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Glucose-dependent regulation of hepatic adipsin controls glucose uptake and tolerance

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Abstract

Background Complement factor D, also known as adipsin, is produced by adipose tissue, and the liver that links metabolic regulation with innate immunity. Despite its established systemic functions, the regulation of hepatic adipsin expression and its contribution to metabolic disease remain poorly defined.

Methods Hepatic adipsin expression was examined in liver biopsies from non-diabetic individuals and patients with type 2 diabetes (T2D), in murine models of type 1 diabetes (T1D) and T2D, during the fasted-to-fed transition, and in primary hepatocytes exposed to glucose. Translational regulation was assessed by glucose stimulation and pharmacological inhibition of mTOR signaling. Hepatocyte-specific adipsin knockdown was performed using liver-targeted shRNA to determine its metabolic function.

Results We show that hepatic adipsin protein abundance is markedly increased in individuals with type 2 diabetes, and positively correlates with glycated hemoglobin, despite unchanged mRNA expression. Concordantly, hepatic adipsin protein levels were elevated in multiple murine models of hyperglycemia, including type 1 diabetes (T1D), type 2 diabetes (T2D), and following fasting–re-feeding transitions. In cultured hepatocytes, glucose exposure induced a rapid, dose-dependent increase in adipsin protein without altering transcript abundance, demonstrating post-transcriptional regulation. Mechanistically, glucose stimulates adipsin translation via dephosphorylation of eukaryotic initiation factor 2α (eIF2α), and activation of the mammalian target of rapamycin, mediated by the 5′ untranslated region of *Adipsin* mRNA. Functionally, hepatocyte-specific depletion of adipsin impaired postprandial glucose tolerance, with reduced glucose uptake and a marked downregulation of glucose transporter type 2 (GLUT2).

Conclusions These findings identify hepatic adipsin as a glucose-responsive translational target that couples nutrient availability to metabolic adaptation, revealing previously unrecognized regulation with potential relevance to diabetes pathogenesis.

Keywords Adipsin, Diabetes, Liver, MTOR, EIF2α, GLUT2

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Background

Adipsin, or CFD, resides at the nexus of metabolic regulation (Flier et al. 1987), immune function (Forneris et al. 2010), and endocrine signaling pathways (Lo et al. 2014). Adipsin is a serine protease (Volanakis and Narayana 1996) that catalyzes the rate-limiting step in the alternative complement pathway and has lately been shown to have biological significance beyond classical immunology (Rosen et al. 1989). Accumulating studies over the past decade have identified it as a metabolically active protein (Kong et al. 2024) that is predominantly secreted by adipose tissue (Cook et al. 1987; White et al. 1992) and macrophages (Xu et al. 2001). Adipsin has been shown to be instrumental in the regulation of systemic inflammation (Jing et al. 1999), systemic energy balance (Gomez-Banoy et al. 2019), and chronological aging (Maity 2024), suggesting that this dual involvement of adipsin, both in innate immunity and metabolic homeostasis, positions it as a crucial link between the complement pathway and metabolic disease pathogenesis.

The liver, an immunologically complex and central metabolic organ produces up to 90% of the fluid-phase complement proteins (Thorgeresen et al. 2019). Adipsin, however, often believed not to be significantly produced nor secreted by hepatocytes. However, immunoblot analysis from primary culture of human hepatocytes (Kitano and Kitamura 2000), and a human hepatocyte cell line, HepG2 (Barnum and Volanakis 1985) exhibited that adipsin had a similar molecular weight compared to the factor D from normal human serum and U937 cells (Barnum and Volanakis 1985). Additionally, recent studies have shown adipsin expression is altered during alcohol associated liver disease (ALD) (McCullough et al. 2018), and metabolic dysfunction-associated liver disease (MASLD) (Pan et al. 2023). These observations highlight multifaceted and is context dependent role of adipsin in liver. Several cross-sectional studies have reported elevated serum adipsin levels in patients with MASLD (Zhao et al. 2022), correlating with metabolic burden and hepatic steatosis severity. However, paradoxically, other studies have shown inverse associations between adipsin levels and NAFLD risk, particularly in obese populations, and this association remained significant after adjusting for multiple metabolic confounders (Zhang et al. 2021). A prospective clinical study of a 3-year follow-up also showed that higher baseline adipsin levels were independently associated with lower odds of NAFLD remission (Gu et al. 2022). Taken together, serum adipsin might serve as a biomarker in MASLD. The tissue-specific regulation of adipsin, particularly within the liver, might have a distinct role that is not completely understood by measuring circulating levels alone.

In this study, we aim to explore the expression pattern of adipsin in both human and mice liver during

various metabolic diseases and to elucidate the molecular mechanisms governing its regulation. Furthermore, we sought to define the functional role of liver-derived adipsin during hepatic metabolic perturbations. Our findings establish that hepatic adipsin is regulated by glucose through eIF2 α - and mTOR-dependent translational control. Functionally, adipsin promotes glucose uptake via GLUT2, and its depletion impairs glycemic control. Together, these data define a glucose–eIF2 α –mTOR–adipsin feedback loop linking nutrient sensing to hepatic glucose metabolism.

Methods

Human subjects

A total of 30 biopsy-proven patients diagnosed with MASLD were recruited for this study. Following participants' written consent, liver biopsies were collected from patients in the Department of Gastroenterology at the Indian Institute of Liver and Digestive Sciences (IILDS), West Bengal, India (IEC reference No. HREC, IILDS/2023-R11). Study participants' inclusion criteria were with or without diabetes, and exclusion criteria included: any liver disease other than MASLD (i.e., alcoholic liver disease, hepatitis B or C, autoimmune hepatitis, hemochromatosis, Wilson disease, or drug-induced hepatitis). Histopathological parameters, including steatosis score, hepatocellular ballooning, fibrosis stage, and NAS, were determined by a single pathologist. Fasting blood samples were collected from the patient for HbA1c, and plasma glucose, insulin, total triglycerides, cholesterol, AST, and ALT were measured. HOMA-IR was calculated as (fasting insulin $\times$ fasting glucose)/22.5; and HOMA- β was calculated as $(20 \times \text{fasting insulin}) / (\text{fasting glucose} - 3.5)$. Plasma and liver adipsin levels were detected by an ELISA kit (R&D Systems) according to the manufacturer's protocol.

Animal experiments

Six- to eight-week-old C57BL/6 male mice were kept in ventilated cages at ambient temperature with a 12:12-h light–dark cycle at 22 ± 1 °C, with unrestrained access to food and water. All experiments and protocols were carried out in accordance with the Institutional Animal Ethics Committee at the CSIR-IICB (approved by the CPCSEA, Ministry of Environment & Forest, and Government of India). After acclimatization, for each experiment, mice were weighed and then randomized accordingly to ensure similar mean body weight between groups. For the Streptozotocin (STZ) experiment, mice were treated with a single intraperitoneal dose of STZ (150 mg/kg of body weight). After the fifth day, subcutaneous insulin (0.75U/kg, Actrapid, Novo Nordisk Pharmaceuticals Ltd., Auckland) was administered twice daily for the next seven days to achieve glycaemic control. Mice

were fed ad libitum with 60% high fat diet (HFD; D12492, Research Diets, Inc., 20 Jules Lane, New Brunswick, NJ 08901 USA) or a chow diet for 16 weeks. A 24 h fasting in mice was performed with total food deprivation and ad libitum access to water from 1200 to 1200 h of the following day, following which one group of mice was refed for 5 h and then sacrificed. For the insulin experiment, mice were fasted for 6 h and then randomly divided into two groups. The insulin-treated groups received 0.75 IU/kg of body weight of insulin and were sacrificed after 30 min.

Adenovirus

For liver-specific knockdown of *Adipsin*, six to eight-week-old male mice (C57BL/6) were intravenously injected with purified shRNA construct or shGFP at a titer of 9.8×10^{12} pfu/mouse, generated by BLOCK-iT Adenoviral Expression System (Invitrogen) using the sequence #5'-CACCGCCTGATGTCTGCATCAAC TCGAAAGTTGATGCAGGACATCAGGC-3'. Mouse CFD-HA adenovirus was purchased from ABM (#159,690,540,200) and used for overexpression of adipsin in cells and mouse liver.

Glucose tolerance test (GTT) and glucose-stimulated insulin secretion (GSIS)

GTT was performed as described by the Mouse Metabolic Phenotyping Centers (MMPC). Briefly, mice were fasted for 6 h with free access to water, and fasting blood glucose levels were measured using a glucometer (SD CodeFree). Mice were then injected with 2 g/kg of glucose, and blood glucose levels were measured at 15, 30, 60, and 120 min after glucose injection. For GSIS, insulin was measured at 0, 30, and 60 min.

Cell culture

Primary hepatocytes were isolated from 6–8-week-old C57BL/6 mice as described (Charni-Natan and Goldstein 2020). Briefly, the liver was perfused with HBSS without calcium, followed by a pre-warmed solution of type I Collagenase (1 mg/mL, Roche) in HBSS with calcium. Cell suspension was centrifuged at 50 g for 2 min at 4 °C to pellet down the parenchymal cells, and seeded onto a collagen-coated tissue culture plate. Human hepatoma cell line HepG2 was cultured in Minimum Essential Medium (HiMedia, India) supplemented with 10% Fetal Bovine Serum (Gibco, USA) and 1% Penicillin–Streptomycin. Cells were treated with L-Leucine (L8000, Sigma Aldrich, USA), Insulin Human Recombinant (#91077C, Sigma Aldrich, USA), Torin 1 (#4247, Tocris Biosciences, USA), Rapamycin (#13,346, Cayman chemicals, USA), and 2-DG (#14,325, Cayman chemicals).

Microsome isolation

HepG2 cells were scraped into ice-cold PBS and centrifuged at 600 g for 5 min at 4 °C. The cells were then resuspended in hypotonic buffer (10 mM HEPES, 1 mM EGTA, 25 mM KCl) and centrifuged at 600 g at 4 °C for 5 min. The cells were resuspended in isotonic buffer (10 mM HEPES, 1 mM EGTA, 25 mM KCl, 250 mM sucrose) and homogenized. Following which centrifugation was performed at 1000 g, 4 °C for 10 min. The supernatant was transferred to a new tube and then centrifuged at 12000 g, 4 °C for 15 min to collect the mitochondrial fraction. The supernatant was then transferred, 7.5 times CaCl_2 (8 mM) was added, and the sample was placed in a slow rotor for 45 min. Centrifugation was then performed at 8000 g at 4 °C for 10 min to collect the microsomes. RNA isolation was then performed from the microsomes.

Quantitative RT-PCR

Total cellular and tissue RNA was isolated using TRIzol reagent (Invitrogen). The iScriptc DNA Synthesis Kit prepared cDNA, and gene expression was quantified by using SYBR green mix (SYBR® Green supermix Bio-Rad) in the Light Cycler 96 real-time PCR System (Roche, Switzerland). The sequences of primers used-Human *Adipsin*: Forward 5'-CTACAGCTGTCGGA GAAG-3', Reverse 5'-CCGCGTGGTTGACTATG-3'; Mouse *Adipsin*: Forward 5'-GGTATGATGTGCAGA GTGTAG-3', Reverse: 5'-GGTTCCACTTCTTTGTC CTC-3'; Mouse *Pck1*: Forward 5'-GAAGAGGACTTT GAGAA -3', Reverse: 5'- GTCAGTTCAATACCAATC -3'; Mouse *Creb1*: Forward 5'-CTCCCACTGTAACT TAGT-3', Reverse: 5'-CTGTTTGGACTTGTGGAG-3'; Mouse *Fbp1*: Forward 5'-CAAAGCCATCTCGTCTG-3', Reverse: 5'-GTATGTCCAGCTTCTTTACT-3'; Mouse *Fbp2*: 5'-GAATGTGACAGGAGATGAG-3', Reverse 5'-C ACCGCTCTTTATTCTC-3'; Mouse *G6pc1*: Forward 5'-CTGTGGGCATCAATCTC-3' Reverse 5'-GCTGTTG CTGTAGTAGTC-3'; Mouse *G6pc3*: Forward 5'-GGCT CAACCTTGCTTTC-3', Reverse 5'-AGGGAAGTGGTG AATCT-3'; Mouse *Mdh1*: Forward 5'-ACGGACAAAGA AGAGATTG-3', Reverse 5'-TCACATTGGCTTTTCAGTA G-3'; Mouse *Mdh2*: Forward 5'-CCAGATTGCCTCAAA GG-3', Reverse 5'-GTAGCGTTGGTGTGAA-3'; Mouse *Pck1*: Forward 5'-CGCAAGCTGAAGAAATAG-3', Reverse 5'-GCTCTTGGGTGATGATG-3'; Mouse *Pck2*: Forward 5'-CTGAGAACTGCCATAC-3', Reverse: 5'- ATCACCGTCTTGCTTTC-3'; Mouse *Pfkfb1*: Forward 5'-GTGAGCTACAGGAAGTATG-3', Reverse 5'- CACG GCTGAGATACTTATG-3'; Mouse *Ppara*: Forward 5'-A CCTGGAAAGTCCCTTAT-3', Reverse 5'-TCCTAAGTA CTGGTAGTCTG-3'; Human RN 18S: Forward 5'-GTAA CCCGTTGAACCCCAT-3', Reverse 5'-CCATCCAATC GGTAGTAGCG-3'. Mouse RN 18S: Forward 5'-GTTGG

TTTTCGGAAGTGGAGG-3', Reverse: 5'- GTAGCCACC ACCTCCCAGTA-3'.

Immunoblotting

Cellular and liver protein extracts in lysis buffer [50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and 1% Triton X-100 along with protease- and phosphatase inhibitor cocktail (Roche, Basel, Switzerland)] was resolved using SDS-PAGE followed by transfer to PVDF Membrane (Immobilon-P, Merck Life Sciences, NJ, USA). Membranes were incubated overnight with primary antibodies, followed by incubation with peroxidase-conjugated secondary antibodies, and the bands were visualized with a ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA). Following primary antibodies were used: Human Adipsin (#A21110), Mouse Adipsin (#A8117), PCK1 (#A22172) was purchased from ABClonal (MA, USA), pS6 Ribosomal Protein (#4858), S6 Ribosomal Protein (#2217), Raptor (#2280), Rictor (#2114), pAKT (#4060), AKT (#4691), pEIF2 α (#3398), eIF2 α (#5324), p4E-BP1 (#2855), 4E-BP1 (#9644), BiP (#3183), GAPDH (#2118) were purchased from Cell Signalling Technology (Minneapolis, USA). β -actin (#A2228) was purchased from Sigma-Aldrich (St. Louis, Missouri, United States).

Histology, immunohistochemistry (IHC), Periodic Acid-Schiff stain (PAS)

The liver tissue was excised immediately and immersed in 10% formalin. The tissue blocks were then sectioned using a rotary microtome with a thickness of 5 μ m. Deparaffinization was performed by baking the tissue section at 80 °C for 20 min and then rehydrating and processing for hematoxylin & eosin (H&E), and PAS, while for IHC it was followed by antigen retrieval in Tri-sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0). The slides were incubated with the primary antibody, then with a diluted biotinylated secondary antibody, and stained with anti-rabbit VECTASTAIN ABC KIT.

5'UTR construct, transfection, and Luciferase assay

Secondary structures of human and mouse adipsin 5'UTR were predicted by using the RNAfold webserver (Gruber et al. 2008). To obtain Firefly Luciferase (FL) expression from a CMV promoter, the CMV promoter region was amplified by using *KpnI*-*NheI* sites (*KpnI*: 5'-GGGGTACCGACATTGATTATTGACTAGT-3'; *NheI*: 5'-CGGCTAGCAGCTCTGCTTATATAGACCT-3') from pcDNA3.1 myc-His B plasmid. It was then inserted into the pGL3-Basic plasmid to get pGL3-CMV-FL. To generate the 5'UTR of *Adipsin* mRNA, cDNA from mice primary hepatocytes was amplified (pre-incubation:

95 °C 10 min, denaturation at 95 °C for 10 s, annealing at 64.5 °C for 30 s, extension at 72 °C for 45 s for 30 cycles) using *NheI*-*HindIII* sites (*NheI*: 5'-CTAGCTAGCAGG GGCAGGAGGTAAG AG -3' and *HindIII*: 5'-CCCAA GCTTTCTGACAGCAGGCACTG -3'). The amplified product was then inserted downstream of CMV in pGL3-FL-CMV to generate pGL3-CMV-FL-5'UTR. Constructs were transfected in HEK293T cells using Lipofectamine™ 2000 Transfection Reagent (Thermo Fisher Scientific, MA, USA) for 48 h. Luciferase activity was detected with the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol.

Glucose uptake assay

Glucose uptake assay was performed by treating the HepG2 or AML12 cells with 100 μ M of 2-NBDG (2-(N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2-deoxyglucose) (Invitrogen) for 20 min. The cells were then lysed, and relative fluorescence was measured using a fluorimeter (BioTek Synergy H1 Multimode Reader, Agilent) at an excitation wavelength of 475 nm and an emission wavelength of 550 nm.

Cycloheximide (CHX) Chase assay

The HepG2 cells were treated with either 50 μ g/ml of cycloheximide (Calbiochem, Merck Millipore, MA, USA) alone or in combination with torin 1 and glucose for the required time periods. Cells were then harvested in protein lysis buffer at different time points for Western blotting.

In vitro translation

In vitro translation assay was performed according to the manufacturer's protocol (PROMEGA Rabbit Reticulocyte Lysate System, Nuclease Treated, L4960). Briefly, total cellular RNA was isolated and added to the reticulocyte lysate with amino acids at a final concentration of 200 μ g/mL. In vitro translation was then performed by keeping the tubes at 30 °C for 90 min. Immunoblotting was performed to detect protein synthesis.

Biochemical tests and liver enzymes

Blood samples were centrifuged at 2000 g for 10 min, and plasma/serum were assayed using total triglycerides, cholesterol, glucose, AST, and ALT reagent kit (TR2774, CH2773, GL2797; AS1204, AL2780; Randox Laboratories Limited, UK) and were measured in a semi-auto analyzer (Microlab 300; ELITech Group, France). Human and mouse adipsin ELISA kit (R&D Systems, Cat: DY5430, DY1824) and insulin ELISA (Millipore Cat: EZHI, EZRMI) were performed using the manufacturer's protocol.

RNA interference (RNAi)

siRNA transfection was carried out using Lipofectamine TM RNAiMax Transfection Reagent (Thermo Fisher Scientific, MA, USA) for 72 h. Human Raptor siRNA SMARTPOOL (Cat No.L-004107-00-0005) and Human Rictor siRNA SMARTPOOL (Cat No.L-016984-00-0005) were purchased from Dharmacon, Horizon Discovery (Waterbeach, UK).

Statistics

Unless otherwise stated, data are presented in mean ± SD. Statistical analysis was carried out using GraphPad Prism 8 and RStudio 8.0.2 (263) and RStudio Version 4.3.3. Unpaired t-test with Welch correction and ANOVA were used as appropriate, followed by Bonferroni post-hoc test. Differences were considered significant at $p < 0.05$.

Results

Adipsin protein expression is increased in the livers of T2D patients and diabetes mouse models

Human hepatocytes express adipsin (Barnum and Volanakis 1985 Barnum and Volanakis 1985); however, the factors regulating hepatic adipsin expression are unknown. We assessed adipsin protein and mRNA expressions in consecutive liver biopsy samples from MASLD patients with (n=10) or without (n=20) diabetes. NAS, fibrosis scores, and BMI were comparable between the groups, whereas glycemic parameters such as FBS and HbA1c were expectedly higher in patients with diabetes (Table 1). Although we did not find a difference in *Adipsin* gene expressions (Fig. 1A), protein levels determined by ELISA of tissue homogenate were significantly higher in diabetes patient livers (Fig. 1B).

Table 1 Baseline clinical and biochemical characteristics of study participants

	Non-Diabetes (n=20)	Diabetes (n=10)	P-value
Sex (Male/Female)	11/9	7/3	0.4497
Age (Years)	38.93 ± 10.86	41.55 ± 6.55	0.3320
BMI (kg/m ²)	28.54 ± 5.01	31.65 ± 5.26	0.1200
Fasting Glucose (mmol/L)	5.38 ± 0.73	6.32 ± 1.41	0.0402
HbA1c (%)	5.52 ± 0.41	7.15 ± 0.66	< 0.0001
Fasting Insulin (µIU/mL)	11.76 ± 4.33	15.42 ± 7.95	0.2230
ALT (U/L)	70.07 ± 33.11	68.5 ± 35.85	0.9500
AST (U/L)	56.99 ± 25.39	52.55 ± 22.85	0.6170
GGT (U/L)	34.58 ± 27.73	46 ± 31.84	0.1173
Total Triglycerides (mg/dL)	176.04 ± 81.99	201.4 ± 63.16	0.1280
Total Cholesterol (mg/dL)	187.65 ± 45.79	187.65 ± 45.79	0.6640
NAFLD Activity Score	2.58 ± 1.47	1.9 ± 1.2	0.1350
Steatosis score	1.58 ± 0.9	1.2 ± 0.79	0.1610
Fibrosis score	1.23 ± 1.21	1.7 ± 1.42	0.3300
HOMA-IR	3.43 ± 1.39	5.53 ± 3.74	0.1970
HOMA-B	138.56 ± 59.92	115.38 ± 58.35	0.2970

Adipsin protein identified by immunohistochemistry also revealed enhanced expression in diabetes patients (Fig. 1C). Interestingly, pericentral stain was more prominent and Kupffer cells had the maximum intensity. Next, we performed correlation analysis to identify how liver adipsin is associated with the parameters of glycemia and MASLD. Only HbA1c was found to be significantly correlated with adipsin protein levels ($r = 0.53$, $p = 0.003$), suggesting long-term hyperglycemia could induce hepatic adipsin (Fig. 1D). In contrast to liver adipsin, we did not find a significant difference in plasma adipsin levels between the groups (Fig. 1E). We next examined whether hyperglycemia and its therapy could modulate liver adipsin levels in experimental animal models of T1D and T2D. First, we used streptozotocin (STZ) to induce T1D, followed by insulin treatment (0.75U/kg) for seven days (Begum and Draznin 1992) (Fig. 1F). Whilst STZ caused rapid hyperglycemia, insulin therapy normalized it (Fig. 1G). Body and liver weight, and serum adipsin levels were unaltered by insulin treatment (Fig. 1H-J). *Adipsin* gene expression in liver showed no significant change following STZ or insulin administration (Fig. 1K). In contrast, adipsin protein levels were intriguingly enhanced following STZ injection, and was markedly diminished by insulin treatment (Fig. 1L). IHC also revealed a consistent pattern of adipsin expression (Fig. 1M). In a separate experimental design, a T2D model was established through high fat diet (HFD) feeding for 12 weeks (Supplementary Fig. 1A). HFD-fed mice exhibited increased body weight, hepatic mass, and epididymal adipose tissue mass, accompanied by elevated serum triglycerides, cholesterol, and hepatic transaminases (AST and ALT) (Supplementary Fig. 1B-H). Fasting hyperglycemia and elevated plasma insulin concentrations in HFD-fed mice confirmed the development of insulin resistance (Fig. 2N, O). Notably, these mice also displayed increased circulating adipsin levels (Fig. 1P). Hepatic *Adipsin* mRNA expression in these animals was variable and did not show a consistent trend (Fig. 1Q); however, hepatic adipsin protein content was significantly increased (Fig. 1R,S). Collectively, these data indicate that hyperglycemia per se induces upregulation of hepatic adipsin protein expression in murine models of diabetes.

Fasting refeeding cycle modulates adipsin protein levels in mice liver

We investigated whether postprandial elevations in blood glucose exert a similar regulatory effect on hepatic adipsin expression. For this purpose, mice were subjected to a 24 h fasting period (Jensen et al. 2013), followed by refeeding with a standard chow diet for 5 h (Fig. 2A). As anticipated, refeeding resulted in a pronounced increase in circulating glucose and insulin concentrations (Fig. 2B, C), accompanied by a modest elevation in

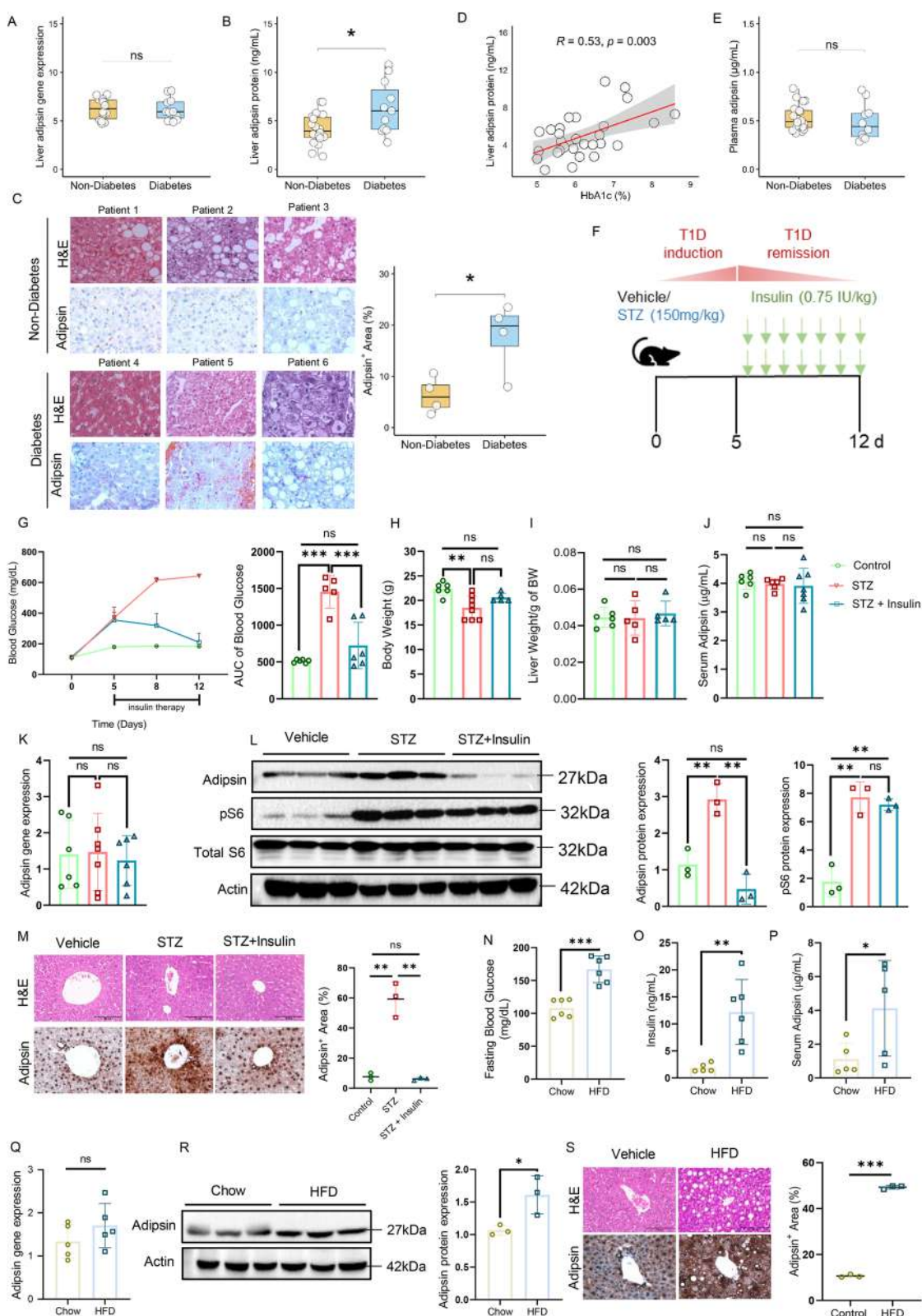


Fig. 1 (See legend on next page.)

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Fig. 1 Hepatic adipsin protein expression is elevated in T2D patients, murine models of diabetes and correlates with chronic hyperglycemia. **A** Hepatic *ADIPSIN* mRNA expression in non-diabetes ($n = 10$) and diabetes ($n = 20$) MASLD patients. **B** Adipsin protein levels in liver homogenates from MASLD patients with or without diabetes. **C** Left panels: H&E, immunohistochemistry of adipsin in independent liver biopsy. Right panels: Hepatic adipsin positive area (%) was quantified using ImageJ software ($n = 4$ /group, 6 fields/sample). **D** Correlation between hepatic adipsin protein and HbA1C. **E** Plasma adipsin concentrations in non-diabetes and diabetes patients. **F** Experimental design for STZ-induced type 1 diabetes (T1D) followed by glycemic correction using insulin treatment. **G** Blood glucose levels and corresponding area under the curve (AUC). **H–J** Body weight, liver weight, and serum adipsin concentrations. **K** Hepatic Adipsin mRNA expression in control, STZ-treated, and STZ plus insulin-treated mice. **L** Immunoblot of adipsin, phosphorylated S6 (pS6), and total S6 in liver homogenates. Right panel: Densitometric analysis of adipsin and pS6 ($n = 3$). **M** H&E, immunohistochemistry of adipsin on liver sections. Hepatic adipsin positive area (%) quantified ($n = 3$ /group, 6 fields/sample). **N–O** Fasting blood glucose and plasma insulin concentrations in the high fat diet (HFD)-induced type 2 diabetes (T2D) model. **P** Circulating adipsin concentrations in HFD-fed mice. **Q** Hepatic Adipsin mRNA expression. **R** Immunoblot of adipsin from liver homogenates. Bottom panel: Densitometric analysis of adipsin ($n = 3$). **S** H&E, immunohistochemistry of adipsin on liver sections. Mice $n = 6$ (control), $n = 8$ (STZ), $n = 7$ (STZ + insulin), $n = 6$ (Chow), $n = 6$ (HFD). Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, statistical significance was determined using the Mann–Whitney U test, Spearman correlation analysis was performed to assess associations between variables, and unpaired two-tailed t-test or one-way ANOVA followed by Bonferroni's multiple comparison test, as appropriate. ns, not significant; GGT, γ -glutamyl transferase; STZ, streptozotocin; HFD, High Fat Diet; BW, body weight. Scale bar, 100 μ m

liver mass, without appreciable changes in body weight or epididymal adipose tissue weight (Fig. 2D–F). Hepatic expression of the rate-limiting gluconeogenic gene *Pck-1* was markedly suppressed under the re-fed condition, whereas *Adipsin* mRNA abundance remained unaltered (Fig. 2G, H). Plasma triglycerides level increased while plasma adipsin concentrations were unaffected by the fasting–refeeding transition (Fig. 2I, J). Immunoblotting further revealed a striking drop in hepatic PCK1 protein in the re-fed state, in contrast to a significant elevation in hepatic adipsin protein levels (Fig. 2K). Consistently, histological and immunohistochemical analyses showed a significant increase in both adipsin levels and glycogen content in the fed state (Fig. 2L). Collectively, these findings indicate that hepatic adipsin protein abundance is increased not only under pathological hyperglycemia associated with diabetes but also during physiological fluctuations in glucose availability.

Glucose controls adipsin protein translation via eIF2 α and mTOR

Because hyperglycemia increased hepatic adipsin expression, whereas adipsin depletion impaired glucose tolerance, we next asked how glucose availability regulates adipsin production. Given that glucose deprivation induced eIF2 α phosphorylation and suppressed mTOR signalling (Muaddi et al. 2010; Roberson et al. 2023) (Fig. 3A), suggesting that adipsin expression might be controlled at the level of translation through coordinated regulation of these nutrient-sensing pathways. To formally test whether adipsin is subject to cell-autonomous translational control, primary hepatocytes and HepG2 cells were transiently deprived of media and subsequently re-exposed to increasing concentrations of glucose. Media withdrawal led to a rapid decline in adipsin protein levels (Fig. 3B; Supplementary Fig. 2A), which were restored in a dose-dependent manner upon glucose reintroduction (Fig. 3C; Supplementary Fig. 2B, D), without changes in mRNA abundance (Supplementary Fig. 2C), indicating post-transcriptional regulation. Consistently,

glucose deprivation enhanced eIF2 α phosphorylation and reduced mTORC1 activity, as evidenced by diminished 4E-BP1 phosphorylation (Fig. 3B). Glucose supplementation reversed these effects, suppressing eIF2 α phosphorylation while augmenting 4E-BP1 phosphorylation (Fig. 3C). Adipsin abundance increased in a concentration-dependent manner and approached a plateau at higher glucose concentrations, with a best-fit EC50 of approximately 4.27 mM glucose ($R^2 = 0.84$) (Fig. 3D). To determine whether glucose metabolism is required for adipsin induction, cells were pre-treated with 2-deoxy-D-glucose (2-DG), a glycolytic inhibitor. 2-DG markedly attenuated glucose-induced adipsin protein expression, concomitant with increased eIF2 α phosphorylation and reduced 4E-BP1 phosphorylation (Fig. 3D), demonstrating that metabolic flux, rather than glucose sensing alone is necessary. Given that insulin activates the AKT–mTOR axis, we next assessed whether insulin alone is sufficient to induce adipsin. Interestingly, insulin treatment in primary hepatocytes did not increase adipsin expression (Supplementary Fig. 2D). Although insulin robustly activated AKT and mTOR signaling in the absence of glucose, it failed to restore adipsin protein expression (Fig. 3E). Notably, insulin did not suppress eIF2 α phosphorylation under glucose deprivation. In vivo, insulin administration under fasting conditions reduced blood glucose (Fig. 3F), and activated hepatic AKT–mTOR signaling without altering eIF2 α phosphorylation or adipsin expression (Fig. 3G). Similarly, in STZ-diabetic mice, restoration of blood glucose by insulin treatment (Fig. 1G) caused an increase in eIF2 α phosphorylation (Supplementary Fig. 2G). Collectively, these data demonstrate that glucose availability is both necessary and sufficient to induce hepatocyte adipsin expression through coordinated regulation of the eIF2 α and eIF4E translational nodes. In contrast, insulin selectively activates the mTOR arm but is insufficient to drive adipsin synthesis in the absence of glucose, underscoring a dominant role for metabolic control of translation in governing hepatocytes derived adipsin expression.

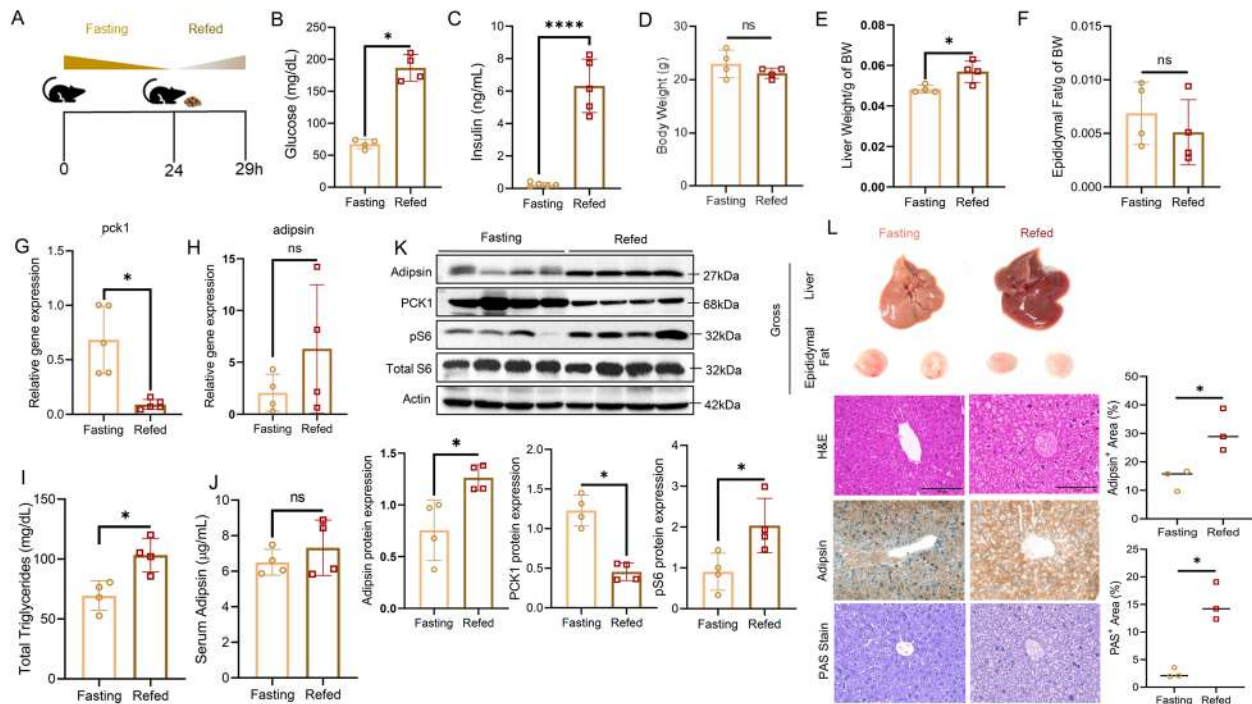


Fig. 2 Regulation of hepatic adipsin expression in fasting-refeeding cycle. **A** Experimental design of 24-h fasting followed by 5-h refeeding. **B–C** Plasma glucose and insulin concentrations during fasting and refeeding. **D–F** Body weight, liver weight, and epididymal adipose tissue weight during fasting and refeeding. **G–H** Hepatic adipsin and *Pck1* mRNA expression during fasting and refeeding. **I–J** Plasma triglyceride and circulating adipsin concentrations during fasting and refeeding. **K** Immunoblots of hepatic adipsin and PCK1 in liver homogenates. Bottom panels: Densitometric analysis of adipsin, PCK1 and pS6 levels ($n=4$). **L** Gross appearance of liver and epididymal adipose tissue depot. H&E, immunohistochemistry and Periodic Acid-Schiff (PAS) on liver sections. For immunohistochemistry, hepatic adipsin-positive area (%) was quantified from representative sections ($n=3$ mice per group, 6 fields per sample). Mice $n=8$ (fasting), $n=8$ (refed)). Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, statistical significance was determined using unpaired two-tailed t-test. ns, not significant; BW, body weight Scale bar, 100 μ m

Structured 5'UTR mediates mTOR-dependent, glucose-responsive translation of hepatocyte derived adipsin

Given the central role of mTOR in nutrient sensing (Simcox and Lamming 2022; Sabatini 2017), we hypothesised that it might be involved in the regulation of hepatocyte derived adipsin protein synthesis. Pharmacological inhibition of mTOR by torin 1 attenuated the glucose-induced adipsin protein expression in hepatocytes, with unaltered mRNA levels (Fig. 4A; Supplementary Fig. 2E, F). Treatment of rapamycin, a selective mTORC1 inhibitor, also suppressed glucose induced adipsin expression (Fig. 4B). Interestingly, glucose stimulation suppressed eIF2 α phosphorylation even in presence of torin 1 and rapamycin and blockade of the mTOR arm of translational regulation was sufficient to inhibit adipsin expression (Fig. 4A, B). Glucose mediated induction of adipsin was further augmented by the concurrent activation of mTORC1 with amino acid leucine (Fig. 4C). Consistently, silencing the key mTOR complex components by siRNA against Raptor and Rictor blunted the glucose-mediated induction of adipsin protein levels (Fig. 4D). Taken together, our results strongly indicate that mTOR mediates the glucose-dependent protein expression in hepatocytes.

To investigate the mechanisms by which mTOR signaling enhances adipsin protein expression, we first assessed the stability of the adipsin protein following mTOR inhibition. Cycloheximide (CHX) chase experiments revealed that neither torin 1 nor glucose treatment significantly altered adipsin protein stability (Fig. 4E, F), indicating that glucose regulates adipsin levels through a distinct mechanism. Given that secretory proteins such as adipsin are synthesized on the endoplasmic reticulum (ER)-associated ribosome for subsequent trafficking via Golgi networks (Brumfield 2017; Chakrabarti et al. 2008), we hypothesized that glucose may influence adipsin translation by modulating the association of its mRNA with ER-bound ribosomes. To test this, we isolated cellular microsomal fractions and quantified *ADIPSIN* mRNA levels (Fig. 6G). Glucose deprivation led to an increased enrichment of *ADIPSIN* mRNA in the microsomal fraction, an effect reversed by glucose repletion. Notably, this glucose-dependent reduction in microsomal *ADIPSIN* mRNA was blocked by both torin 1 (Fig. 6H), indicating that inhibition of mTOR stalls *ADIPSIN* mRNA in the microsomal fraction, rendering a slower rate of translation. mTOR signaling is known to promote translation of mRNAs with structured 5' untranslated regions (5'UTRs)

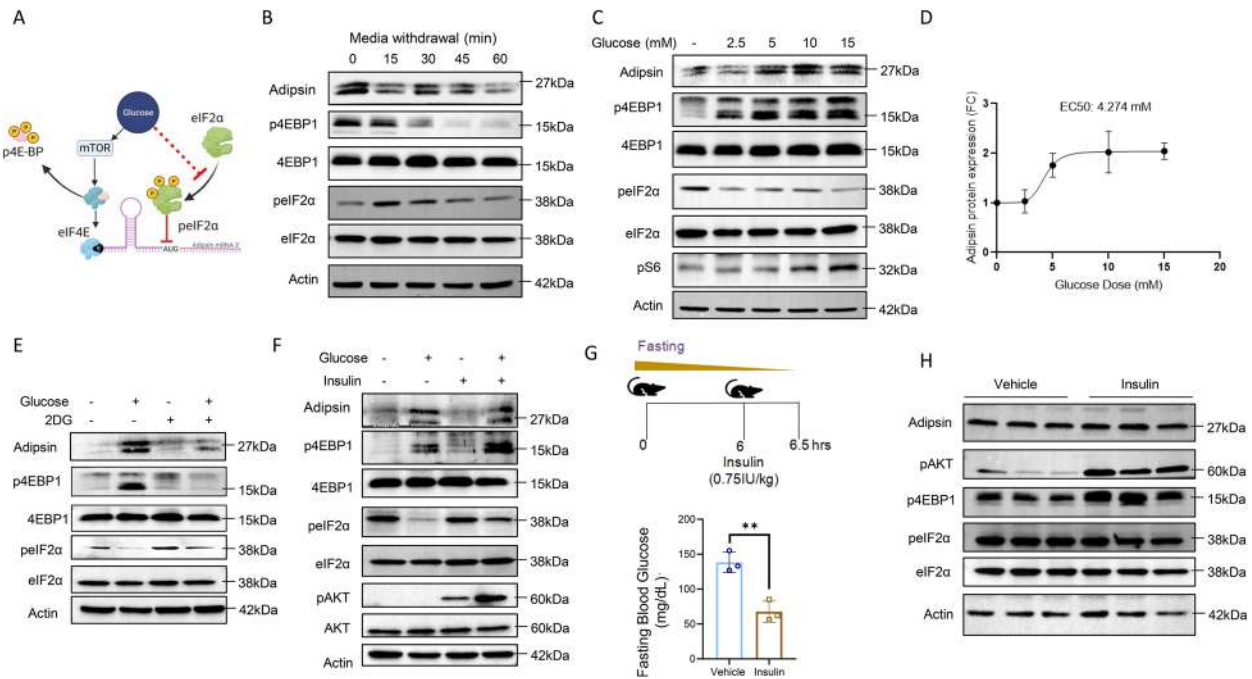


Fig. 3 Glucose regulates hepatic adipsin protein translation via eIF2 α and mTOR. **A** Experimental hypothesis illustrating that glucose availability is required for hepatic adipsin protein expression through coordinated regulation of pelfF2 α and eIF4E-dependent translation. **B–C** Immunoblots from HepG2 cells following media withdrawal for indicated time points (**B**), and after glucose re-addition in a dose dependent manner (**C**). HepG2 cells were starved in HBSS for 6 h prior to treatments. **D** Densitometric analysis of adipsin for different concentrations of glucose for determination of EC50. **E** Cells were pre-treated with glycolytic inhibitor 2-deoxy-D-glucose (2-DG) for 30 min. **F** Cells were treated with insulin for 30 min followed by glucose for 30 min to assess induction of adipsin protein expression. **G** Experimental design of mice: 6 h fasting followed by insulin (0.5 IU/kg) treatment for 30 min. Plasma glucose from tail vein blood in control and insulin treated mice. **H** Immunoblots of adipsin, phosphorylated eIF2 α (pelfF2 α), total eIF2 α , phosphorylated 4EBP1 (p4EBP1), total 4EBP1, pS6, phosphorylated AKT (pAKT) and total AKT in mice liver lysates. **H** Immunoblots of pelfF2 α , total eIF2 α from vehicle, STZ and STZ + Insulin-treated mice liver lysates. Mice n=6 (control), n=6 (insulin). Data are represented as mean $\pm$ SD. * P < 0.05, ** P < 0.01, *** P < 0.001, statistical significance was determined using unpaired two-tailed t-test or one-way ANOVA followed by Bonferroni's multiple-comparison test as appropriate. ns, not significant

by regulating the translation initiation machinery (Benham 2012). Consistent with this, RNA secondary structure prediction revealed that the 5'UTR of both murine and human *ADIPSIN* mRNAs forms a stable stem-loop structure ($\Delta G = -25$ kcal/mol, and $\Delta G = -5.74$ kcal/mol) (Hofacker 2003) (Fig. 4I). To directly assess the functional role of the adipsin 5'UTR in translation, we generated a luciferase reporter construct containing the adipsin 5'UTR upstream of the firefly luciferase coding sequence (Fig. 4I). Expression of this construct led to increased luciferase activity in an mTOR-sensitive manner (Fig. 4J). Furthermore, glucose treatment enhanced luciferase activity, whereas torin 1 treatment attenuated the glucose-induced response (Fig. 4K), supporting the notion that mTOR activation facilitates adipsin translation via its 5'UTR. Finally, we validated these findings in a cell-free translation system. Consistent with cellular data, glucose addition enhanced in vitro translation of adipsin, whereas torin 1 suppressed this effect (Fig. 4L). Together, these results indicate that mTOR promotes adipsin protein synthesis by enhancing translation of its structured 5'UTR in a glucose-dependent manner.

mTOR inhibition attenuates feeding-induced hepatic adipsin protein expression

We next sought to determine whether in vivo inhibition of mTOR affects hepatic adipsin expression during the fasting refeeding cycle. To that end, mice were fasted for 12 h and subsequently administered with torin 1 (10 mg/kg body weight, intraperitoneal) for 4 h (Liu et al. 2012). A subset of mice was refed during the final hour of this period (Fig. 5A). As expected, torin 1-treated animals exhibited elevated fasting blood glucose levels compared to controls in both fasted and refed conditions, accompanied by a concurrent increase in circulating insulin levels (Fig. 5B, C). Western blot analysis revealed that hepatic adipsin protein levels were markedly reduced in both fasted and refed groups following torin 1 treatment, paralleling decreased phosphorylation of p4EBP1, a downstream target of mTORC1 (Fig. 5D). *Adipsin* mRNA levels were expected to remain unaltered (Fig. 5E). Immunohistochemical analysis further confirmed the reduction in hepatic adipsin protein expression (Fig. 5F). We also assessed circulating adipsin levels and found a significant decrease in serum adipsin concentrations

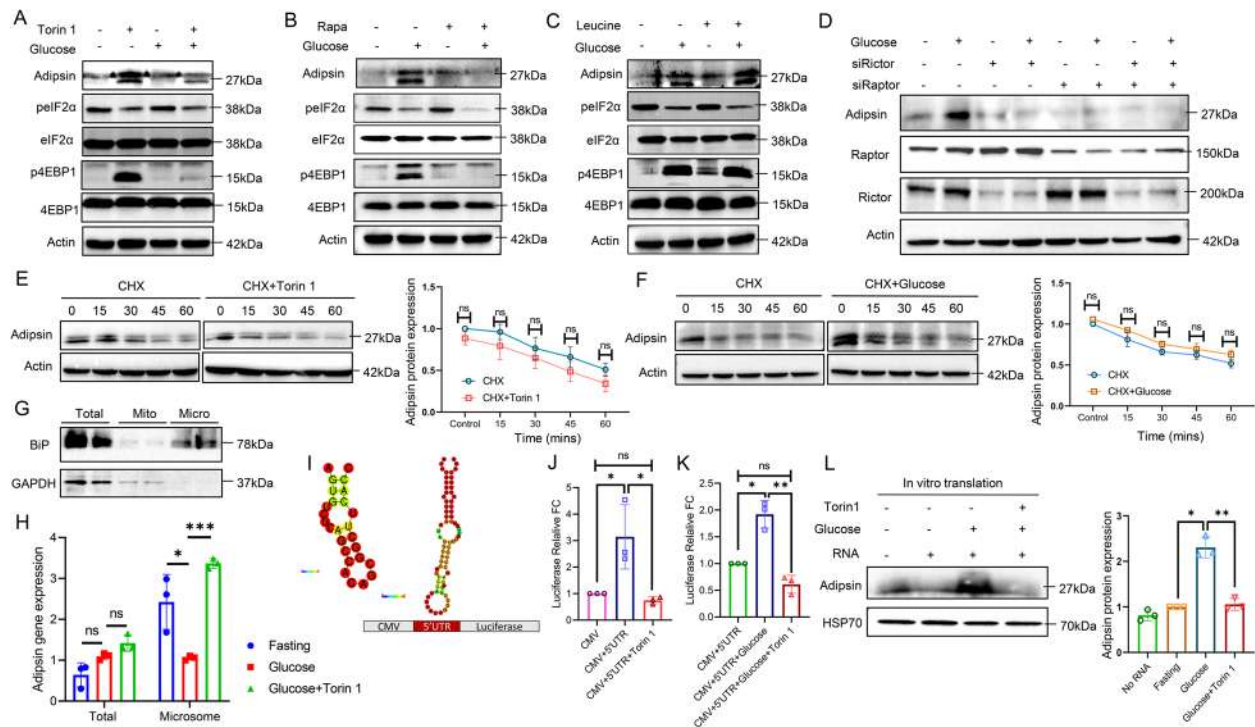


Fig. 4 mTOR promotes adipsin translation through its structured 5'UTR in a glucose-dependent manner. **A–C** HepG2 cells were starved in HBSS for 6 h prior to treatments. Cells were pre-treated with mTOR inhibitor Torin 1 (**A**), rapamycin (Rapa) (**B**), mTOR activator Leucine (**C**) for 30 min, followed by addition of glucose for the next 30 min to access the induction of adipsin protein expression. **D** siRNA-mediated knockdown of mTOR complex components (Raptor and Rictor) on glucose-induced adipsin protein expression. **E–F** Immunoblot analysis of HepG2 cells for adipsin protein stability assessed by cycloheximide (CHX) chase assay in the presence or absence of torin 1 (**E**), and glucose (**F**). **G** Mitochondrial and microsomal fractions from HepG2 cell lysates were isolated by differential centrifugation. Purity of microsome isolation determined by ER resident BiP by immunoblotting. **H** Quantification of *ADIPSIN* mRNA expressions in total cellular and microsomal fraction under glucose deprivation, glucose stimulation, and glucose stimulation with torin 1. **I** Predicted secondary structures of murine and human 5'UTR of adipsin mRNA, and schematic of the firefly luciferase reporter construct containing the CMV promoter. **J** HEK293T cells were transfected with luciferase reporter constructs with and without 5'UTR of *ADIPSIN* mRNA. After 48 h, cells were treated with torin 1 and cellular luciferase activity was determined. **K** Luciferase activity in response to glucose stimulation with and without torin 1 treatment. **L** Immunoblot from a cell-free translation assay showing adipsin protein synthesis in the presence or absence of glucose and torin 1. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, statistical significance was determined using unpaired two-tailed t-test or one-way ANOVA followed by Bonferroni's multiple-comparison test, as appropriate. ns, not significant; CMV, cytomegalovirus

in torin1-treated mice, suggesting that mTOR inhibition suppresses adipsin production in both hepatic and adipose tissues (Fig. 5G). Importantly, no significant differences were observed in body weight, liver weight, or adipose tissue mass across treatment groups (Fig. 5H, I). Collectively, these findings demonstrate that hepatic adipsin protein expression is positively regulated by mTOR signaling during the postprandial phase, primarily through translational mechanisms rather than transcriptional changes.

Depletion of adipsin in the mouse liver promotes glucose intolerance

To investigate the physiological role of hepatic adipsin in metabolic homeostasis, we selectively silenced its expression in the liver using adenovirus-mediated shRNA (Supplementary Fig. 3A, B). As expected, *Adipsin* mRNA expression (Fig. 6A) was significantly lower in the adenovirus-mediated shAdipsin group compared

to the shGFP. Consistently, histological and immunohistochemical analyses showed a significant downregulation in adipsin levels (Supplementary Fig. 3C). We first examined whether hepatic adipsin depletion affects basal metabolic parameters. Interestingly, hepatic adipsin knockdown did not alter fasting blood glucose levels and circulating insulin concentrations (Fig. 6B, C). However, glucose tolerance test (GTT) revealed a markedly reduced glucose clearance in the shAdipsin group, as reflected by a higher area under the curve (AUC) compared with shGFP-treated mice (Fig. 6D). To determine whether this impaired glucose tolerance is due to defects in insulin secretion, we performed glucose-stimulated insulin secretion (GSIS). Despite the known role of adipsin in improving β -cell function (Lo et al. 2014; Gomez-Banoy et al. 2019), insulin secretion in response to glucose challenge was not altered in the shAdipsin group (Fig. 6E). We next assessed insulin responsiveness, and found that insulin-dependent glucose lowering

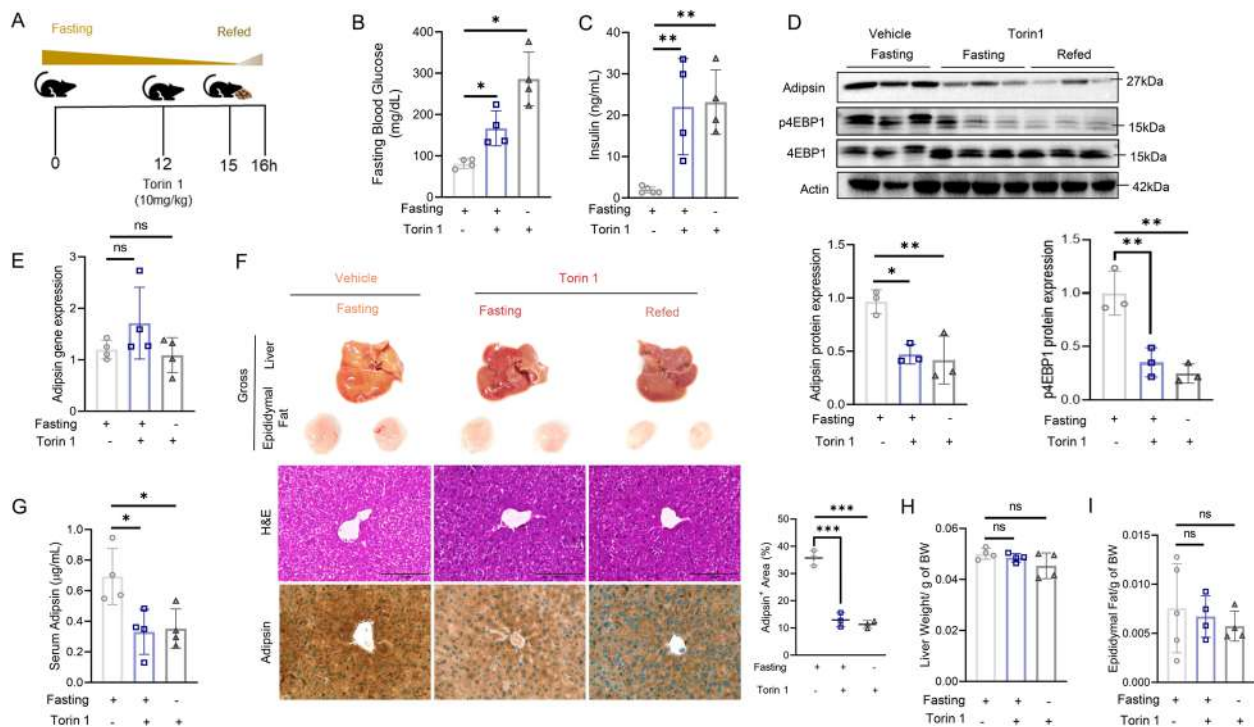


Fig. 5 Pharmacological inhibition of mTOR suppresses hepatic adipsin expression during the fasting–re-feeding cycle. **A** Experimental design depicting 12 h fasting followed by 4 h treatment with torin 1 (10 mg/kg, intraperitoneal), with a subset of mice refed during the final hour. **B–C** Plasma glucose and insulin concentrations in vehicle- and torin 1-treated mice under fasted and refed conditions. **D** Immunoblot analysis of hepatic adipsin and phosphorylated S6 (pS6) protein levels in response to torin1 treatment. **E** Quantification of hepatic *Adipsin* mRNA expression following torin 1 treatment. **F** Gross liver and epididymal adipose tissue, and liver sections for H&E, immunohistochemistry of adipsin. Adipsin positive area (%) was quantified (n = 3/group, 6 fields/sample). **G** Serum adipsin concentrations in vehicle and torin 1-treated mice. **H–I** Body weight, liver weight, and epididymal adipose tissue depot across experimental groups. Mice n=6(vehicle), n=6(torin 1, fasting), n=6 (torin1, refed). Data are presented as mean $\pm$ SD. * P < 0.05, ** P < 0.01, *** P < 0.001, statistical significance was determined using one-way ANOVA followed by Bonferroni's multiple-comparison test. Scale bar, 100 μ m. ns, not significant

and downstream signaling remained unchanged (Fig. 6F, Supplementary Fig. 3D). We further evaluated whether changes in body composition or lipid metabolism could explain the observed phenotype. Body weight, liver weight, epididymal adipose tissue, and total triglyceride levels were comparable between groups (Supplementary Fig. 3E–H), indicating that the impaired glucose tolerance is not due to alterations in overall metabolic status. Since hepatic glucose production is a major determinant of systemic glucose levels (Sharabi et al. 2015), we next examined gluconeogenesis. We observed that expression of gluconeogenic genes and proteins remained unchanged (Supplementary Figs. 3I and 3J), suggesting that hepatic glucose output is not affected by adipsin depletion. Having excluded alterations in insulin secretion, insulin sensitivity, body composition, and gluconeogenesis, we hypothesized that impaired hepatic glucose uptake may underlie the observed phenotype, and examined the hepatic glucose transporters. Expressions of glucose transporters, specifically glucose transporter type 2 (GLUT2), were significantly downregulated upon adipsin knockdown (Fig. 6G, H). Consistently, in vitro glucose

uptake assays showed a marked reduction in glucose uptake in both HepG2 (1.4-fold) and AML12 (4.5-fold) hepatocytes, indicating a cell-autonomous effect. Adipsin silencing in AML12 cells reduced *Glut2* mRNA and protein levels. Conversely, overexpression of adipsin in AML12 cells enhanced glucose uptake (Supplementary Fig. 4A), and increased the protein levels of adipsin and GLUT2 (Supplementary Fig. 4B). To further validate these findings in vivo, we overexpressed hepatic adipsin in mice (Supplementary Fig. 4C). As expected, hepatic adipsin expression was markedly elevated, accompanied by increased circulating adipsin levels. Consistent with our in vitro observations, hepatic GLUT2 protein levels were also increased in adipsin-overexpressing mice (Supplementary Fig. 4D, E). We next assessed metabolic parameters and found no significant changes in fasting blood glucose, circulating insulin levels, body weight, liver weight, total triglycerides, or cholesterol levels between groups (Supplementary Fig. 4F–K). Interestingly, GTT, as reflected by a higher area under the curve (AUC) compared with Ad-eGFP-treated mice, revealed significantly improved glucose clearance in

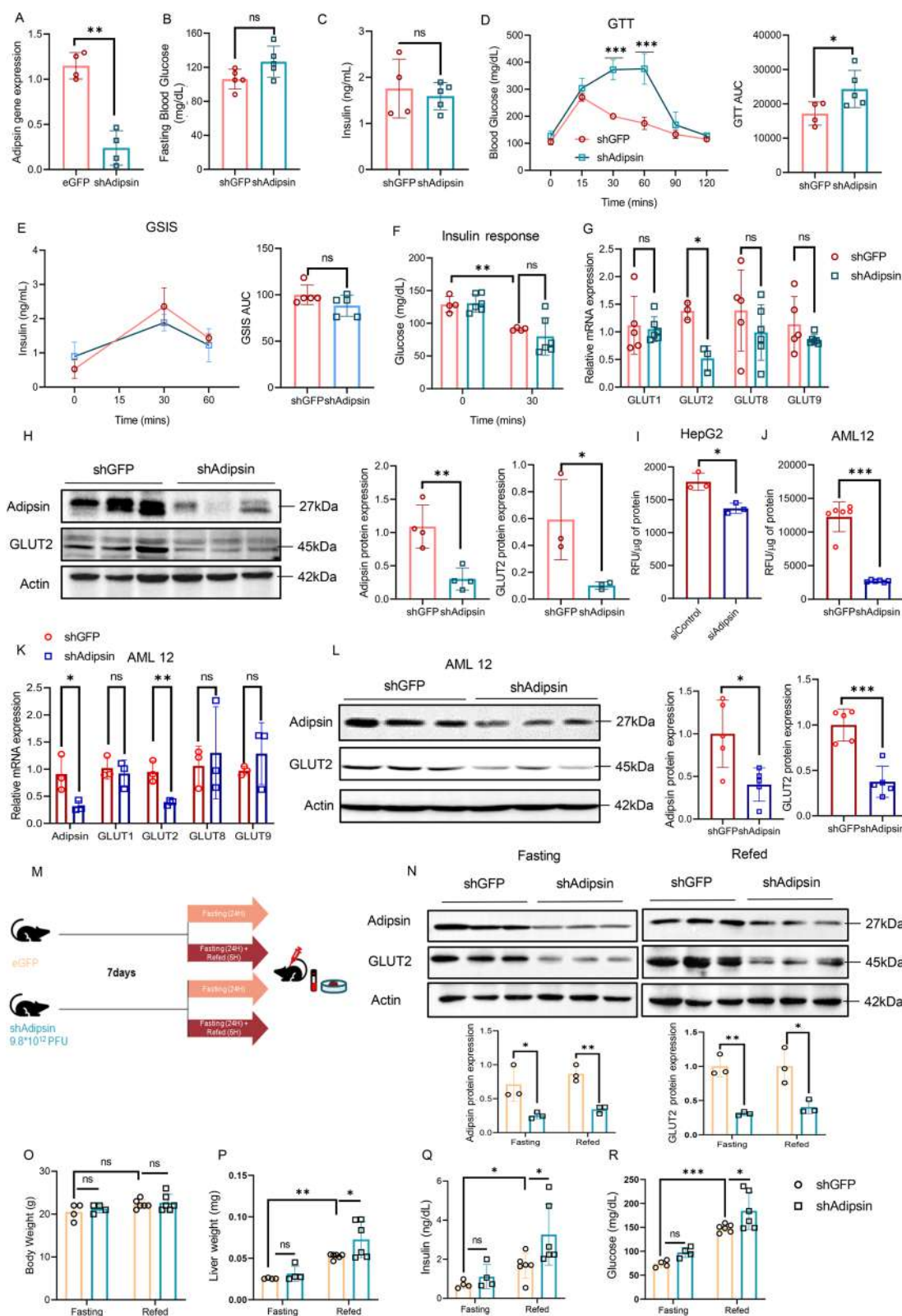


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Fig. 6 Hepatic adipsin deficiency impairs systemic glucose tolerance and postprandial metabolic regulation. **A** mRNA expression of *Adipsin* in liver homogenates following adenovirus-mediated shRNA knockdown of *Adipsin*. **B, C** Fasting blood glucose levels (**B**), plasma insulin concentrations (**C**) in shGFP and shAdipsin-treated mice. **D–G** Glucose tolerance test (GTT), and corresponding area under the curve (AUC) (**D**), Glucose-stimulated insulin secretion (GSIS) (**E**), insulin tolerance test (ITT) (**F**), mRNA expression of *Glut1*, *Glut2*, *Glut8*, and *Glut9* genes (**G**) in shGFP and shAdipsin-treated mice. **H** Immunoblot analysis of adipsin and GLUT2 protein in hepatic homogenates following adenovirus-mediated shRNA knockdown of Adipsin. Right panel: Densitometric analysis of Adipsin and GLUT2. **I** Glucose uptake assay in HepG2 cells following siRNA-mediated knockdown of adipsin. **J** Glucose uptake assay in AML12 cells following adenovirus-mediated shRNA knockdown of Adipsin. **K** mRNA expression of *Adipsin*, *Glut1*, *Glut2*, *Glut8*, and *Glut9* genes in shGFP and shAdipsin-treated AML12 cells. **L** Immunoblot analysis of adipsin and GLUT2 protein in hepatic homogenates following adenovirus-mediated shRNA knockdown of Adipsin in AML12 cells. Right panel: Densitometric analysis of Adipsin and GLUT2 (n=6). **M** Experimental design for the fasting–refeeding protocol. **N** Immunoblot analysis of hepatic adipsin and GLUT2 expression following fasting and refeeding. Right panel: Densitometric analysis of adipsin and GLUT2 expression (n=3). **O–R** Quantification of body weight (**O**), liver weight (**P**), plasma insulin (**Q**) and plasma glucose concentrations (**R**) in shGFP and shAdipsin-treated mice subjected to fasting–refeeding. Mice n=10 (shGFP), n=10 (shAdipsin), n=10 (fasting, shGFP), n=10 (fasting, shAdipsin), n=10 (refed, shGFP), n=10 (refed, shAdipsin). Data are presented as mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, statistical significance was determined using unpaired two-tailed t-test or one-way ANOVA followed by Bonferroni’s multiple-comparison test, as appropriate. ns, not significant; shGFP, Green Fluorescent Protein

adipsin-overexpressing mice (Supplementary Fig. 4L), supporting a functional role for hepatic adipsin in promoting hepatic glucose uptake and maintaining systemic glucose homeostasis.

Next, we examined whether adipsin knockdown alters glucose excursions during the fasting–refeeding cycle. Mice with hepatic adipsin silencing were subjected to a fasting–refeeding protocol (Fig. 6M). As expected, adipsin and GLUT2 protein levels were significantly reduced in the adipsin knockdown group (Fig. 6N). Interestingly, adipsin silencing increased adipose tissue mass (Supplementary Figs. 4 M, N), whereas body weight and liver weight (Figs. 6O, P), as well as circulating triglyceride and cholesterol levels, remained unaltered between groups (Supplementary Fig. 4O, P), thus suggesting that hepatic adipsin depletion does not markedly disrupt systemic lipid metabolism responses. Refeeding increased circulating insulin and glucose levels in adipsin-deficient mice (Figs. 6Q, R). In conjunction with impaired glucose tolerance during GTT and reduced GLUT2 expression and hepatocyte glucose uptake, these findings indicate that hepatic adipsin depletion disrupts postprandial glucose handling and impairs systemic glucose tolerance. Collectively, our data support a model in which the glucose–mTOR axis governs hepatic adipsin translation, while hepatic adipsin, in turn, facilitates efficient glucose uptake and systemic glycemic control, forming a reciprocal regulatory loop.

Discussion

The immunometabolic functions of adipsin have been implicated in multiple metabolic disorders (Ma et al. 2024; Moreno-Navarrete and Fernandez-Real 2019; Milek 2022; Zhang et al. 2021), although prior studies have yielded inconsistent results (Zhang et al. 2021; Gu et al. 2022; Liu et al. 2023; Zhou et al. 2018; Kyohara et al. 2020). These discrepancies underscore the necessity of delineating its tissue-specific expression patterns and regulatory mechanisms. In this study, we uncover previously unrecognized aspects of hepatic adipsin biology

in the context of glucose homeostasis. We demonstrate that adipsin protein abundance is increased in human and murine liver during diabetes and in the postprandial state, despite largely unchanged mRNA abundance. While this hepatic adipsin pool may reflect local contributions from parenchymal cells (Kitano and Kitamura 2000; Barnum and Volanakis 1985; Barnum and Volanakis 1985), and from non-parenchymal cells, or circulating adipsin, our experiments in primary hepatocytes and hepatocyte-derived cells establish that hepatocytes themselves exhibit cell-autonomous glucose-dependent regulation of adipsin protein expression. Furthermore, we identify a mechanistic link whereby glucose promotes adipsin translation in hepatocytes through coordinated regulation of eIF2 α and mTOR signaling, with the 5'UTR of *Adipsin* mRNA conferring mTOR-dependent translational control. Importantly, hepatocyte-specific depletion of adipsin impaired systemic glucose tolerance, particularly in the postprandial state. This defect was associated with reduced hepatic glucose uptake, accompanied by a marked downregulation of GLUT2. Collectively, these findings establish hepatic adipsin as a glucose-responsive regulator that links nutrient sensing to hepatic glucose handling (Fig. 7).

Our data further demonstrate that liver-derived adipsin is not merely a by-product of metabolic stress but functions as an active regulator of systemic glucose homeostasis (Gomez-Banoy et al. 2019). Genetic ablation of hepatic adipsin resulted in impaired glucose tolerance without alterations in insulin secretion or signaling, indicating that the observed phenotype is not driven by defects in β -cell function or insulin responsiveness. Adipsin depletion reduced both GLUT2 mRNA and protein abundance, suggesting hepatic SLC2A2 expression is regulated through promoter-bound transcription factors and enhancer-promoter interactions (Kim and Ahn 1998). The observed changes could therefore reflect SLC2A2 transcription (Im et al. 2005), altered GLUT2 mRNA stability, or indirect metabolic and signaling consequences of adipsin depletion. While adipose-derived

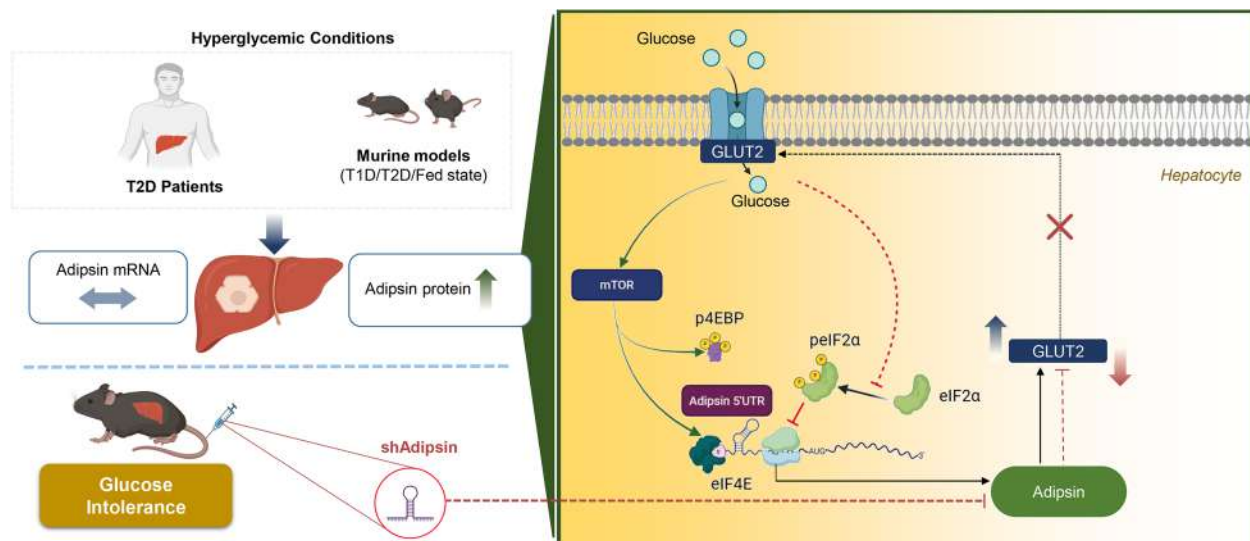


Fig. 7 Schematic of glucose dependent hepatic adipsin expression, and its implication in diabetes and glucose metabolism. In human patients with diabetes, in murine models of both type 1 and type 2 diabetes, and during refeeding, hepatic Adipsin protein abundance is increased despite unchanged Adipsin mRNA levels, indicating predominant post-transcriptional regulation. Mechanistically, glucose uptake by hepatocytes through GLUT2 activates mTOR signaling, leading to 4E-BP phosphorylation and enhanced eIF4E-dependent translation mediated by the Adipsin 5'UTR. In parallel, glucose attenuates eIF2α phosphorylation, thereby relieving translational repression and further promoting Adipsin protein expression. Functionally, hepatic Adipsin supports GLUT2 expression and glucose handling, as liver-specific Adipsin depletion reduces both SLC2A2/GLUT2 mRNA and protein abundance, impairs hepatic glucose uptake, and results in glucose intolerance. Collectively, these findings identify glucose-induced translational upregulation of hepatocyte Adipsin as an adaptive mechanism that helps maintain GLUT2 expression and hepatic glucose homeostasis during diabetes

adipsin has largely been considered a systemic endocrine factor that enhances β -cell function via complement C3a-mediated mitochondrial support (Lo et al. 2014; Gomez-Banoy et al. 2019; Pereira Lima et al. 2025; Park 2024), our findings extend this paradigm by identifying a tissue-specific role for hepatocyte adipsin in facilitating glucose uptake. Additionally depletion or overexpression of Adipsin do not establish whether the effect on hepatocyte GLUT2 is mediated by secreted adipsin, alternative-complement signaling, a complement-independent extracellular pathway, or an intracellular consequence of adipsin synthesis and processing. Our findings establish adipsin-dependent regulation of GLUT2 abundance but do not demonstrate that adipsin directly regulates SLC2A2 transcription. These results reconcile previously conflicting reports on circulating adipsin and metabolic liver disease (Zhou et al. 2018) by demonstrating that hepatic adipsin is subject to post-transcriptional regulation and exerts local metabolic effects that may not be reflected by circulating levels. Such context-dependent regulation may explain reports associating elevated adipsin levels with increased MASLD severity (Pan et al. 2023), as well as studies describing inverse relationships (Gu et al. 2022). Together, these observations suggest that circulating adipsin concentrations may not reliably reflect its tissue-specific function and that its role may vary across disease stages, potentially acting as a compensatory factor during early metabolic stress but becoming dysregulated in chronic disease (Aaron et al.

2021; Kershaw and Flier 2004). The precise consequences of hepatocyte-derived adipsin on the liver microenvironment and overall hepatic physiology, however, remain to be fully elucidated.

The differential effects of acute and chronic insulin treatment further indicate that hepatic adipsin abundance may depend on both the duration of glycemic exposure and the associated nutrient-signaling state. In fasted mice, acute insulin administration rapidly lowered blood glucose but without alteration in hepatic adipsin, suggesting that this interval may be insufficient for changes in adipsin synthesis to substantially affect the existing protein pool. In contrast, sustained correction of hyperglycemia in STZ-diabetic mice was accompanied by reduced hepatic adipsin abundance. Notably, although acute insulin robustly activated hepatic AKT–mTOR-signaling, it did not produce a corresponding change in eIF2α phosphorylation, suggesting that insulin signaling alone does not fully recapitulate the coordinated nutrient-responsive translational state associated with glucose-dependent adipsin regulation.

Mechanistically, our data indicate that glucose availability coordinates eIF2α- and mTOR-associated translational pathways that regulate adipsin synthesis. Consistent with this nutrient-responsive regulation, adipsin abundance increased with glucose concentration and approached saturation at higher glucose levels, indicating a graded response to glucose availability. Furthermore, glucose deprivation rapidly induces eIF2α

phosphorylation (Muaddi et al. 2010), leading to inactivation of the eIF2 trimer, and its interaction with the initiator tRNA and engagement with the start codon (Gilani et al. 2024). Concurrently, inhibited mTOR complex hinders uncoupling of eIF4E from its binding partner 4E-BP1, and binding to 5' cap of *Adipsin* mRNA, thereby suppressing translation initiation (Sonenberg and Hinnebusch 2009). Consistent with a contribution of glucose metabolism to these responses, 2-deoxy-D-glucose (2-DG) attenuated glucose-induced adipsin expression, accompanied by increased eIF2 α phosphorylation and reduced 4E-BP1 phosphorylation (Xi et al. 2011). However, because 2-DG can also disrupt N-linked glycosylation and induce ER stress, these effects cannot be attributed exclusively to inhibition of glycolytic flux. Of these two controlling arms, the glucose-mTOR signalling axis promotes adipsin protein translation via the 5'UTR of *Adipsin* mRNA. Potential mechanisms may involve secondary RNA structures or internal ribosome entry sites (IRES) within the 5'UTR that integrate nutrient-sensitive signalling cascades, as has been described for other mTOR-dependent transcripts (Hinnebusch 2011; Thoreen et al. 2012). The adipsin 5'UTR may therefore contain structural or sequence features that confer nutrient-sensitive translational regulation, although the specific regulatory element remains to be defined.

Functionally, our findings place hepatic adipsin at a critical node linking nutrient sensing to glucose metabolism. Despite preserved insulin secretion and signalling, adipsin depletion impaired glucose tolerance and reduced hepatic glucose uptake, revealing a previously unappreciated link between adipsin and GLUT2-dependent glucose handling, although the molecular mechanism connecting adipsin to GLUT2 remains to be defined. Ribosome profiling and reporter-based assays will be instrumental in identifying such elements and in clarifying how nutrient signaling intersects with adipsin synthesis. Furthermore, the downstream metabolic functions of adipsin—as both an endocrine factor and a tissue-specific modulator—remain incompletely understood. Future studies should address the relative contributions of alternative complement activation versus direct modulation of hepatocellular pathways, including glucose transport, in mediating the metabolic effects of adipsin.

Conclusions

This study identifies liver-derived adipsin as a nutrient-responsive protein whose translation is governed by the glucose–eIF2 α –mTOR axis and that plays a direct functional role in systemic glucose homeostasis. By linking glucose-dependent translational regulation of adipsin to hepatic glucose uptake and GLUT2 expression, our findings provide a mechanistic basis for its contribution to glucose intolerance. These results demonstrate that the

hepatic adipsin pool is dynamically regulated through post-transcriptional mechanisms, while our studies in primary hepatocytes and hepatocyte-derived cells establish a cell-autonomous glucose-responsive component of this regulation. Collectively, our work positions hepatic adipsin as a tissue-specific regulator of glucose metabolism with potential therapeutic relevance in metabolic liver disease and diabetes.

Abbreviations

ALD	Associated Liver Disease
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
CFD	Complement Factor D
CHX	Cycloheximide
eIF2 α	Eukaryotic Initiation Factor 2 α
GSIS	Glucose-Stimulated Insulin Secretion
GTT	Glucose Tolerance Test
HbA1c	Glycated Hemoglobin
HepG2	Human Hepatocellular Carcinoma Cell Line
HFD	High-Fat Diet
HOMA-B	Homeostatic Model Assessment of β -cell Function
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IHC	Immunohistochemistry
MASLD	Metabolic Dysfunction-Associated Steatotic Liver Disease
mRNA	Messenger Ribonucleic Acid
mTOR	Mechanistic Target of Rapamycin
NAFLD	Non-Alcoholic Fatty Liver Disease
PAS	Periodic Acid–Schiff Stain
shRNA	Short Hairpin Ribonucleic Acid
STZ	Streptozotocin
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
GLUT2	Glucose Transporter Type 2

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s10020-026-01620-3>.

Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

SKM: Methodology, experimentation, data analysis, visualization, writing the original draft; AS: Generation of plasmid construct and experimentation. TD, AB, and AS: biochemical and in vivo experiments. SM and AC: patient recruitment, clinical classification, liver biopsy, and analysis of histology. PC: Conceptualization, designed study, researched data, methodology, writing & editing, project administration, funding acquisition; PC is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All participants provided consent before liver biopsies were collected from patients in the Department of Gastroenterology at the Indian Institute of Liver and Digestive Sciences (IILDS), West Bengal, India (IEC reference No. HREC, IILDS/2023-R11).

Consent for publication

Not Applicable.

Competing interests

The authors declare no conflicts of interest.

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