinoma cells. *Chemico-Biological Interactions*, **2019**, 307: 51–57. https://doi.org/10.1016/j.cbi.2019.04.028

[31] Liu, H., Song, Z., Liao, D. G., et al. Anticonvulsant and sedative effects of Eudesmin isolated from *Acorus tatarinowii* on mice and rats. *Phytotherapy Research*, **2015**, 29: 996–1003. https://doi.org/10.1002/ptr.5337

[32] Nam, K. H., Yi, S. A., Lee, J., et al. Eudesmin impairs adipogenic differentiation via inhibition of S6K1 signaling pathway. *Biochemical and Biophysical Research Communications*, **2018**, 505: 1148–1153. https://doi.org/10.1016/j.bbrc.2018.09.188

[33] Nurcahyanti, A. D. R., Jap, A., Lady, J., et al. Function of selected natural antidiabetic compounds with potential against cancer via modulation of the PI3K/AKT/mTOR cascade. *Biomedicine & Pharmacotherapy*, **2021**, 144: 112138. https://doi.org/10.1016/j.biopha.2021.112138

[34] Yan, J., Wang, C., Jin, Y., et al. Catalpol ameliorates hepatic insulin resistance in type 2 diabetes through acting on AMPK/NOX4/PI3K/AKT pathway. *Pharmacological Research*, **2018**, 130: 466–480. https://doi.org/10.1016/j.phrs.2017.12.026

[35] Gao, C., Fei, X., Wang, M., et al. Cardamomin protects from diabetes-induced kidney damage through modulating PI3K/AKT and JAK/STAT signaling pathways in rats. *International Immunopharmacology*, **2022**, 107: 108610. https://doi.org/10.1016/j.intimp.2022.108610

[36] Klein, S., Gastaldelli, A., Yki-Järvinen, H., et al. Why does obesity cause diabetes. *Cell Metabolism*, **2022**, 34: 11–20. https://doi.org/10.1016/j.cmet.2021.12.012

[37] Hutchison, A. L., Tavaglione, F., Romeo, S., et al. Endocrine aspects of metabolic dysfunction associated steatotic liver disease (MASLD): beyond insulin resistance. *Journal of Hepatology*, **2023**, 79: 1524–1541. https://doi.org/10.1016/j.jhep.2023.08.030

[38] Han, H. S., Kang, G., Kim, J. S., et al. Regulation of glucose metabolism from a liver-centric perspective. *Experimental and Molecular Medicine*, **2016**, 48: e218. https://doi.org/10.1038/emm.2015.122

[39] Shen, X., Luo, K., Yuan, J., et al. Hepatic DDAH1 mitigates hepatic steatosis and insulin resistance in obese mice: involvement of reduced S100A11 expression. *Acta Pharmaceutica Sinica B*, **2023**, 13: 3352–3364. https://doi.org/10.1016/j.apsb.2023.05.020

[40] Kumar, S., Chhimwal, J., Kumar, S., et al. Phloretin and phlorizin mitigates inflammatory stress and alleviate adipose and hepatic insulin resistance by abrogating PPARγ S273-Cdk5 interaction in type 2 diabetic mice. *Life Sciences*, **2023**, 322: 121668. https://doi.org/10.1016/j.lfs.2023.121668

[41] Long, F., Bhatti, M. R., Kellenberger, A., et al. A low-carbohydrate diet induces hepatic insulin resistance and metabolic associated fatty liver disease in mice. *Molecular Metabolism*, **2023**, 69: 101675. https://doi.org/10.1016/j.molmet.2023.101675

[42] Alemayehu, B., Liu, J., Rajpathak, S., et al. Healthcare resource use and associated costs of hypoglycemia in patients with type 2 diabetes prescribed sulfonylureas. *Journal of Diabetes and Its Complications*, **2017**, 31: 1620–1623. https://doi.org/10.1016/j.jdiacomp.2017.07.012

[43] Grytsai, O., Myrgorodska, I., Rocchi, S., et al. Biguanides drugs: past success stories and promising future for drug discovery. *European Journal of Medicinal Chemistry*, **2021**, 224: 113726. https://doi.org/10.1016/j.ejmech.2021.113726

[44] Wu, D., Eead, V., Undi, R. B., et al. A novel peroxisome proliferator-activated receptor gamma ligand improves insulin sensitivity and promotes browning of white adipose tissue in obese mice. *Molecular Metabolism*, **2021**, 54: 101363. https://doi.org/10.1016/j.molmet.2021.101363

[45] Vinoth, K. T., Lakshmanasenthil, S., Geetharamani, D., et al. Fucoidan–a *α-D*-glucosidase inhibitor from *Sargassum wightii* with relevance to type 2 diabetes mellitus therapy. *International Journal of Biological Macromolecules*, **2015**, 72: 1044–1047. https://doi.org/10.1016/j.ijbiomac.2014.10.013

[46] Manaithiya, A., Alam, O., Sharma, V., et al. GPR119 agonists: novel therapeutic agents for type 2 diabetes mellitus. *Bioorganic Chemistry*, **2021**, 113: 104998. https://doi.org/10.1016/j.bioorg.2021.104998

[47] Joshi, D., Prashant, G. J., Gohsh, S., et al. TRC150094, a novel mitochondrial modulator, reduces cardio-metabolic risk as an add-on treatment: a phase-2, 24-week, multi-center, randomized, double-blind, clinical trial. *Diabetes Metabolic Syndrome and Obesity*, **2022**, 15: 615–631. https://doi.org/10.2147/DMSO.S330515

[48] Demonbreun, A. R., Posey, A. D., Heretis, K., et al. Myoferlin is required for insulin-like growth factor response and muscle growth. *FASEB Journal*, **2010**, 24: 1284–1295. https://doi.org/10.1096/fj.09-136309