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ORIGINAL ARTICLE

LRP1 facilitates hepatic glycogenesis by improving the insulin signaling pathway in HFD-fed mice

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Abstract

Background: LDL receptor-related protein-1 (LRP1) is a cell-surface receptor that functions in diverse physiological pathways. We previously demonstrated that hepatocyte-specific LRP1 deficiency (hLRP1KO) promotes diet-induced insulin resistance and increases hepatic gluconeogenesis in mice. However, it remains unclear whether LRP1 regulates hepatic glycogenesis.

Methods: Insulin signaling, glycogenic gene expression, and glycogen content were assessed in mice and HepG2 cells. The pcDNA 3.1 plasmid and adeno-associated virus serotype 8 vector (AAV8) were used to overexpress the truncated β-chain (βΔ) of LRP1 both in vitro and in vivo.

Results: On a normal chow diet, hLRP1KO mice exhibited impaired insulin signaling and decreased glycogen content. Moreover, LRP1 expression in HepG2 cells was significantly repressed by palmitate in a dose- and time-dependent manner. Both LRP1 knockdown and palmitate treatment led to reduced phosphorylation of Akt and GSK3β, increased levels of phosphorylated glycogen synthase (GYS), and diminished glycogen synthesis in insulin-stimulated HepG2 cells, which was restored by exogenous expression of the βΔ-chain. By contrast, AAV8-mediated hepatic βΔ-chain overexpression significantly improved the insulin signaling pathway, thus activating glycogenesis and enhancing glycogen storage in the livers of high-fat diet (HFD)-fed mice.

Conclusion: Our data revealed that LRP1, especially its β-chain, facilitates hepatic glycogenesis by improving the insulin signaling pathway, suggesting a new therapeutic strategy for hepatic insulin resistance-related diseases.

KEYWORDS
glycogenesis, insulin resistance, insulin signaling pathway, LRP1

Xingxian Guo and Jiangxia Pu contributed equally to this work.

1 | INTRODUCTION

Insulin resistance is a pathological condition in which insulin target tissues, including liver, muscle, and adipose tissues, are less sensitive to the metabolic actions of circulating insulin.[1–3] Hepatic insulin resistance mostly precedes the development of peripheral insulin resistance, which is increasingly regarded as a major initiating factor for metabolic disorders such as type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD).[4,5] Hepatic insulin resistance is characterized by increased endogenous glucose production, decreased glycogen synthesis, and overstimulation of lipogenesis processes in hepatocytes, eventually leading to hyperglycemia and disruption of whole-body energy homeostasis.[6,7] Although enhanced gluconeogenesis is the major contributor to hyperglycemia in T2D patients, the dysregulation of glycogen metabolism in the pathogenesis of hepatic insulin resistance has attracted increasing attention.[5,8]

LDL receptor-related protein-1 (LRP1) is an endocytic and signaling receptor containing an extracellular α-chain and a membrane-anchored cytoplasmic β-chain that functions in the regulation of various biological processes, including lipoprotein metabolism and vascular homeostasis, endocytosis and signal transduction. The extracellular domain of LRP1 can undergo shedding via regulated intramembrane proteolysis (RIP), whereby the intracellular β-chain can be cleaved by γ-secretase to yield the intracellular C-terminal domain (ICD), which relocates to the nucleus to regulate gene transcription.[9–11] As a novel integrator of adipocyte differentiation and fat storage signals, LRP1 is required for adipolysis and stimulates fatty acid synthesis.[12] Moreover, LRP1 has been demonstrated to play an important role in the intracellular processing of lipids in hepatocytes after fatty acid internalization.[13] Gene–diet interactions analysis in human obesity supported LRP1 as a potential obesity candidate for modulating sensitivity to saturated fat intake.[14] On the other hand, the LIPGENE study revealed that the LRP1 rs4759277 SNP was correlated with fasting insulin concentration and insulin resistance in patients with metabolic syndrome.[15] These findings imply that LRP1 may be involved in the pathogenesis of insulin resistance. Previously, we demonstrated that hepatic LRP1 deficiency (hLRP1KO) promoted high-fat diet (HFD)-induced insulin resistance in mice, as evidenced by impaired insulin signal transduction and increased gluconeogenesis in the liver.[16] However, whether and how LRP1 regulates hepatic glycogenesis in the liver remain largely unclear.

In the present study, we employed gain- and loss-of-function approaches to uncover a hitherto unreported link between LRP1 and glycogenesis, highlighting the important role of LRP1 in the regulation of glycogenesis to maintain glucose homeostasis by improving the hepatic insulin signaling pathway.

2 | METHODS

2.1 | Animals

Lrp1^{em1Cflox} mice (CKOCMP-16971-*Lrp1*-B6N-VA) on a congenic C57BL/6 background were purchased from Cyagen Biosciences (Suzhou, China). hLRP1KO mice were generated by crossing *Lrp1*^{em1Cflox} mice with Albumin-Cre mice. hLRP1$^{+/+}$ littermates were used as wild-type (WT) control mice. Five- to six-week-old male C57BL/6J mice were housed at 22–24°C with a 12-hour light/12-hour dark cycle, and fed with a HFD containing 60% fat (D12492, Research Diets) for at least 16 weeks.

2.2 | Cell culture and treatment

HEK293T cells and the human hepatocellular carcinoma cell line HepG2 (preserved at Chongqing Key Laboratory for Lipid and Glucose Metabolism) were maintained in DMEM supplemented with 4.5 g/L glucose and 10% FBS and 1% antibiotics in a humidified atmosphere of 5% CO_2 at 37°C. HepG2 cells were starved overnight in the absence or presence of 0.25 mM palmitate and treated with 100 nM insulin for 5 min.

2.3 | Reagents and antibodies

Antibodies against Akt, glycogen synthase kinase 3β (GSK3β), glycogen synthase (GYS), phospho-Akt (p-Akt), p-Ser9-GSK3β and p-GYS were purchased from Cell Signaling Technology. An antibody against GSK3β was purchased from BBI (Shanghai, China) and an antibody against LRP1 was purchased from Selleck Company (USA). Antibodies against β-actin, HRP-conjugated AffiniPure goat anti-mouse IgG (H+L), and goat anti-rabbit IgG (H+L) were purchased from Proteintech (Rosemont, USA). A periodic acid-Schiff (PAS) stain kit was purchased from Beyotime (Shanghai, China). A glycogen content assay kit was purchased from Boxbio (Beijing, China).

2.4 | Plasmids and lentivirus infection

A pLKO.1 lentiviral vector carrying small hairpin RNA (shRNA) with the functional sequence CCGGCGCCGGATGTATAAATGT AAACTCGAGTTTAC ATTTATACA TCCGGCGTTTTTG to target the human *Lrp1* gene sequence (CGCCGGATGTATAAATGTAAA) was produced as previously described.[17] The scrambled shRNA was a gift from David Sabatini, and the hairpin sequence was as follows: CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACT TAACCTTAGG.[18] The lentiviral particles were generated by co-transfection of HEK293T cells with lentiviral packaging mix (psPAX2, pMD2.G; Addgene) and the lentiviral plasmid pLKO.1 using FuGENE 6 Transfection Reagent (Roche, Hamburg, Germany).

To overexpress the truncated β-chain (βΔ) of LRP1 overexpression, a fragment encoding the β-chain (amino acids 4195–4544) of human LRP1 was amplified from the *hLrp1*-pcDNA3.1 plasmid, and the PCR product was inserted into the pcDNA 3.1-3×Flag plasmid to express the Flag-tagged βΔ-chain (which is insensitive to *hLrp1* shRNA). The *hLrp1*-pcDNA3.1 plasmid was a gift from Dr Joachim Herz (UT Southwestern Medical Center, USA). The adeno-associated virus serotype 8 vector (AAV8) expressing the βΔ-fragment was obtained from Obio Technology (Shanghai, China). The HFD-fed mice

were randomly divided into two groups and subjected to tail vein injection of 10^{10} virus particles/mouse of AAV8-βΔ-chain or AAV8-GFP. Four weeks after the administration of AAV8, the mice were sacrificed, and liver samples were collected.

2.5 | Immunoblot analysis

Cells and liver tissues were lysed using RIPA buffer, separated by SDS-PAGE, transferred to polyvinylidene fluoride (PVDF, Millipore) membranes and incubated with the indicated antibodies. The membranes were visualized with SuperSignal West Pico chemiluminescence reagents and analyzed using ChampChemi™ TOP 610.

2.6 | Periodic acid-Schiff staining

The glycogen content in HepG2 cells was detected by PAS staining with some modifications.[19] In brief, cells were cultured in glucose and serum-free medium supplemented with 0.5% BSA for 24 hours, after which 5.5 mM glucose with or without 100 nM insulin was added to the medium. After 12 h, the cells were fixed with 70% alcohol, treated with 1% periodic acid, and stained with the Schiff reagent. The micrographs were captured under a microscope at 200× magnification.

2.7 | Measurement of glycogen content

Mice were sacrificed after 24 h of fasting followed by 6 hours of refeeding. Glycogen levels in liver tissues were measured according to the manufacturer's instructions.

2.8 | Statistical analysis

The results are reported as means ± SEM. Statistical analysis was performed using the GraphPad Prism 5 software. The unpaired two-tailed Student's *t* test was used to compare differences between two groups. Differences among three or more groups were analyzed using one- or two-way ANOVA with Tukey's or Sidak's post hoc multiple comparisons test. Differences were considered statistically significant at $p < 0.05$ between two groups, as denoted by a single asterisk, and at $p < 0.01$, as denoted by a double asterisk.

3 | RESULTS

3.1 | Hepatic LRP1 deficiency impairs insulin signaling and glycogenesis in the liver

Our previous study demonstrated that HFD-fed hLRP1KO mice exhibit severe hepatic insulin resistance, leading to incomplete suppression of hepatic gluconeogenic genes, reduced surface insulin receptor (IR) expression, and impaired insulin-induced phosphorylation of IR.[16] We then investigated the effects of LRP1 deficiency on insulin signaling molecules and GYS in livers isolated from WT and hLRP1KO mice shortly after intraperitoneal insulin injection. As shown in Figure 1A, phosphorylated Akt (p-Akt) and p-GSK3β levels in liver tissues were significantly lower in hLRP1KO mice than in WT controls. Consistently, GYS phosphorylation was increased in the livers of hLRP1KO mice. Moreover, hLRP1KO mice showed significantly reduced PAS-positive staining and decreased glycogen content in liver tissues (Figure 1B,C). These results indicate that LRP1 deficiency impairs insulin signaling, thereby attenuating the activating effect of insulin on GYS activity and abating glycogen storage in the liver.

3.2 | LRP1 knockdown leads to blunted insulin signaling and glycogenesis in HepG2 cells

We further explored the effect of LRP1 knockdown on the insulin signaling pathway in HepG2 cells using shRNA-mediated RNA interference of the *Lrp1* gene. As shown in Figure 2A, western blot analysis confirmed that LRP1 expression was effectively silenced in *Lrp1*-shRNA lentivirus-infected HepG2 cells ($LRP1^{KD}$ group). Then the expression of Akt and its downstream target molecules GSK3β and GYS was determined. Under basal conditions, p-GYS levels were significantly elevated in $LRP1^{KD}$ cells despite no difference in the expression of p-Akt and p-GSK3β between the two groups. Upon insulin stimulation, p-Akt and p-GSK3β levels were reduced, whereas p-GYS was increased in the $LRP1^{KD}$ group compared with the control group (Figure 2A).

To assess the effects of LRP1 deficiency on hepatic glycogen synthesis, we determined intracellular glycogen accumulation in control and $LRP1^{KD}$ HepG2 cells by PAS staining both in the basal state and in response to glucose + insulin stimulation. Along with impaired insulin signaling, quantification of PAS staining demonstrated that glycogen deposition in the $LRP1^{KD}$ group was obviously less than that in the control group in both the presence and absence of insulin (Figure 2B). Together, these results demonstrate that LRP1 knockdown suppresses insulin signaling and impairs glycogen synthesis in HepG2 cells.

3.3 | The LRP1 βΔ-chain improves the LRP1 knockdown-mediated suppression of insulin signaling and glycogenesis in HepG2 cells

After cleavage by γ-secretase upon signaling activation, the β-chain of LRP1 releases the intracellular domain (ICD) from the membrane, which translocates to the cytoplasm and nucleus.[10,20,21] Given that LRP1-ICD transcriptionally and post-translationally affects the activity of various functional proteins, we investigated whether LRP1-ICD plays a role in the regulation of

insulin signaling and glycogenesis. As shown in Figure 2C, overexpression of the βΔ-chain led to the recovery of insulin-stimulated Akt and GSK3β phosphorylation in LRP1^{KD} cells, which was accompanied by decreased p-GYS levels. Correspondingly, a reversal of the inhibitory effects of LRP1 knockdown on glycogen storage was observed in LRP1-deleted cells transfected with the βΔ-chain expression vector (Figure 2D). Therefore, these results strongly indicate that exogenously expressed βΔ-chain can fully correct the effects of LRP1 silencing on insulin signaling and glycogenesis in LRP1^{KD} cells.

3.4 | Palmitate suppresses insulin signaling and glycogenesis in HepG2 cells

Excessive free fatty acid (FFA) flux into the liver plays a pathogenic role in the development of hepatic insulin resistance.[22,23] We then investigated the effect of FFAs on insulin action in HepG2 cells treated with palmitate, one of the most abundant FFAs in human plasma. As shown in Figure 3A, exposure of cultured HepG2 cells to palmitate caused a marked decrease in p-AKT and p-GSK3β levels and increased p-GYS levels under insulin-stimulated conditions. Similarly, glycogen deposition in the palmitate group was dramatically reduced in both the presence and absence of insulin compared with that in the control group (Figure 3B).

Given that palmitate treatment and LRP1 knockdown exerted similar effects on insulin signaling and glycogen storage in HepG2 cells, we wanted to identify whether the palmitate-induced impairment of insulin signaling was associated with alterations in LRP1 expression. HepG2 cells were co-cultured with step-increasing concentrations of palmitate (from 100 to 500 μM) for 24 h, and LRP1 expression was determined by western blotting. As shown in Figure 3C, palmitate contributed to a dose-dependent decrease in LRP1 expression at an initial dose of 100 μM. In the time-course experiments, HepG2 cells were exposed to 250 μM palmitate for 0, 4, 12 or 24 h, after which the LRP1 expression levels were analyzed. Similarly, LRP1 expression was decreased significantly with increasing palmitate treatment time (Figure 3D).

3.5 | The LRP1 βΔ-chain restores palmitate-mediated inhibition of insulin signaling and glycogenesis in HepG2 cells

To further verify whether LRP1 is involved in the aberrant insulin resistance and glycogenesis triggered by dietary fatty acids, we

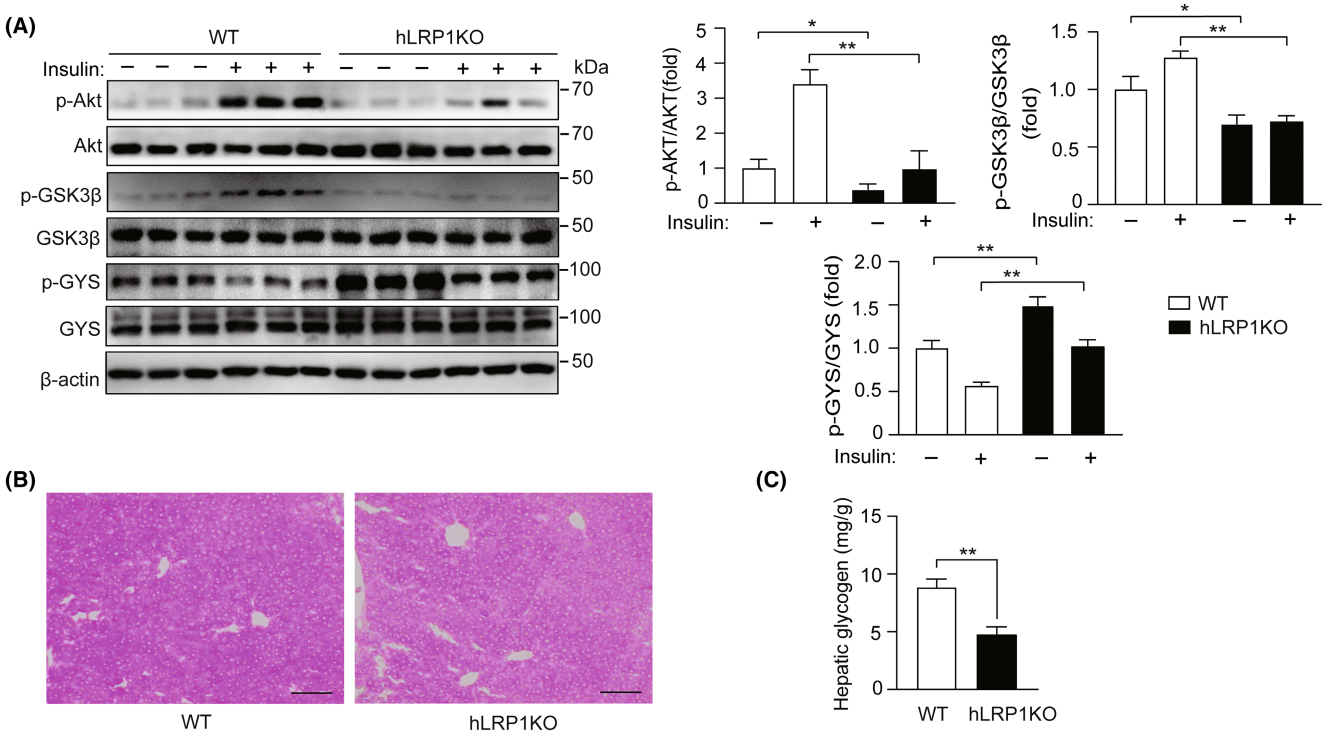
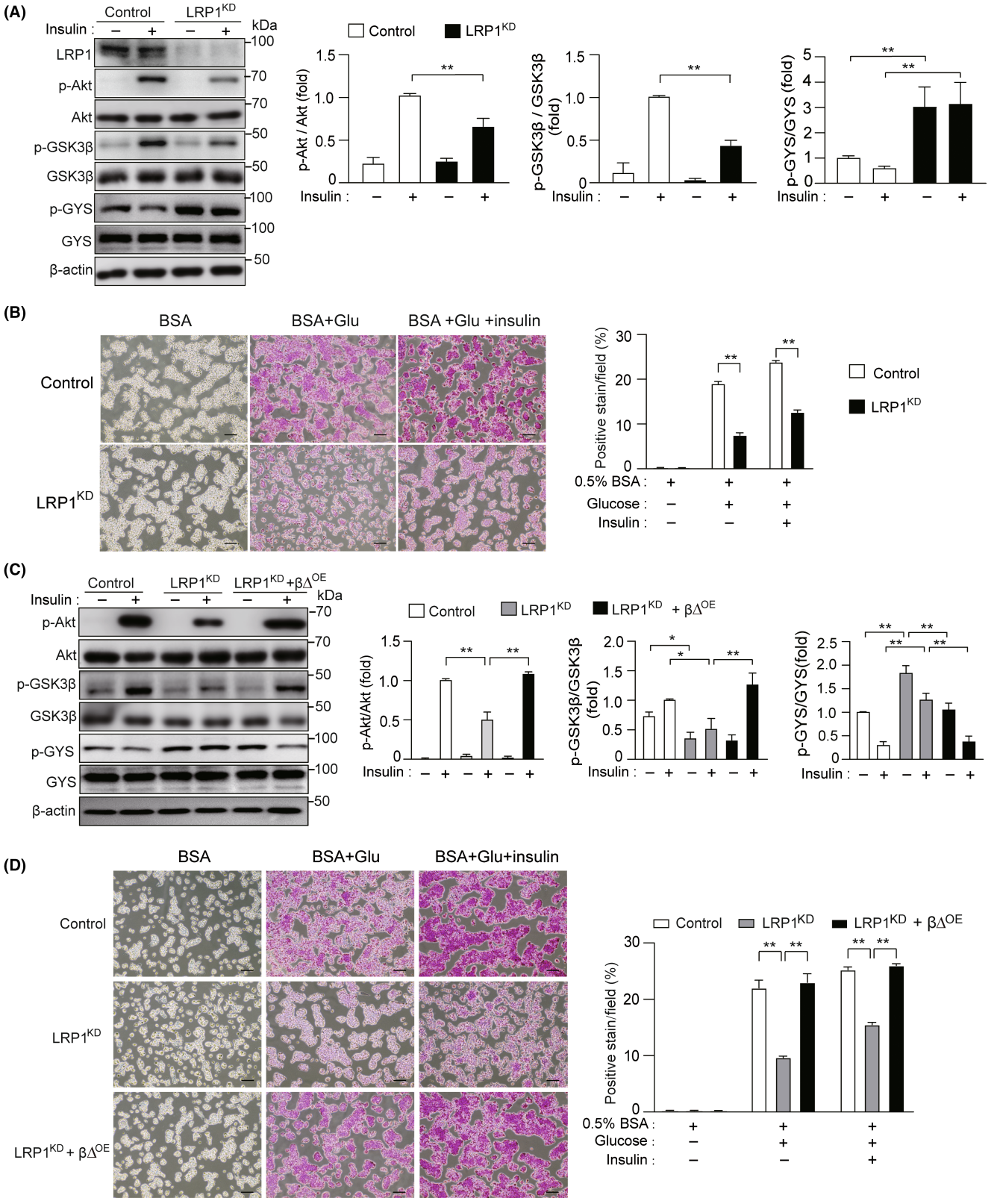


FIGURE 1 LRP1 deficiency leads to impaired hepatic insulin signaling and glycogenic dysfunction in mice. (A), Mice were fasted overnight and intraperitoneally administered insulin at a dose of 1 IU/kg. After 30 min, the mice were sacrificed and liver tissues were harvested. The expression and phosphorylation of Akt, GSK3β and GYS in total liver extracts were assessed by western blotting. The data are presented as the means ± SEM and were analyzed using two-way ANOVA with Sidak's multiple comparisons test (*$p < 0.05$; **$p < 0.01$). (B), Mice were fasted overnight, followed by refeeding for 6 h. Representative PAS-stained liver sections from WT and hLRP1KO mice are shown (scale bar, 100 μm). (C), The glycogen content in the liver tissues was determined. The data are presented as the mean ± SEM and analyzed using an unpaired two-tailed Student's *t* test (**$p < 0.01$).



investigated the effects of βΔ-chain overexpression in palmitate-treated cells. The inhibitory effects of palmitate on insulin signaling and GYS activity were reversed in LRP1-deleted cells when the βΔ-chain was overexpressed (Figure 3E). Furthermore, βΔ-chain expression dramatically blocked the inhibitory effects of palmitate on glycogen synthesis (Figure 3F). These data suggest that the βΔ-chain of LRP1 can reverse palmitate-induced insulin resistance and promote glycogenesis in hepatocytes.

FIGURE 2 LRP1 βΔ-chain overexpression prevents LRP1 silencing-induced impairment of insulin signaling and glycogenesis in HepG2 cells. (A), Control and $LRP1^{KD}$ HepG2 cells were treated with or without insulin (100 nM) for 5 min, and whole-cell lysates were assayed for the expression and phosphorylation of Akt, GSK3β, and GYS via western blotting. β-actin was used as a loading control. Western blotting data obtained from three independent experiments were quantified in the right panel. The data are presented as the means ± SEM and were analyzed using two-way ANOVA with Sidak's multiple comparisons test (**$p < 0.01$). (B), Control and $LRP1^{KD}$ HepG2 cells were subjected to PAS staining after treatment with 0.5% BSA, 0.5% BSA + 5.5 mM glucose and 0.5% BSA + 5.5 mM glucose +100 nM insulin. Representative PAS staining and average positive staining normalized to the field area were presented in the left and right panels (scale bar, 500 μm). The data are presented as the means ± SEM from four independent experiments and were analyzed using two-way ANOVA with Sidak's multiple comparisons test (**$p < 0.01$). (C), Western blotting was performed to determine p-Akt, p-GSK3β, p-GYS, Akt, GSK3β, and GYS levels in the control, $LRP1^{KD}$, and $LRP1^{KD} + \beta\Delta^{OE}$ HepG2 cells under basal and insulin-stimulated conditions. β-actin was used as a loading control. Quantification analysis of three independent experiments was presented in the right panel. The data are presented as the means ± SEM and analyzed using two-way ANOVA with Tukey's multiple comparisons test (*$p < 0.05$; **$p < 0.01$). (D), PAS staining was performed to detect glycogen content in control, $LRP1^{KD}$, and $LRP1^{KD} + \beta\Delta^{OE}$ HepG2 cells treated with 0.5% BSA, 0.5% BSA + 5.5 mM glucose and 0.5% BSA + 5.5 mM glucose +100 nM insulin. The results of the imaging analysis were presented in the right panel (scale bar, 500 μm). The values shown were expressed as the means ± SEM of four independent experiments and analyzed using two-way ANOVA with Tukey's multiple comparisons test (**$p < 0.01$).

3.6 | Hepatic βΔ-chain expression improves insulin signaling and increases glycogenesis in the livers of HFD-fed mice

To confirm the beneficial effects of βΔ-chain on insulin signaling and glycogenesis, mice were fed a HFD for 16 weeks to induce insulin resistance and then exogenous βΔ-chain was introduced into the liver of the mice via AAV8 (Figure 4A). We detected long-term and highly stable expression of the FLAG-βΔ-chain in the livers of AAV8-βΔ-chain-injected mice (Figure 4B). Strikingly, hepatic overexpression of the βΔ-chain resulted in a marked increase in p-Akt and p-GSK3β levels while reducing p-GYS levels in the liver with and without insulin stimulation (Figure 4C). Moreover, both PAS-positive staining and glycogen content were significantly increased in the liver tissues of the AAV8-βΔ-chain group compared to those in the AAV8-GFP group (Figure 4D,E). Taken together, these results highlight the important role of the LRP1 β-chain in ameliorating glycogenic dysfunction in HFD-fed mice by improving the hepatic insulin signaling pathway.

4 | DISCUSSION

In this study, we demonstrated that LRP1 plays a critical role in the regulation of glycogenesis by modulating the Akt/GSK3β/GYS pathway (Figure 5). In the absence of LRP1 or palmitate treatment, the insulin-induced phosphorylation of Akt and GSK3β was decreased, which consequently increased the phosphorylation of GYS and decreased glycogen synthesis. By contrast, overexpression of the LRP1 βΔ-chain reversed the effects of LRP1 silencing and palmitate treatment on insulin signaling and glycogen synthesis in HepG2 cells.

Growing evidence suggests that an intact insulin signaling pathway in the liver is a prerequisite for maintaining blood glucose levels within a narrow normal glycemic range through coordinated effects on glucose production, uptake, and storage.[24,25] Generally, insulin exerts its inhibitory effects on glucose production by activating the Akt/Forkhead box protein O1 (FoxO1) signaling pathway, thereby downregulating phosphoenolpyruvate carboxykinase (PCK1) and glucose-6 phosphatase (G6PC) expression.[26] On the other hand, insulin promotes glycogen storage through the Akt-mediated phosphorylation of Ser9 of GSK3β, which results in the inactivation of GSK3β and activation of GYS.[27] Under pathological conditions, such as obesity and T2D, insulin fails to inhibit hepatic gluconeogenesis, even in the postprandial state, leading to hepatic insulin resistance.[4,5] In addition, hepatic glycogen content and glycogenesis were demonstrated to be reduced in T2D patients, which was correlated with excessive glucose production and postprandial hyperglycemia.[28] In contrast, GSK3β inhibitors improved insulin resistance in HFD-induced insulin resistant animal models by increasing whole-body insulin sensitivity and hepatic glycogen synthesis.[29]

Emerging research has demonstrated that LRP1 is involved in the regulation of glucose metabolism.[9,30] Our previous study demonstrated that LRP1 deletion in the liver promoted diet-induced hepatic insulin resistance in mice, suggesting a protective role for LRP1 against HFD-induced insulin resistance.[16] Interestingly, another study by Wu et al. provided evidence that hepatic LRP1 expression levels were reduced in Zucker diabetic fatty (ZDF) rats during the development of HFD-induced diabetes, whereas berberine treatment enhanced LRP1 expression in the liver and effectively attenuated hepatic insulin resistance and inflammatory responses in diabetes mellitus.[31] However, this intriguing finding did not prove a cause-and-effect relationship between LRP1 expression and insulin resistance.

Although the overconsumption of saturated fatty acids (SFAs) has been well accepted as a risk factor for insulin resistance and metabolic disorders, the mechanisms by which excessive NEFAs in circulation lead to insulin resistance in peripheral tissues remain incompletely understood.[6,32] In this study, we found that palmitate inhibited LRP1 expression in a dose- and time-dependent manner. It appears more likely that palmitate either inhibits LRP1 mRNA expression or promotes the proteolytic degradation of LRP1 at the post-translational level. Interestingly, LRP1 deficiency recapitulated the effects of palmitate on insulin signaling and glycogenesis in HepG2 cells and mice, whereas overexpression of the LRP1 βΔ-chain was sufficient to reverse the impaired hepatic insulin signaling and glycogenic dysfunction in both LRP1-knockdown hepatocytes and

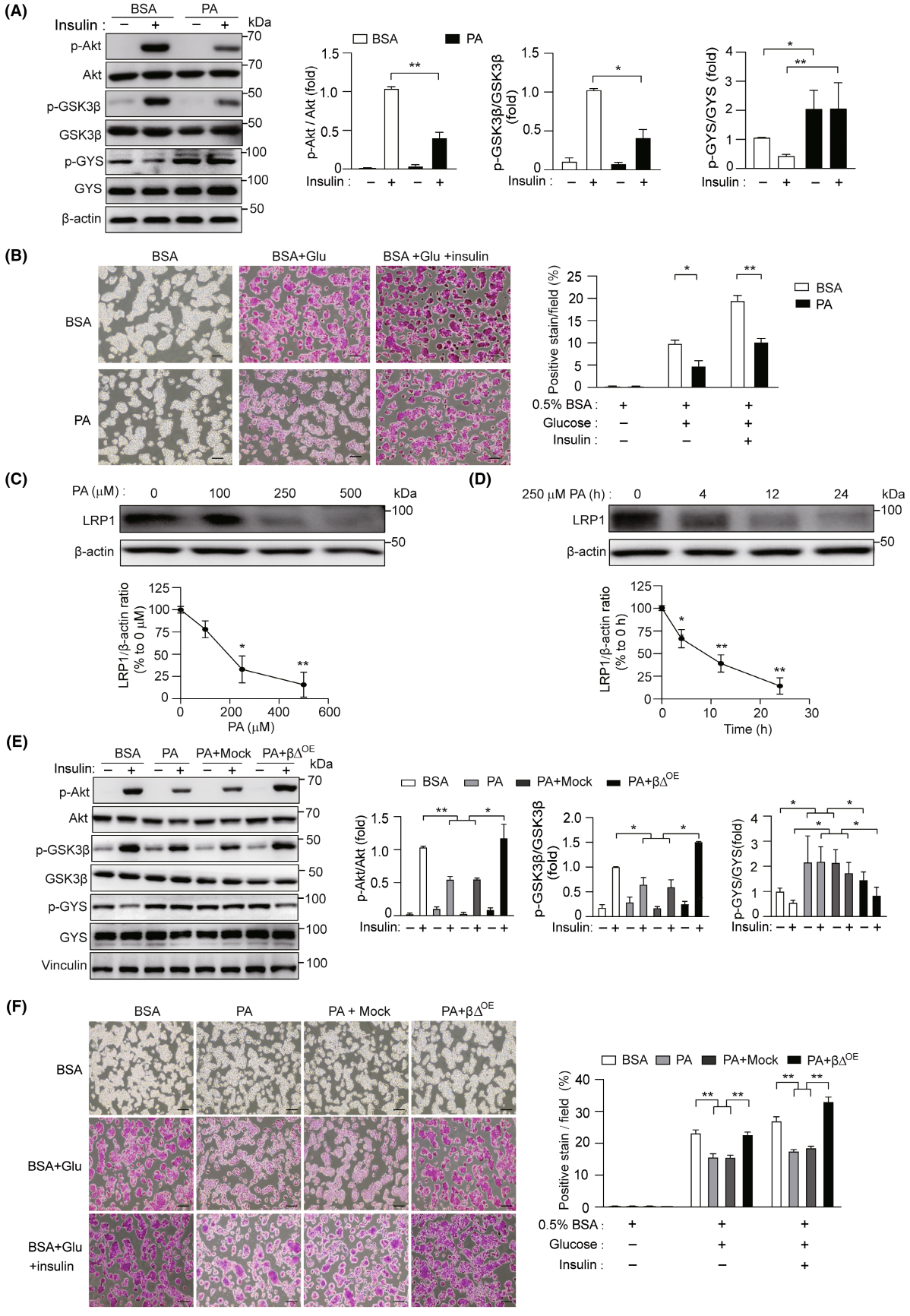

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FIGURE 3 βΔ-chain reverses palmitate-induced insulin resistance in HepG2 cells. (A), HepG2 cells were treated with either 0.5% BSA or 0.25 mM palmitate (PA) for 24 h, followed by incubation with or without insulin (100 nM) for 5 min, and the phosphorylation and expression levels of Akt, GSK3β, and GYS were determined by immunoblotting. Western blot data from three independent experiments were quantified in the right panel (*$p < 0.05$; **$p < 0.01$). (B), The cells were incubated with 0.5% BSA or 0.25 mM PA for 24 h and then subjected to PAS staining after treatment with 0.5% BSA, 0.5% BSA + 5.5 mM glucose, or 0.5% BSA + 5.5 mM glucose +100 nM insulin. Representative PAS staining and average positive staining normalized to the field area from four independent experiments are presented in the left and right panels (*$p < 0.05$; **$p < 0.01$). Scale bar, 500 μm. (C), The cells were treated with the indicated concentrations of PA, and the LRP1 expression levels were determined by immunoblotting, normalized to the β-actin levels, expressed as a percentage of the vehicle-treated control cultures and represented as the means ± SEM of three independent experiments (*$p < 0.05$; **$p < 0.01$). (D), The cells were treated with 250 μM PA for 0, 4, 12 and 24 h. LRP1 expression levels were determined by immunoblotting, normalized to β-actin levels, and expressed as a percentage of the vehicle-treated control cultures and represented as the means ± SEM of three independent experiments (*$p < 0.05$; **$p < 0.01$).(E), Western blotting was performed to determine the p-Akt, p-GSK3β, p-GYS, Akt, GSK3β, and GYS levels in HepG2 cells under basal and insulin-stimulated conditions. Quantification of three independent experiments is presented in the right panel (*$p < 0.05$; **$p < 0.01$). Vinculin was used as a loading control. (F), PAS staining was performed to detect glycogen content in control, $LRP1^{KD}$, and $LRP1^{KD} + \beta\Delta^{OE}$ HepG2 cells treated with 0.5% BSA, 0.5% BSA + 5.5 mM glucose, and 0.5% BSA + 5.5 mM glucose +100 nM insulin. The results of the imaging analysis are presented in the right panel ($n = 3$ independent experiments. **$p < 0.01$). Scale bar, 500 μm.

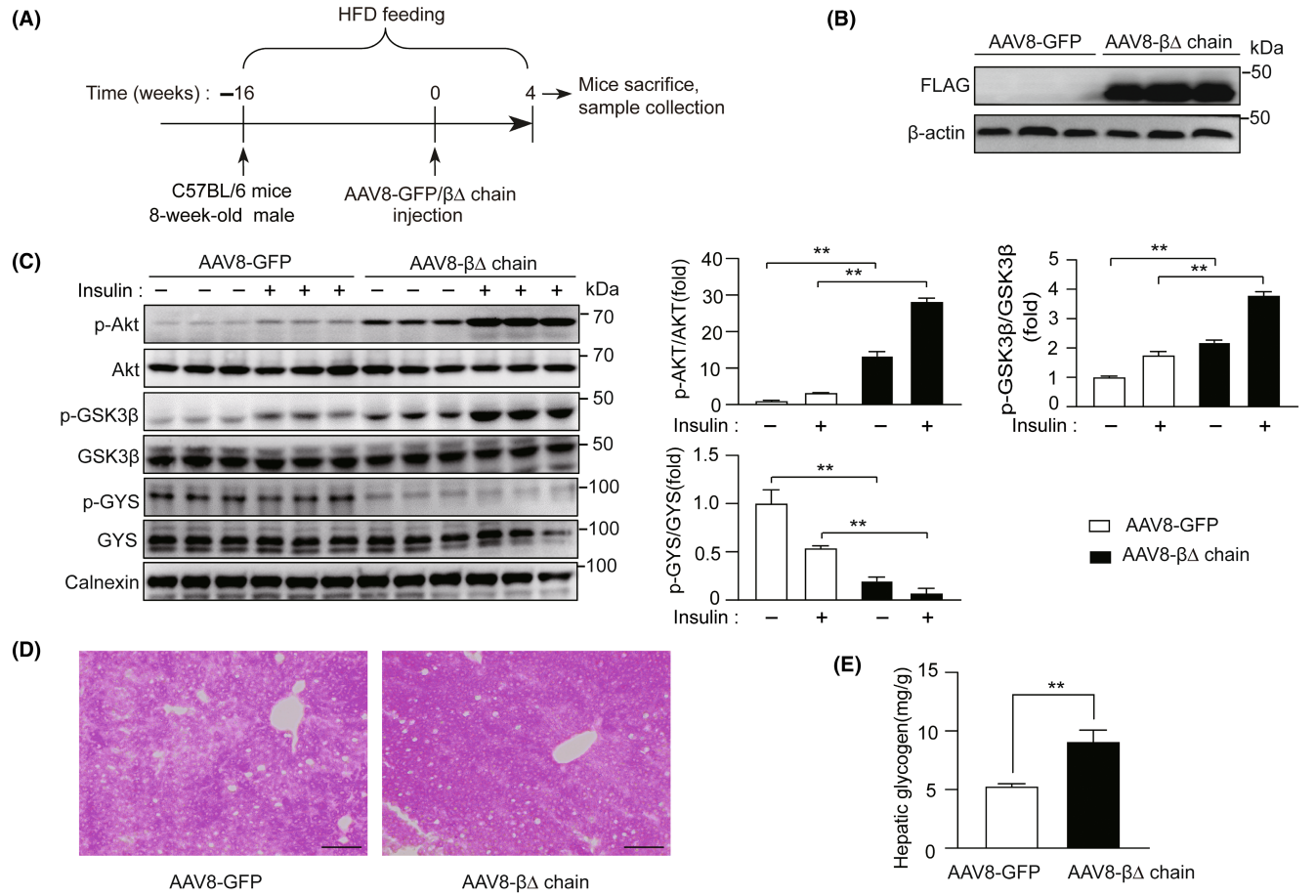

FIGURE 4 Overexpression of the LRP1 βΔ-chain rescues impaired hepatic insulin signaling and glycogenic dysfunction in HFD-induced insulin-resistant mice. (A), The experimental procedure was designed to determine the effects of hepatic βΔ-chain overexpression in HFD-fed mice. (B), Protein expression of F tagged βΔ-chain in the liver was determined in AAV8-GFP- and AAV8-βΔ chain-injected mice at the end of the experiment. (C), Western blotting was performed to determine the p-Akt, p-GSK3β, p-GYS, Akt, GSK3β, and GYS levels in HepG2 cells under basal and insulin-stimulated conditions. Quantification of three independent experiments is presented in the right panel (**$p < 0.01$). Calnexin was used as a loading control. (D), Representative PAS staining of liver sections from starved-refed mice is shown (scale bar, 100 μm). (E), The glycogen content in the liver tissues was determined. The data are presented as the means ± SEM and analyzed using unpaired two-tailed Student's *t* test (**$p < 0.01$).

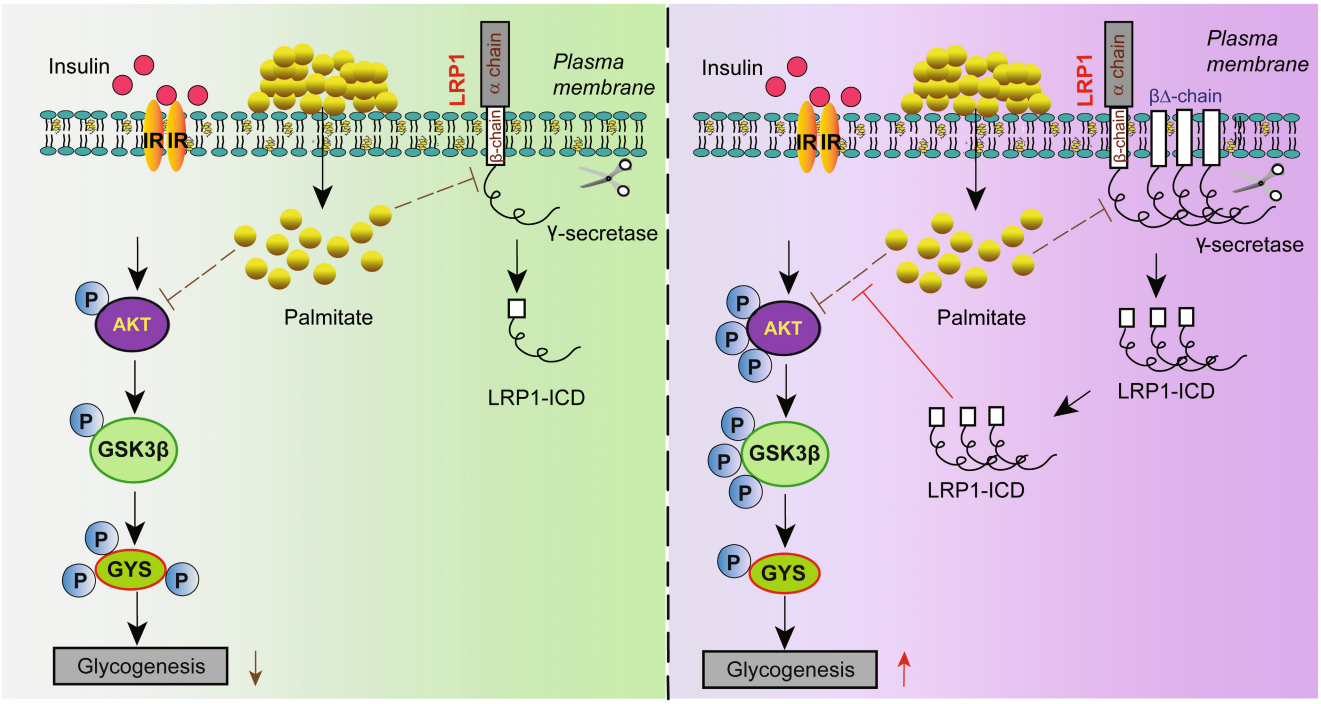

FIGURE 5 Summary of the mechanism by which LRP1 regulates insulin signaling and glycogen synthesis. Upon activation of signaling, the β-chain of LRP1 is cleaved by γ-secretase and releases the intracellular domain (ICD) from the membrane, which enhances insulin-stimulated activation of the Akt/GSK3β/GYS pathway and glycogenesis. Palmitate suppresses LRP1 expression, leading to impaired insulin signaling and glycogenesis in hepatocytes. Overexpression of βΔ-chain overwhelms the suppression of palmitate and significantly improves the insulin signaling pathway, thus activating glycogenesis and enhancing glycogen storage in the liver.

liver tissues of HFD-fed mice. Collectively, our data strongly suggest that alterations in LRP1 expression act as an important trigger in palmitate-induced hepatic insulin resistance, which may provide new insights into the treatment of insulin resistance-related diseases.

Consequently, impairment of the Akt/GSK3β pathway contributed to decreased glycogen storage. These results raise the question of how LRP1 deficiency causes the inhibition of insulin signaling pathway and glycogenesis. As shown in our previous study, hepatic LRP1 inactivation caused defective insulin signaling, including reduced expression and phosphorylation of IR and decreased glucose transporter 2 (GLUT2) translocation.[16] In addition to its general functions as a transmembrane receptor, LRP1 can be cleaved to liberate its ICD into the cytoplasm.[33] Upon signaling activation, LRP1-ICD translocates into the nucleus and influences gene transcription.[17] Lin et al. demonstrated that LRP1-ICD interacted with plasminogen activator inhibitor-1 (PAI-1) to downregulate the expression of SMAD family member 4 (SMAD4) by binding to the *SMAD4* promoter, and to enhance the ubiquitin-mediated degradation of SMAD4.[10] However, further studies are needed to elucidate the detailed mechanism through which the β-chain of LRP1 coordinates with insulin to regulate the insulin signaling pathway.

There was evidence that high concentrations of glucose or glucosamine treatment suppressed the activation of GYS by insulin.[34,35] Another study showed that the activity of GYS could be regulated by O-linked β-N-acetylglucosamine (O-GlcNAc) modification.[36] Moreover, excess hexosamine biosynthesis pathway (HBP) flux or elevated O-GlcNAc levels led to defective Akt activation and decreased glycogen synthesis upon insulin stimulation given that GSK3β was not efficiently phosphorylated, which inhibited glycogen synthase and thereby increased the release of glucose-6-phosphate from glycogen.[37,38] Therefore, we hypothesized that an O-GlcNAcylation-related mechanism might be involved in the regulation of the insulin signaling pathway and glycogenesis by LRP1 in response to nutritional stimuli.

In summary, we revealed that palmitate substantially represses LRP1 expression in hepatocytes, and that through its β-chain, LRP1 regulates insulin activity and subsequently affects hepatic glycogenesis, suggesting that LRP1 plays an important role for LRP1 in modulating insulin signaling to maintain glycogen homeostasis, especially under HFD-induced insulin-resistant conditions. Given the beneficial effects of βΔ-chain, an endogenous peptide, on insulin signaling and glycogenesis in HFD-fed mice, our study may shed light on the development of new therapeutic strategies against hepatic insulin resistance and related diseases.

AUTHOR CONTRIBUTIONS

Xingxian Guo, Jiangxia Pu and Yinyuan Ding conceived and designed the research; Xingxian Guo, Jiangxia Pu, Ziqi Tang and Can Jia conducted the experiments; Xingxian Guo and Jiangxia Pu

contributed equally to this work; Xingxian Guo and Jiangxia Pu performed the data analysis; Ziqi Tang and Can Jia provided reagents; Fan Yang and Tianyi Liu conducted the statistical analysis; Xingxian Guo and Jiangxia Pu wrote the original draft; Yinyuan Ding supervised the study and reviewed and edited the manuscript. All the authors have read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest in the current work.

DATA AVAILABILITY STATEMENT

All data supporting the conclusions are included in the article by the authors, and further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

All animal experimental protocols were guided and approved by the Medical Experimental Animal Care Commission of Chongqing Medical University (Approval No. IACUC-CQMU-2023-0072) and were in compliance with the National Institutes of Health guidelines.

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