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TSH-stimulated hepatocyte exosomes modulate liver-adipose triglyceride accumulation via the TGF-β1/ATGL axis in mice

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Abstract

Subclinical hypothyroidism (SCH) contributes to obesity, with the liver acting as a crucial metabolic regulator. Thyroid-stimulating hormone (TSH) affects systemic lipid balance, potentially linking SCH to obesity. While the direct impact of TSH on hepatic lipid metabolism has been extensively documented, its role in modulating lipid dynamics in peripheral organs through liver-mediated pathways remains insufficiently understood. This study identifies TSH-stimulated hepatocyte-derived exosomes (exosomes^{TSH}) as key mediators in liver-adipose communication, promoting triglyceride accumulation in adipocytes via the transforming growth factor-beta 1 (TGF-β1)/adipose triglyceride lipase (ATGL) axis. Exosomes^{TSH} enhance lipid storage in adipocytes, significantly increasing triglyceride content and lipid droplet formation while reducing lipolysis, effects that are dependent on TSH receptor (TSHR) activation in hepatocytes. In vivo, exosomes^{TSH} induce weight gain and adipose tissue expansion, impairing glucose metabolism in both chow- and high-fat diet-fed mice. Mechanistically, exosomes^{TSH} upregulate TGF-β1 and downregulate ATGL in adipocytes, establishing the TGF-β1/ATGL pathway as essential for exosome-mediated lipid accumulation. Further, miR-139-5p is identified as a modulator of TGF-β1 expression within this pathway, with overexpression of miR-139-5p alleviating exosomes^{TSH}-induced lipid accumulation in adipocytes. This study elucidates a novel miR-139-5p-dependent mechanism through which TSH modulates lipid metabolism via liver-derived exosomes, highlighting the pivotal role of miR-139-5p in linking SCH to adipose lipid accumulation through the TGF-β1/ATGL signaling axis.

Keywords Thyrotropin, Obesity, Exosomes, Lipid metabolism

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Introduction

Subclinical hypothyroidism (SCH) has increasingly been recognized as a key contributor to metabolic disorders, particularly obesity [1–3]. Elevated thyroid-stimulating hormone (TSH) levels, a hallmark of SCH, are closely associated with increased adiposity and lipid metabolism abnormalities [4, 5]. While TSH primarily regulates thyroid function, emerging evidence suggests that it also directly impacts lipid metabolism by promoting lipid synthesis and accumulation in the liver, thereby contributing to systemic metabolic dysregulation [2, 6]. This establishes a potential link between SCH, obesity, and lipid homeostasis disturbances. As a result, the liver is considered a central metabolic organ through which TSH influences systemic lipid balance, potentially mediating the relationship between SCH and obesity [7, 8]. However, despite well-documented hepatic effects of TSH, its role in regulating lipid metabolism in peripheral organs through liver-derived factors remains largely unexplored.

The liver-adipose axis has emerged as a critical pathway for inter-organ communication, particularly in maintaining lipid homeostasis [9–11]. Through this axis, the liver regulates lipid storage, mobilization, and overall metabolic function in adipose tissue, thereby playing a pivotal role in systemic energy balance [12]. Extracellular vesicles, especially exosomes, function as essential mediators facilitating liver-peripheral tissue interactions [13, 14]. These nanoscale, lipid bilayer-enclosed vesicles are capable of transporting various bioactive components, such as proteins, lipids, and nucleic acids, delivered to target cells [15]. This molecular signaling capability enables exosomes to exert metabolic regulatory effects beyond the liver, influencing processes in distant tissues [16–18]. Recent findings emphasize that exosomes are crucial components of the liver-adipose axis, actively contributing to lipid metabolism and playing a significant role in metabolic disorders, highlighting their promise as potential therapeutic agents for addressing metabolic imbalances [19, 20].

Despite the well-recognized role of the liver-adipose axis in lipid homeostasis, the mechanisms by which TSH influences adipocyte lipid metabolism through hepatocyte-derived exosomes remain unclear. To address this gap, this study investigates the role of TSH-stimulated hepatocyte-derived exosomes (exosomes^{TSH}) in adipose lipid metabolism. Prior multiomics investigations have demonstrated that TSH stimulation induces notable changes in the proteomic profile of hepatocyte-derived exosomes, resulting in an enrichment of proteins that participate in metabolic processes, cellular signaling, programmed cell death, and inflammatory pathways [21–24]. These findings suggest that exosomes^{TSH} may function as mediators of lipid metabolism beyond the liver, yet their precise impact on adipose tissue lipid dynamics and regulatory mechanisms remains undefined.

This study aims to elucidate the function of exosomes^{TSH} in triglyceride accumulation within adipocytes and their broader role in liver-adipose communication. Specifically, the effects of exosomes^{TSH} on lipid deposition in both cellular and animal models are examined to determine their contribution to adipose lipid accumulation and metabolic dysregulation. A key hypothesis of this study is that exosomes^{TSH} facilitate lipid storage in adipocytes through a novel signaling mechanism, distinct from direct hepatic lipid regulation. Through mechanistic investigations, this research identifies the TGF-β1/ ATGL axis as a downstream pathway mediating the metabolic effects of exosomes^{TSH}. By uncovering a previously uncharacterized mechanism linking TSH to adipose lipid accumulation via exosomal signaling, these findings provide new insights into obesity-related metabolic changes and offer potential targets for therapeutic intervention in TSH-related lipid metabolism disorders (Fig. 1).

Materials and methods

Animal experiments

Male mice with adipocyte-specific TGF-β1 conditional knockout and age-matched wild-type controls (7 weeks old) were used in this study. TGFβ1$^{fl/fl}$ -Adipoq-Cre mice were generated by crossing floxed TGFβ1 (TGFβ1$^{fl/fl}$) mice with Adiponectin-Cre transgenic mice, allowing for constitutive, adipocyte-specific deletion of TGFβ1. Genotypes were confirmed by PCR analysis of tail biopsies using specific primers designed to detect the TGFβ1 floxed allele and Cre transgene before experimental procedures. After a one-week acclimatization, they were assigned to either a chow or high-fat diet (HFD). At 8 weeks, mice received intraperitoneal exosomesTSH (30 μg/mouse) every three days for 8 weeks. Body weight was monitored weekly, and upon completion, mice were euthanized for serum, liver, and adipose tissue collection.

Primary cell isolation, culture, and treatment

Preadipocytes were isolated from TGFβ1-CKO and WT mice via enzymatic digestion of adipose tissue in DMEM with 1 mg/mL collagenase at 37 °C for 45 min, followed by filtration and centrifugation. Cells were cultured in DMEM with 5% FBS and 5% newborn calf serum until confluence. Differentiation was induced using troglitazone (0.5 mg/L), dexamethasone (5 μM), insulin (10 ng/mL), and IBMX (0.5 mM) for 48 h, then maintained in insulin-supplemented medium for full adipogenic maturation. HepG2 and 3T3-L1 cells were originally purchased from ScienCell Research Laboratories. Both cell lines were supplied

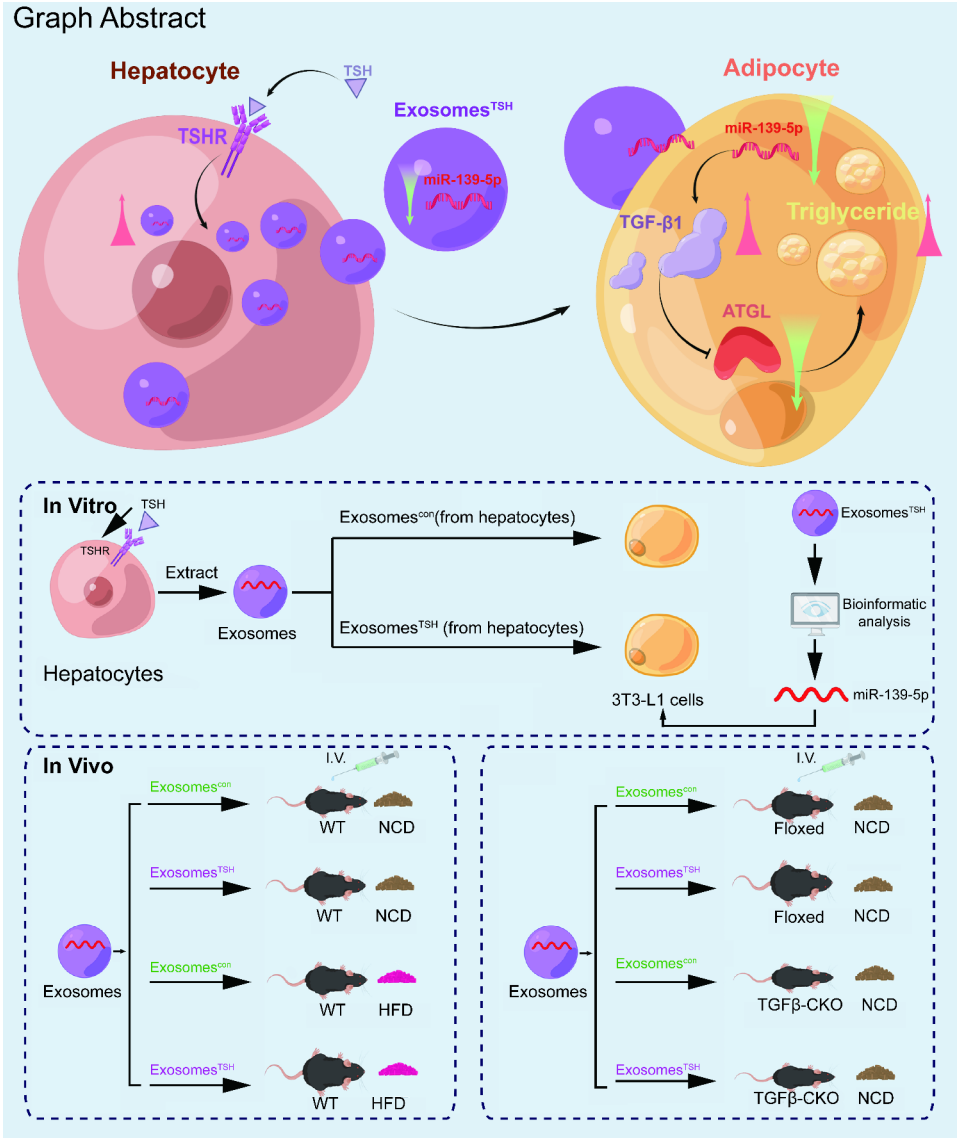

Fig. 1 Schematic overview of the effects of TSH-induced exosomes on hepatocyte-adipocyte communication

with Mycoplasma-free certification, as verified by PCR-based Mycoplasma detection assays conducted by ScienCell Research Laboratories prior to shipment. No additional Mycoplasma testing was performed in the laboratory. 3T3-L1 preadipocytes were maintained and differentiated according to the established protocol. Cells were first expanded in DMEM supplemented with 10% FBS and antibiotics until full confluence was attained. Upon reaching 100% confluence, the differentiation process was initiated. For differentiation initiation, cells were exposed to MEM supplemented with 10% FBS, 10 µM insulin, 0.5 mM isobutylmethylxanthine (IBMX), and 0.25 µM dexamethasone for two days (Differentiation Medium 1). At day 2 (D2), Differentiation Medium 2 (MEM with 10% FBS and 10 µM insulin) was introduced, replacing the initial medium. Cells remained in this medium for another 2–3 days before being transferred to MEM with 10% FBS, where the medium was replenished every 2–3 days. By days 10–12, approximately 80–90% of the cells had differentiated into adipocytes, ready for further analysis. HepG2 cells were treated with 100 mU/L Thyroid-Stimulating Hormone as described previously. This concentration was chosen based on prior studies modeling subclinical hypothyroidism in vitro. The concentration falls within the physiologically relevant range observed in SCH patients and has been demonstrated to activate TSH receptors in hepatocytes. After TSH treatment, cells were transfected with si-TSHR or si-NC to create $HepG2^{si-Tshr}$ and $HepG2^{si-ctrl}$ cells, respectively [21]. 3T3-L1 cells were transfected with

either miRNA (50 nM) using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA).

Exosome isolation

Exosomes were isolated from HepG2 supernatants using ExoQuick-based precipitation combined with differential centrifugation. Sequential spins included 300×g (10 min) for cell removal, 2,000×g (10 min) for debris clearance, 10,000×g (30 min) for large vesicles, and 110,000×g (60 min) for exosome pelleting [21]. The isolated pellets were resuspended in PBS, with exosomes derived from TSH-stimulated HepG2 cells labeled as Exosomes^{TSH} and those from solvent control-stimulated cells labeled as Exosomes^{con}. Exosomes from HepG2$^{si-Tshr}$ cells were designated as Exosomes$^{si-Tshr}$, and those from HepG2^{si-NC} cells as Exosomes$^{si-ctrl}$.

Co-culture of exosomes with adipocytes

Fully differentiated 3T3-L1 adipocytes, along with primary adipocytes derived from TGFβ1-CKO and WT mice, were utilized. Cells were plated in 6-well dishes at approximately 80% confluency. Exosomes^{TSH} or Exosomes$^{si-Tshr}$ were introduced into the culture medium at 25 μg/mL and incubated for 24 h. miR-139-5p mimic or inhibitor was transfected into 3T3-L1 cells 24 h before exosome treatment.

Metabolic analysis

The Columbus Instruments Comprehensive Lab Animal Monitoring System (CLAMS, Columbus, OH, USA) was used to assess oxygen consumption, carbon dioxide and heat production, activity levels, respiratory exchange ratio, and food intake. Prior to data collection, mice underwent a 48-hour acclimatization period in metabolic cages, followed by a 24-hour continuous measurement phase. The following parameters were monitored: Oxygen Consumption (VO_2): Measured continuously to assess energy expenditure. Carbon Dioxide Production (VCO_2): Collected for respiratory quotient calculations. Heat Production: Calculated from VO_2 and VCO_2 readings. Physical Activity: Monitored through infrared beams to record movement counts. Food and Water Intake: Quantified manually at specific intervals.

Oil red O staining and lipid quantification

To assess lipid accumulation, differentiated 3T3-L1 adipocytes were stained with Oil Red O. Cells were fixed in 4% paraformaldehyde for 10 min at room temperature, then incubated with Oil Red O solution (Servicebio, Wuhan, China) in the dark for 30 min. Excess stain was removed, followed by 60% isopropanol differentiation (3–5 s) and three washes with distilled water (5 min each). A final wash ensured complete stain removal. For cells on coverslips, glycerol gelatin mounting medium was used for sealing. Quantitative analysis of triglyceride content was performed using commercial kits (APPLYGEN, Beijing, China).

Statistical analysis

Data are shown as mean ± SD. Normality was tested using the Shapiro-Wilk test before parametric analysis. ANOVA assessed diet and genotype effects. For two-group comparisons, Student's t-test was used if normality was met; otherwise, the Mann-Whitney U test was applied. One-way ANOVA was used for multiple groups, or the Kruskal-Wallis test if normality was not met. Each experiment included three independent batches.

Results

TSH-Stimulated Hepatocyte-derived exosomes enhance lipid accumulation in adipocytes via the TSH/TSHR pathway

To evaluate the effects of TSH-stimulated hepatocyte-derived exosomes (exosomes^{TSH}) on lipid accumulation in adipocytes, 3T3-L1 adipocytes were treated with exosomes^{TSH} and exosomes^{con} derived from non-stimulated HepG2 cells. Oil Red O staining showed a marked increase in lipid droplet formation in adipocytes treated with exosomes^{TSH}, with noticeably intensified staining compared to the exosomes^{con} group (Fig. 2A). Quantitative analysis revealed that exosomes^{TSH} significantly increased lipid droplet formation (Fig. 2B), triglyceride (TG) content (Fig. 2C), and free fatty acid (FFA) levels (Fig. 2D), while reducing glycerol release (Fig. 2E), indicating enhanced lipid accumulation and suppressed lipolysis.

To further assess the involvement of the TSH/TSHR pathway in exosome-induced lipid accumulation, siRNA was used to knock down TSHR in HepG2 cells before TSH stimulation. Exosomes collected from TSHR knockdown cells (exosomes$^{si-Tshr}$) and control siRNA-treated cells (exosomes$^{si-ctrl}$) were subsequently applied to 3T3-L1 adipocytes. Unlike exosomes$^{si-ctrl}$, exosomes$^{si-Tshr}$ failed to facilitate triglyceride accumulation, as evidenced by diminished lipid staining and free fatty acid levels, reduced triglyceride content, and a decreased number of lipid droplets (Fig. 2F and J). These findings indicate that activation of the TSH/TSHR pathway in hepatocytes is essential for the secretion of exosomes that enhance lipid accumulation in adipocytes.

Exosomes^{TSH} promote weight gain and adipose tissue expansion in mouse models

To investigate the in vivo effects of exosomes^{TSH} on body weight and adipose tissue accumulation, exosomes^{TSH} were administered to two groups of mice: one group on a regular chow diet and the other on HFD. Exosomes^{TSH} treatment significantly increased body weight in

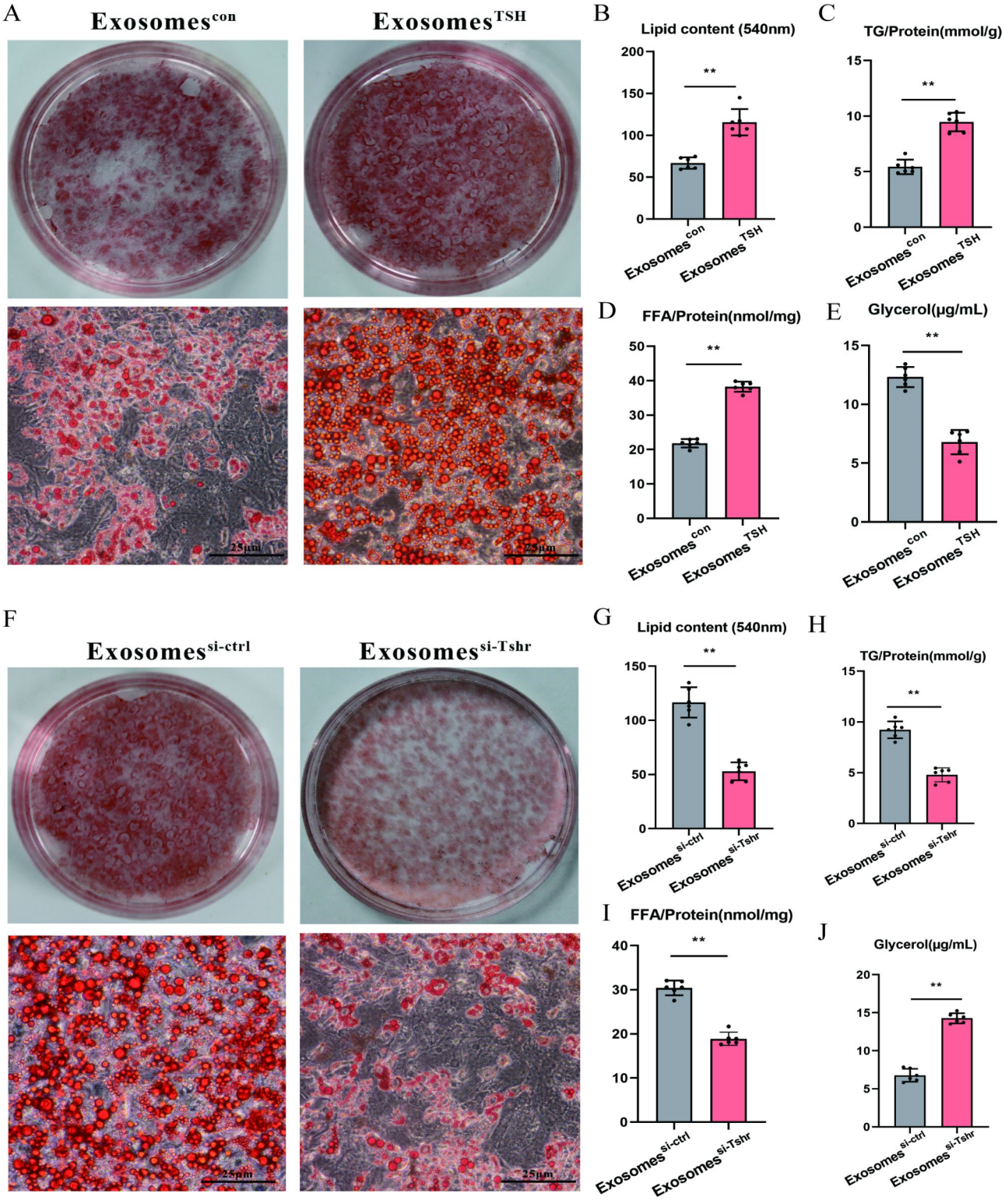

Fig. 2 Exosomes^{TSH} enhance lipid accumulation in 3T3-L1 adipocytes via the TSH/TSHR pathway. (**A-D**) Oil Red O staining (**A**), Lipid droplet count (**B**), Quantification of triglyceride content (**C**), Quantification of FFA content (**D**) and Measurement of glycerol release (**E**) in 3T3-L1 adipocytes treated with exosomes^{TSH} or exosomes^{con}. (**F-J**) Oil Red O staining (**F**), Lipid droplet count (**G**), Quantification of triglyceride content (**H**), Quantification of FFA content (**I**) and Measurement of glycerol release (**J**) in 3T3-L1 adipocytes treated with exosomes$^{si-Tshr}$ and exosomes$^{si-ctrl}$. Statistical significance: ** $P < 0.01$, * $P < 0.05$

both dietary groups compared to controls (SupFigure 1 A-1B). Imaging of adipose depots revealed a notable enlargement of inguinal white adipose tissue (iWAT) and epididymal white adipose tissue (eWAT) in exosomes^{TSH}-treated mice (Fig. 3A and B). Consistently, H&E staining showed adipocyte hypertrophy in both iWAT and eWAT, indicative of enhanced lipid accumulation (Fig. 3C and D). At the molecular level, qPCR analysis demonstrated a significant upregulation of CEBP-α in exosomes^TSH-treated adipose tissues, while PPAR-γ expression remained unchanged (SupFigure 1 C), further supporting the adipogenic effects of exosomes^{TSH}.

Metabolic cage studies revealed differences in energy expenditure and activity levels between exosomes^{TSH}-treated and control mice (Fig. 3E and H and SupFigure 1D-1G). Exosomes^{TSH}-treated mice also exhibited elevated serum triglyceride and cholesterol levels compared to controls (SupFigure 2 A-2B). Furthermore, glucose tolerance tests (GTT) and insulin tolerance tests (ITT) revealed impaired glucose metabolism in exosomes^{TSH}-treated mice across both diets (SupFigure 2 C-2 F). Collectively, these findings suggest that exosomes^{TSH} promote adiposity and metabolic disturbances independent of dietary composition.

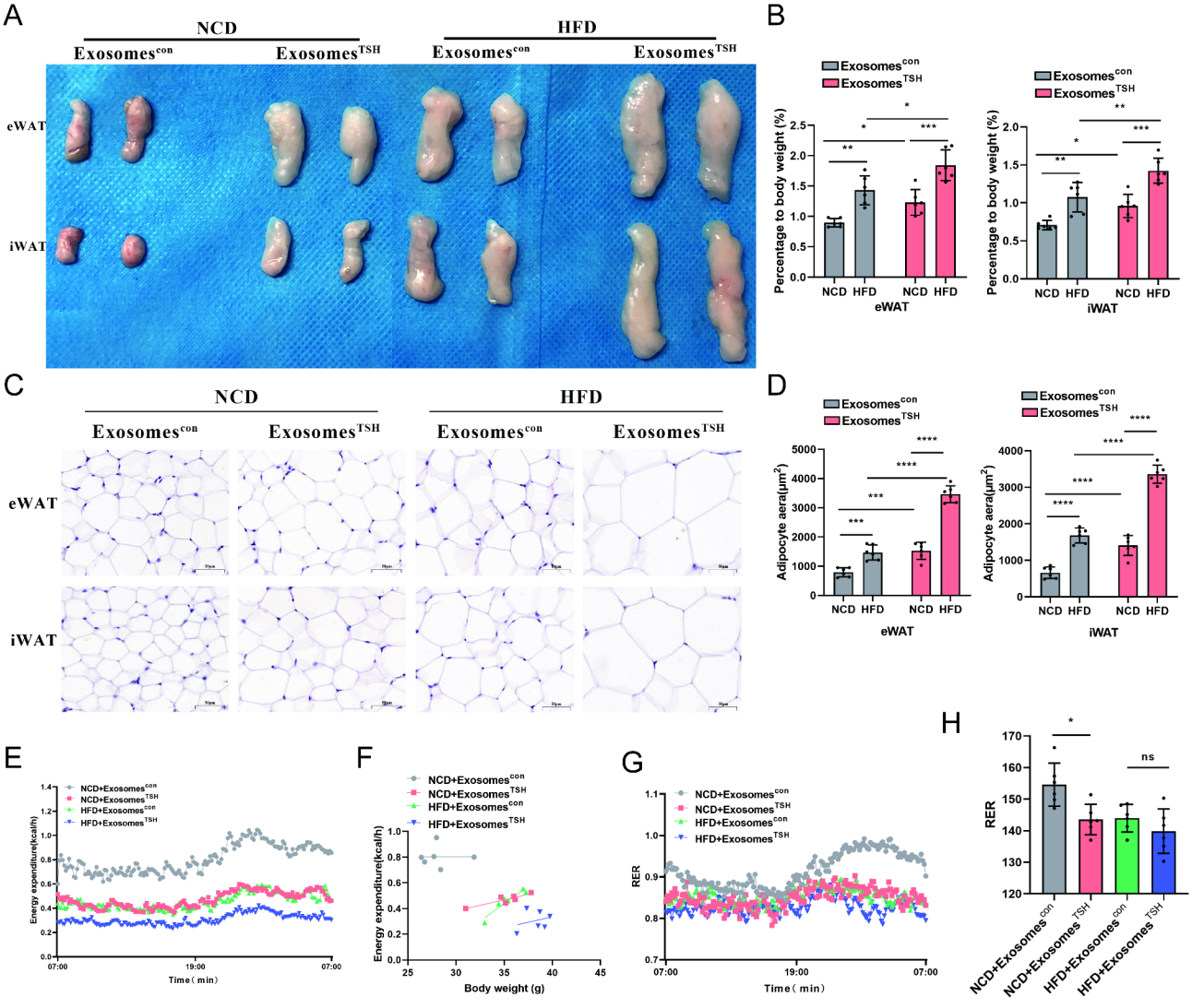

Fig. 3 Exosomes^{TSH} promote weight gain and adipose tissue expansion in mouse models. (**A**) Imaging of iWAT and eWAT depots in NCD mice and HFD mice treated with exosomes^{TSH} or vehicle. (**B**) Ratio of fat weight to body weight for iWAT and eWAT in NCD mice and HFD mice following treatment with exosomes^{TSH} or vehicle. (**C**) H&E staining of iWAT and eWAT tissues in NCD mice and HFD mice () treated with exosomes^{TSH} or vehicle. (**D**) Quantification of adipose tissue area in iWAT and eWAT following H&E staining in NCD mice () and HFD mice treated with exosomes^{TSH} or vehicle. (**E-F**) Energy expenditure (**E**) and Quantification (**F**) over a 24 h period in NCD and HFD mice treated with exosomes^{TSH} or vehicle (**G-H**) RER (**G**) and Quantification (**H**) over a 24 h period in NCD and HFD mice treated with exosomes^{TSH} or vehicle. Statistical significance: ** $P < 0.01$, *$P < 0.05$, ns = not significant

Exosomes^{TSH} downregulate ATGL and upregulate TGF-β1 expression in adipocytes

To explore the molecular mechanisms by which exosomes^{TSH} affect lipid metabolism in adipocytes, key lipid metabolism regulators were examined in 3T3-L1 adipocytes treated with exosomes^{TSH}. Following treatment with exosomes^{TSH}, qPCR and WB were performed to assess the expression of critical lipid metabolism enzymes, including ATGL, HSL, DGAT2, and GPAT3. Among these, ATGL expression was significantly downregulated in exosomes^{TSH}-treated 3T3-L1 adipocytes and primary adipocytes isolated from TGF-β1 knockout and WT mice, while the expression of HSL, DGAT2, and GPAT3 remained unchanged (Fig. 4A and C).

To identify regulatory factors involved in adipose triglyceride lipase (ATGL) downregulation, the expression of upstream molecules potentially modulated by TSH-stimulated hepatocyte-derived exosomes (exosomes^{TSH}). TGF-β1 was markedly upregulated in response to exosomes^{TSH}, while other regulators showed no significant changes (Fig. 4D and F). This pattern was also observed in adipose tissue from exosomes^{TSH}-treated mice, where TGF-β1 upregulation and ATGL suppression were consistently present (SupFigure 3 A-3 C). These findings suggest that the TGF-β1/ATGL pathway may play a pivotal role in mediating lipid accumulation in adipocytes in response to exosomes^{TSH}.

TGF-β1 knockdown inhibits exosomes^{TSH}-Induced triglyceride accumulation in adipocytes

To investigate the roles of TGF-β1 and ATGL in lipid accumulation induced byexosomes^{TSH}, TGF-β1 knockdown and ATGL overexpression models were employed in 3T3-L1 adipocytes. In TGF-β1 knockdown adipocytes, exosomes^{TSH} failed to induce significant triglyceride accumulation, as indicated by reduced lipid droplet formation (Oil Red O staining), fewer lipid droplets, lower triglyceride content, and increased glycerol release compared to control cells (Fig. 5A and E). Conversely, ATGL-overexpressing adipocytes continued to exhibit triglyceride accumulation following exosomes^{TSH} treatment, as shown by sustained lipid droplet formation, an increased number of lipid droplets, elevated triglyceride content, and reduced glycerol release (Fig. 5F and J). These findings suggest that ATGL overexpression alone does not counteract the lipid-accumulating effects induced by exosomes^{TSH}. Together, these findings suggest that exosomes^{TSH} promote triglyceride accumulation in adipocytes primarily through TGF-β1 upregulation, rather than direct modulation of ATGL.

In vivo validation confirms TGF-β1 dependency of exosomes^{TSH}-mediated adipose accumulation

To validate the essential role of TGF-β1 in adipose tissue for the effects of exosomes^{TSH}, adipocyte-specific TGF-β1 knockout mice (TGF-β1-CKO) were generated and administered exosomes^{TSH}. Unlike floxed controls, TGF-β1-CKO mice were resistant to exosomes^{TSH}-induced weight gain and adipose tissue expansion, showing minimal enlargement of iWAT and eWAT depots (Fig. 6A and B, SupFigure 4 A-4B).

Histological analysis (H&E staining) confirmed the absence of adipocyte hypertrophy in TGF-β1-CKO mice, indicating that TGF-β1 deficiency effectively blocks exosomes^{TSH}-mediated adipose accumulation (Fig. 6C and D). Metabolic cage analysis showed no differences in caloric intake or physical activity, excluding behavioral factors as contributors to the observed effects (Fig. 6E and H, SupFigure 4 C-4 F). Additionally, TGF-β1-CKO mice showed no changes in serum triglyceride

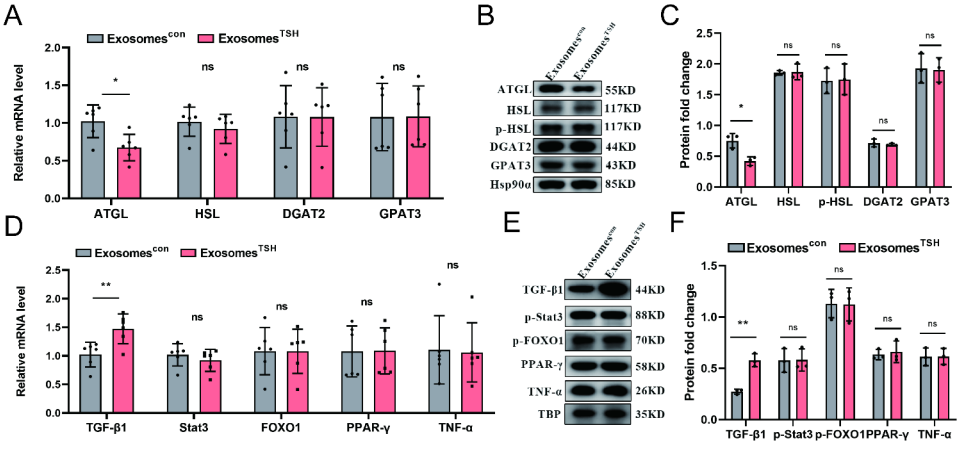

Fig. 4 Exosomes^{TSH} downregulate ATGL and upregulate TGF-β1 expression in adipocytes. (**A-C**) qPCR (**A**), Western blot (**B**) and Quantification of WB (**C**) of ATGL, HSL, DGAT2, and GPAT3 expression in 3T3-L1 adipocytes treated with exosomes^{TSH} or exosomes^{con}. (**D-F**) qPCR (**D**), Western blot (**E**) and Quantification of WB (**F**) of upstream regulatory factors, including TGF-β1, STAT3, FOXO1, PPARγ, and TNF-α, in 3T3-L1 adipocytes treated with exosomes^{TSH} or exosomes^{con}, and in adipose tissue from exosomes^{TSH}-treated mice. Statistical significance: ** $P < 0.01$, * $P < 0.05$

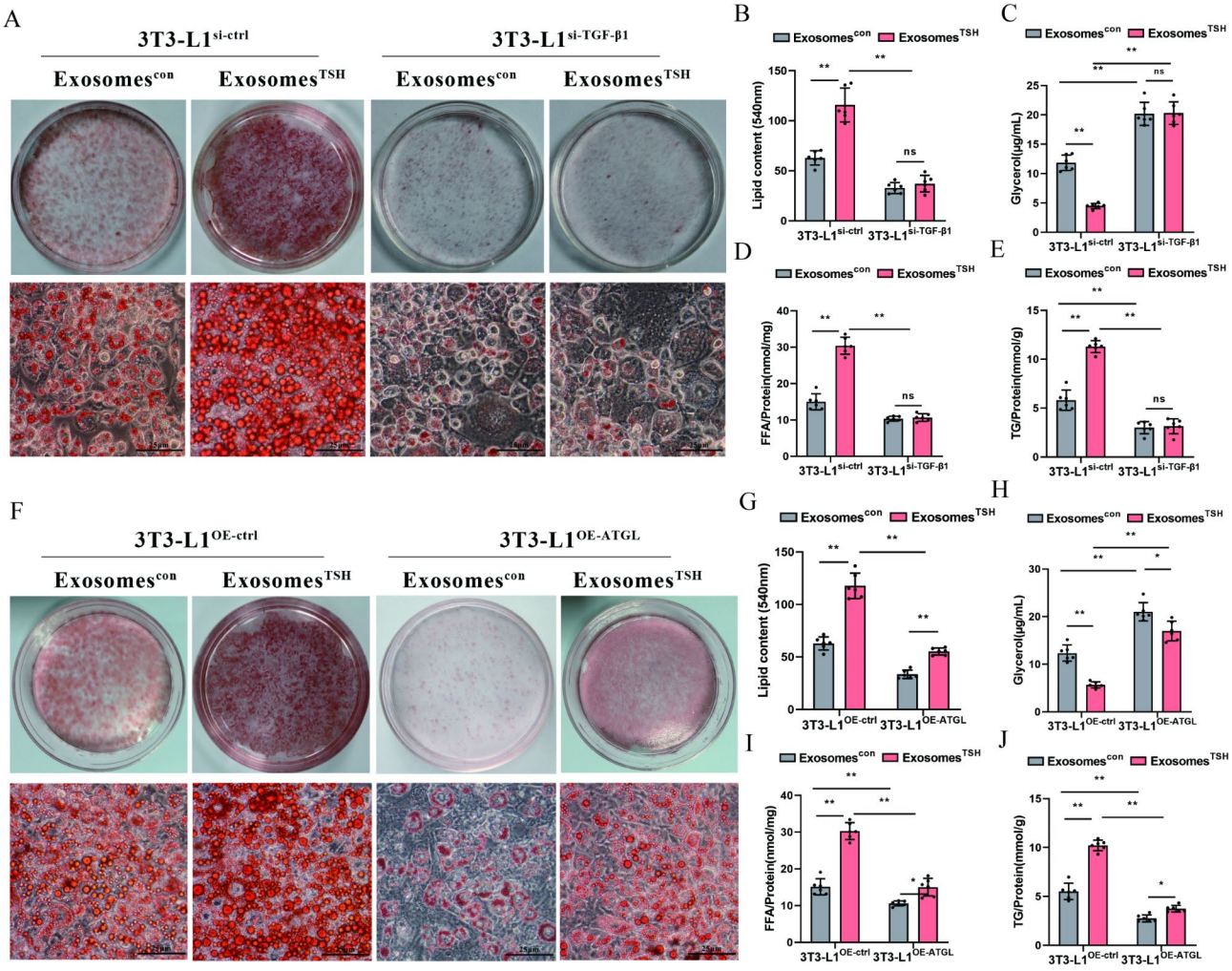

Fig. 5 TGF-β1 knockdown inhibits exosomes^{TSH}-induced triglyceride accumulation in adipocytes. (**A-E**) Oil Red O staining (**A**), Lipid droplet count (**B**), Quantification of triglyceride content (**C**), Quantification of FFA content (**D**) and Measurement of glycerol release (**E**) in TGF-β1 knockdown and control adipocytes treated with exosomes^{TSH}. (**F-H**) Oil Red O staining (**F**), Lipid droplet count (**G**), Quantification of triglyceride content (**H**), Quantification of FFA content (**I**) and Measurement of glycerol release (**J**) in ATGL-overexpressing and control adipocytes treated with exosomes^{TSH}. Statistical significance: ** $P < 0.01$, * $P < 0.05$

or cholesterol levels, nor in glucose metabolism, following exosomes^{TSH} treatment (SupFigure 5). These findings confirm that TGF-β1 in adipose tissue is critical for the lipogenic and metabolic effects of exosomes^{TSH}, effectively blocking adipocyte triglyceride accumulation.

Preliminary identification of miR-139-5p as a regulator of TGF-β1 expression in Exosomes^{TSH}

Exosomes, known for carrying miRNAs that mediate intercellular communication, may influence TGF-β1 expression through specific miRNAs. To investigate this potential regulatory mechanism, a bioinformatic analysis was conducted, identifying 20 candidate miRNAs associated with TGF-β1 from three public databases (SupFigure 6 A). Among these, miR-139-5p was significantly downregulated in exosomes^{TSH} compared to control exosomes, whereas other miRNAs showed no notable changes (Fig. 7A). Luciferase assay showed that miR-139-5p mimics significantly reduced TGF-β1 3'UTR-WT activity but had no effect on 3'UTR-MUT, confirming TGF-β1 as a direct miR-139-5p target (Fig. 7B and SupFigure 6B). miR-139-5p overexpression mitigated exosomesTSH-induced triglyceride accumulation in 3T3-L1 adipocytes, as shown by Oil Red O staining, triglyceride quantification, and lipid droplet analysis (Fig. 7C-G). In contrast, miR-139-5p inhibition exacerbated lipid accumulation (Fig. 7H-L). These findings suggest that miR-139-5p modulates triglyceride accumulation via regulation of TGF-β1, providing a potential mechanism for exosomes^{TSH}-induced adipose tissue expansion.

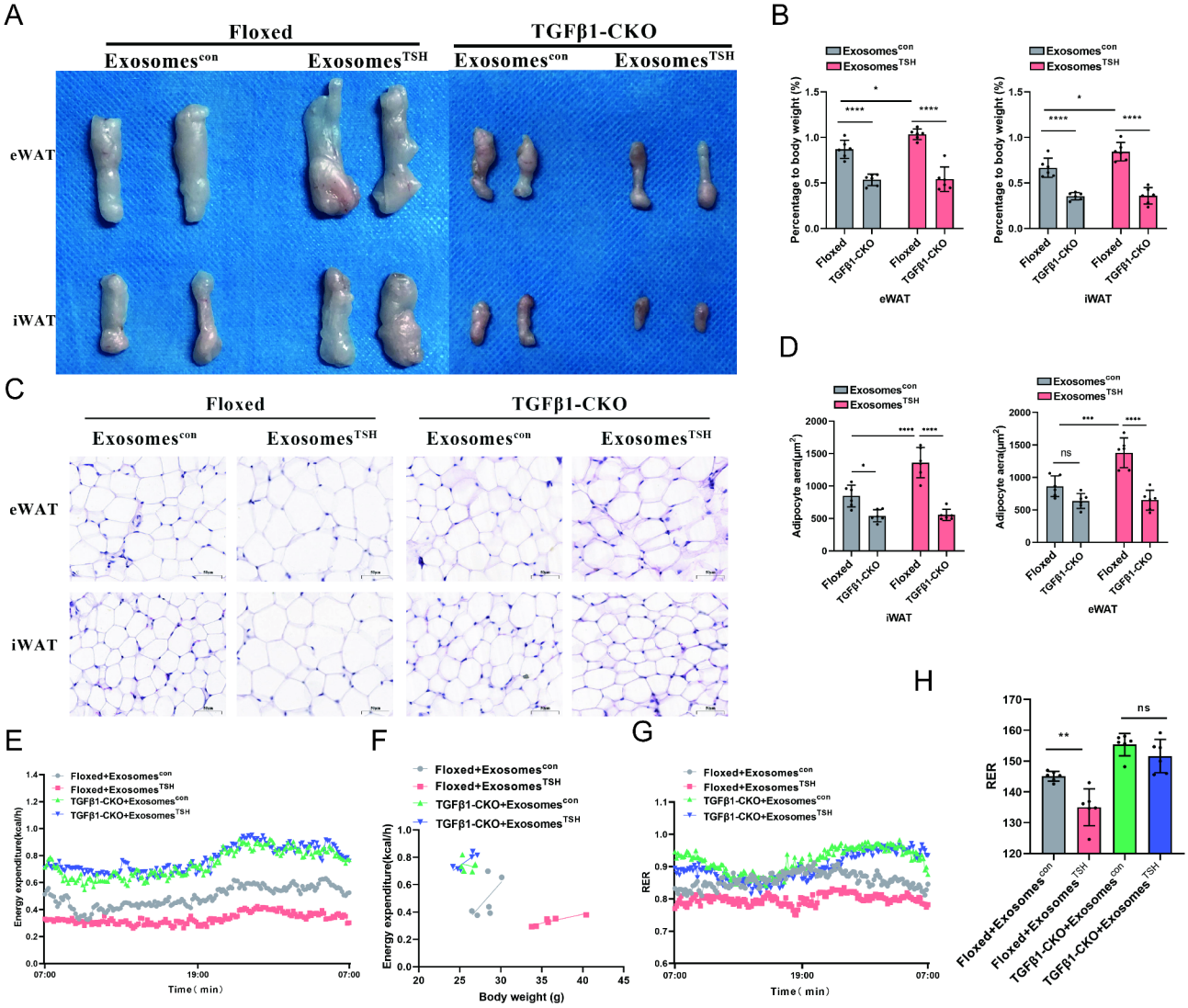

Fig. 6 TGF-β1 dependency of exosomes^{TSH}-mediated adipose accumulation in vivo. (**A**) Imaging of iWAT and eWAT depots in Floxed mice and TGFB1-CKO mice treated with exosomes^{TSH} or vehicle. (**B**) Ratio of fat weight to body weight for iWAT and eWAT in Floxed mice and TGFB1-CKO mice following treatment with exosomes^{TSH} or vehicle. (**C**) H&E staining of iWAT and eWAT tissues in Floxed mice and TGFB1-CKO mice treated with exosomes^{TSH} or vehicle. (**D**) Quantification of adipose tissue area in iWAT and eWAT following H&E staining in WT mice and TGFB1-CKO mice treated with exosomes^{TSH} or vehicle. (**E-F**) Energy expenditure (**E**) and Quantification (**F**) over a 24 h period in Floxed and TGFB1-CKO mice treated with exosomes^{TSH} or vehicle. (**G-H**) RER (**G**) and Quantification (**H**) over a 24 h period in NCD and HFD mice treated with exosomes^{TSH} or vehicle. Statistical significance: ** $P < 0.01$, * $P < 0.05$

Discussion

Summary of key findings

This study explored the impact of exosomes^{TSH} on lipid accumulation in adipocytes, focusing on the TGF-β1/ATGL signaling axis as a mediator of liver-adipose communication. Findings reveal that TSH-stimulated hepatocyte-derived exosomes (exosomesTSH) enhance triglyceride accumulation in adipocytes, as demonstrated by increased lipid droplet formation, elevated triglyceride content, and reduced glycerol release. Mechanistically, the upregulation of TGF-β1 in adipocytes treated with exosomes^{TSH} was identified as a critical driver of lipid accumulation, coinciding with the downregulation of ATGL, a key enzyme responsible for triglyceride breakdown. Knockdown of TGF-β1 effectively blocked the lipid-accumulating effects of exosomes^{TSH}, whereas ATGL overexpression alone was insufficient to reverse these effects, underscoring the pivotal role of TGF-β1 in this pathway. Additionally, miR-139-5p was identified as a regulatory element for TGF-β1 expression, with reduced levels in exosomes^{TSH} potentially lifting miR-139-5p-mediated inhibition on TGF-β1, further supporting TGF-β1 upregulation in adipocytes.

In summary, these findings reveal a novel pathway through which exosomes^{TSH} facilitate lipid accumulation in adipocytes by upregulating TGF-β1 and

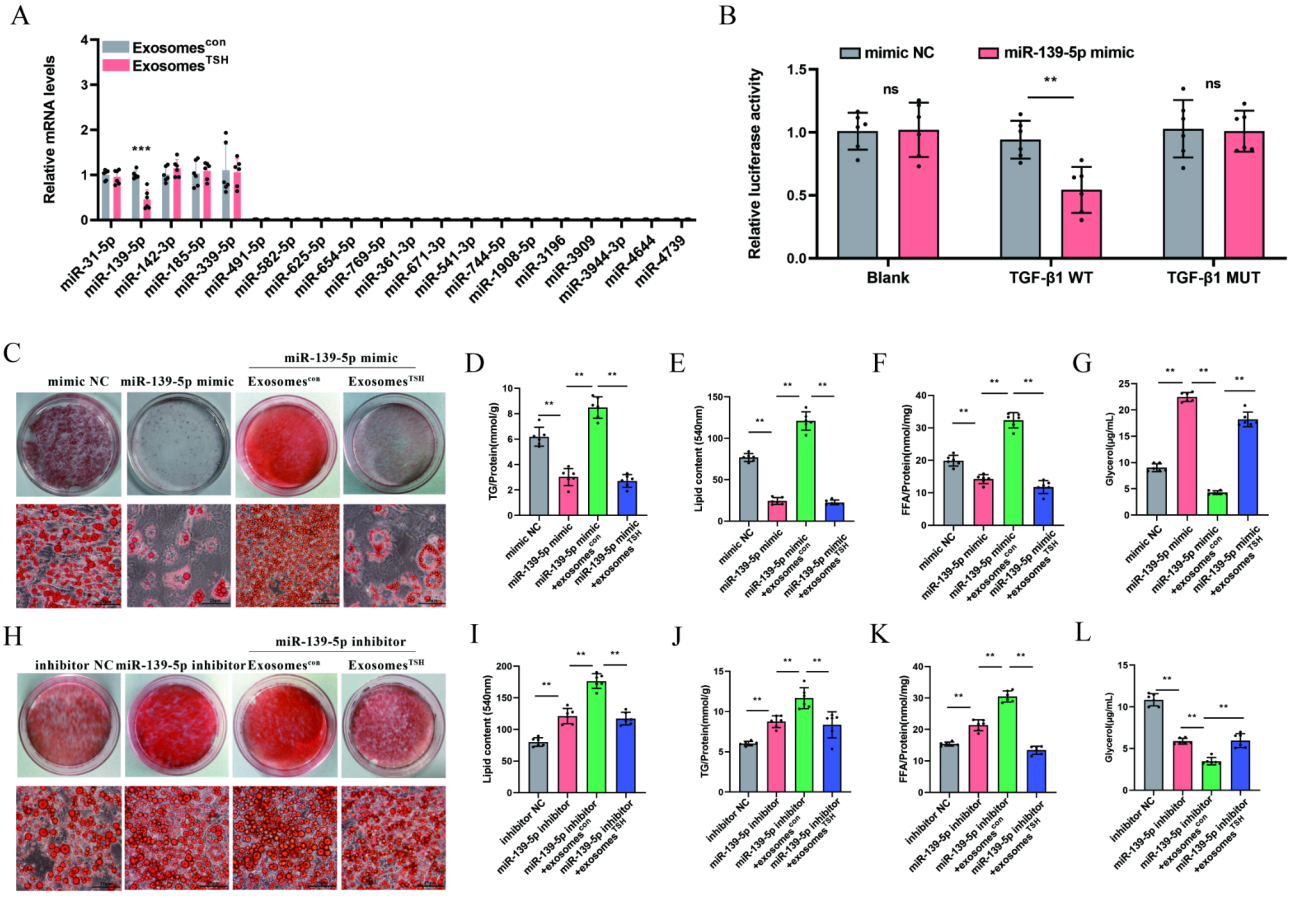

Fig. 7 Preliminary identification of miR-139-5p as a regulator of TGF-β1 expression in exosomes^{TSH}. (**A**) miRNA profiling showing significant downregulation of miR-139-5p in exosomes^{TSH} compared to control exosomes. (**B**) Dual-luciferase reporter assay showing miR-139-5p mimic inhibition of luciferase activity in TGF-β1 3'UTR-WT, with no effect on TGF-β1 3'UTR-MUT. (**C-G**) Oil Red O staining (**C**), Lipid droplet count (**D**), Quantification of triglyceride content (**E**), Quantification of FFA content (**F**) and Measurement of glycerol release (**G**) in 3T3-L1 adipocytes treated with miR-139-5p mimic and exosomes^{TSH}. (**H-L**) Oil Red O staining (**H**), Lipid droplet count (**I**), Quantification of triglyceride content (**J**), Quantification of FFA content (**K**) and Measurement of glycerol release (**L**) in 3T3-L1 adipocytes treated with miR-139-5p mimic and exosomes^{TSH}. Statistical significance: ** $P < 0.01$, *$P < 0.05$

downregulating ATGL. This exosome-mediated liver-adipose signaling axis offers insight into how elevated TSH levels, commonly observed in subclinical hypothyroidism, may contribute to metabolic dysregulation and obesity.

Insights on SCH, TSH, and lipid metabolism

Subclinical hypothyroidism is increasingly recognized as a contributing factor to obesity and lipid metabolism disorders [25–27]. Clinical research has consistently shown positive associations between SCH and various metabolic markers, such as increased BMI, waist circumference, and elevated triglyceride levels [28, 29]. These findings have extended the role of TSH beyond its traditional regulatory function on thyroid hormones, suggesting a broader impact on lipid metabolism and overall energy balance in the body.

Mechanistic studies investigating the pathways through which TSH impacts lipid metabolism have provided valuable insights, with previous research demonstrating that the liver serves as a central mediator of TSH's effects on lipid regulation [30–32]. Specifically, TSH was shown to upregulate hepatic triglyceride content through the activation of sterol regulatory element-binding protein-1c (SREBP-1c), a key regulator of lipid synthesis, and to influence hepatic bile acid homeostasis [7, 8]. These findings highlight the complex role of TSH in modulating hepatic lipid metabolism and suggest that TSH contributes to systemic lipid dysregulation primarily through liver-specific signaling pathways.

This study builds on existing research by identifying the TGF-β1/ ATGL signaling axis as a downstream pathway through which exosomes^{TSH} influence lipid accumulation in adipocytes. By demonstrating that exosomes^{TSH} upregulate TGF-β1 and downregulate ATGL in adipocytes, these findings highlight a previously uncharacterized mechanism of TSH-driven lipid storage beyond the liver. This expands the current understanding of TSH's metabolic effects, linking SCH-associated TSH elevations to adipocyte lipid accumulation, and introduces potential

therapeutic targets within the TSH-driven liver-adipose axis for managing lipid disorders associated with SCH.

Exosome-mediated liver-adipose communication

Exosomes, nanoscale vesicles carrying bioactive molecules like proteins, lipids, and nucleic acids, are key mediators of inter-organ communication [33–35]. Within the liver-adipose axis, exosomes serve as key messengers, transferring molecular cargo from hepatocytes to adipose tissue and influencing metabolic functions [36].

Among exosomal contents, miRNAs have emerged as prominent regulators, due to their ability to modulate gene expression in recipient cells [37, 38]. Exosomal miRNAs can influence a wide range of cellular processes and are thus widely studied in metabolic disease contexts [39–41]. Previous omics analyses identified TSH-stimulated exosomes^{TSH} as carriers of molecules associated with metabolism, immune function, and inflammation, suggesting that exosomes^{TSH} may facilitate metabolic signaling from the liver to adipose tissue.

In the current study, exosomes^{TSH} were demonstrated to drive lipid accumulation in adipocytes through the upregulation of TGF-β1 and the downregulation of ATGL, a key enzyme for triglyceride breakdown. This provides evidence of a TGF-β1/ATGL axis mediated by exosomes^{TSH} in adipocyte lipid accumulation. These findings clarify how SCH-related TSH elevation drives metabolic dysregulation and highlight exosome-mediated pathways as potential therapeutic targets for lipid imbalance.

Role of the miR-139-5p/TGF-β1/ATGL pathway in adipose lipid metabolisms

Lipid metabolism within adipose tissue is a tightly regulated process involving both lipid storage and breakdown, essential for maintaining energy balance [42–44]. ATGL (adipose triglyceride lipase) is pivotal in triglyceride hydrolysis, enabling lipid mobilization [45]. Reduced ATGL expression or activity can impair lipid breakdown, causing triglyceride accumulation and potentially leading to obesity and metabolic dysregulation [46–48].

Conversely, TGF-β1 (transforming growth factor-beta 1) influences adipose tissue expansion and has been associated with lipid storage [49]. TGF-β1's pro-adipogenic effects promote adipocyte hypertrophy and contribute to metabolic stress under conditions of obesity or insulin resistance. Together, TGF-β1 and ATGL represent critical but opposing forces in adipocyte lipid metabolism: while ATGL facilitates lipid mobilization, TGF-β1 encourages lipid storage [50].

This study demonstrates that exosomes^{TSH} modulate the TGF-β1/ATGL axis in adipocytes, leading to enhanced lipid accumulation. By upregulating TGF-β1 and downregulating ATGL, exosomes^{TSH} shift the metabolic balance toward lipid storage, providing a potential mechanistic link between elevated TSH levels and adiposity. This exosome-mediated modulation of adipocyte lipid metabolism represents a novel pathway through which SCH-associated TSH elevations may contribute to obesity, with therapeutic implications for targeting the TGF-β1/ATGL axis in metabolic disorders.

Study limitations and future directions

While this study provides valuable insights into the role of exosomes^{TSH} in adipocyte lipid accumulation, several limitations must be acknowledged. First, these findings are based primarily on cellular and animal models, which, while essential for mechanistic studies, may not fully capture human physiology. HepG2 cells, a human hepatocyte cancer cell line, were used to generate exosomes, which may not fully represent the behavior of normal hepatocytes. Although a mock extracellular vesicle (EV) control was used to minimize the potential impact of TSH contamination, future studies should validate these findings using primary hepatocytes or human liver models to confirm their relevance in humans.

Additionally, the in vivo experiments were conducted exclusively in male mice, and as hypothyroidism affects more women than men, it is crucial to explore potential sex-specific differences in the response to TSH and exosome treatment. Future studies should incorporate both male and female models to assess sex-associated variations in adipocyte metabolism, lipid accumulation, and overall metabolic outcomes. This will provide a more comprehensive understanding of the role of TSH-exosome signaling in adipose tissue metabolism and its implications for sex-specific metabolic disorders.

Moreover, the dietary control used in this study was a standard chow diet rather than a purified low-fat control diet (D12450J). The chow diet was chosen due to its availability and prior validation in similar metabolic studies; however, D12450J would have been a more appropriate control, as it is precisely matched to the high-fat diet (HFD, D12492) in terms of protein content, fat source, and lipid composition. Future studies should incorporate D12450J or other nutritionally matched diets to better isolate the effects of dietary fat content on metabolic outcomes.

Furthermore, while this study primarily focused on white adipose tissue (WAT), it remains unclear whether exosomes^{TSH} also influence brown adipose tissue (BAT) metabolism and thermogenesis. Given BAT's critical role in energy expenditure and thermoregulation, future studies should examine whether exosomes^{TSH} impact BAT function, lipid metabolism, and mitochondrial activity. These investigations

should incorporate histological and molecular analyses to evaluate BAT morphology, UCP1 expression, and metabolic activity. Additionally, exploring these effects under thermoneutral conditions would provide further insight into the physiological relevance of TSH-exosome signaling in energy balance and adipose tissue function.

Lastly, while the experiments suggest a role for exosomes^{TSH} in modulating adipocyte lipid accumulation, the exact mechanism by which these exosomes influence adipocyte differentiation remains unclear. Further studies are needed to investigate whether exosome treatment directly promotes adipogenesis, or whether it primarily affects lipid storage without influencing adipocyte differentiation.

Conclusion

This study uncovers a novel pathway in which TSH-stimulated exosomes drive lipid accumulation in adipocytes by modulating the TGF-β1/ATGL axis, linking elevated TSH levels with adipocyte lipid storage and metabolic imbalance. These findings highlight the TGF-β1/ ATGL axis as a key component in liver-adipose communication in subclinical hypothyroidism, providing insights into the broader role of TSH in lipid metabolism. These results suggest potential therapeutic targets within the exosome-mediated liver-adipose signaling pathway, offering new avenues for managing metabolic dysfunction associated with SCH.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

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Author contributions

S.Z.M. secured funding and oversaw project administration. Y.Y.W., S.T.F., W.L.J., M.L.S., and M.Z.J. conducted the investigation and contributed to data acquisition. M.Z. and W.K.B. were responsible for project administration, investigation, and contributed to the manuscript's review and editing. D.M.W. conceptualized the study, designed the methodology, conducted formal analysis, curated and validated the data, and created visualizations. D.M.W. also drafted the manuscript and contributed to its review and editing. All authors reviewed and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The animal study was reviewed by the Ethics Committee of Shandong Provincial Hospital (NSFC: NO.2019 – 131).

Consent for publication

All authors agreed to submission of the article.

Competing interests

The authors declare no competing interests.

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