Society for Endocrinology
European Society of Endocrinology

Endocrine Connections (2024) **13** e230302
https://doi.org/10.1530/EC-23-0302

Received 27 July 2023
Accepted 21 June 2024
Available online 21 June 2024
Version of Record published 15 July 2024

RESEARCH

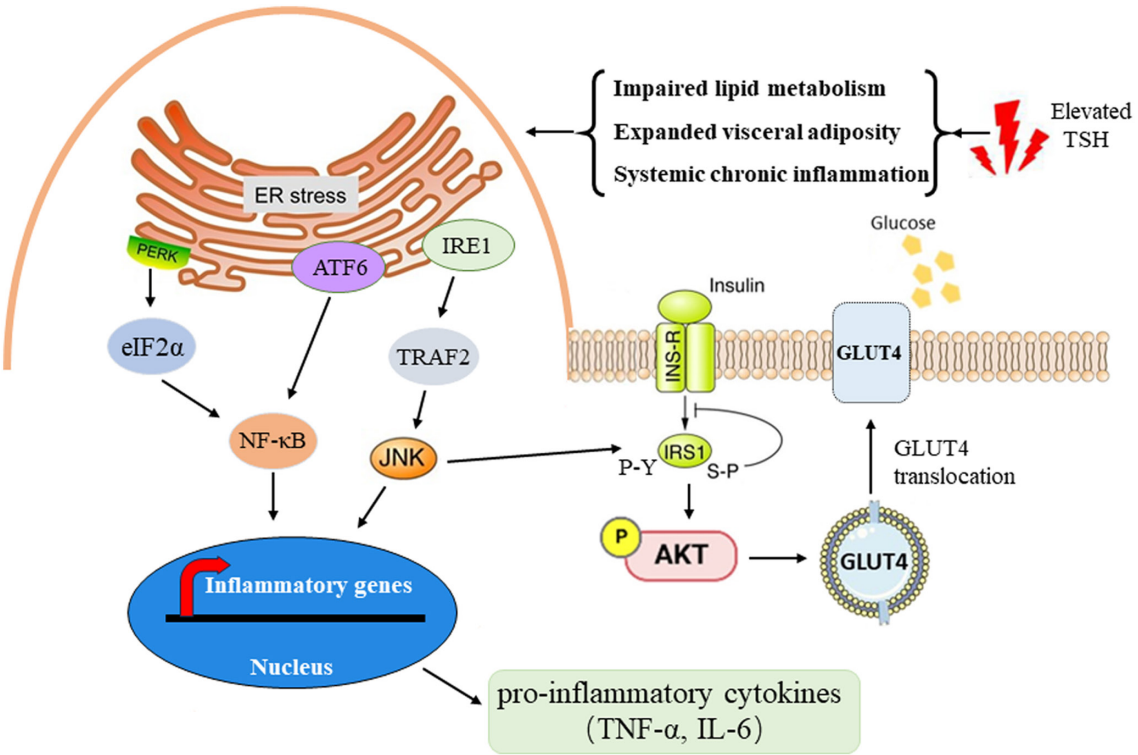
Thyroid-stimulating hormone induces insulin resistance in adipocytes via endoplasmic reticulum stress

Qing Zhou[1], Li Yong Zhang[2], Mei Feng Dai[3], Zhen Li[1], Chao Chun Zou[4] and Hui Liu[1]

[1]Department of Endocrinology, Fujian Maternity and Child Health Hospital, Fujian Children's Hospital, Fuzhou, China
[2]Department of Thyroid Surgery, Minimal Invasive Center, Fujian Medical University Union Hospital, Fuzhou, China
[3]Department of Clinical Lab, Fujian Maternity and Child Health Hospital, Fuzhou, China
[4]Department of Endocrinology, The Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China

Correspondence should be addressed to H Liu or C Zou: zmjia2019@163.com or zcc14@zju.edu.cn

Graphical abstract



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Endocrine Connections (2024) **13** e230302
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Abstract

Subclinical hypothyroidism (SCH) is closely related to insulin resistance, and thyroid-stimulating hormone (TSH) level is an independent factor for insulin resistance associated with subclinical hypothyroidism. This study aims to explore the effects of TSH levels on insulin signal transduction in adipocytes and to establish the role of endoplasmic reticulum (ER) stress in this process. In this study, the SCH mouse model was established, and 3T3-L1 adipocytes were treated with TSH or tunicamycin (TM), with or without 4-phenylbutyric acid (4-PBA), an inhibitor of ER stress. Subclinical hypothyroidism mice exhibited impaired glucose tolerance, inactivation of the IRS-1/AKT pathway, and activation of the IRE1/JNK pathway in adipose tissue, which can all be alleviated by 4-PBA. Supplementation with levothyroxine restored the TSH to normal, alongside alleviated ER stress and insulin resistance in SCH mice, which is characterized by improved glucose tolerance, decreased mRNA expression of IRE1, and decreased phosphorylation of JNK in adipose tissue. In 3T3-L1 adipocytes, TSH induces insulin resistance, leading to a decrease in glucose uptake. This effect is mediated by the downregulation of IRS-1 tyrosine phosphorylation, reduced AKT phosphorylation, and inhibited GLUT4 protein expression. Notably, all these effects can be effectively reversed by 4-PBA. Moreover, TSH induced TNF-α and IL-6 production and upregulated the expression of ER stress markers. Similarly, these changes can be recovered by 4-PBA. These findings indicate that TSH has the capability to induce insulin resistance in adipocytes. The mechanism through which TSH disrupts insulin signal transduction appears to involve the ER stress–JNK pathway.

Keywords: adipocytes; endoplasmic reticulum stress; insulin resistance; thyroid-stimulating hormone

Introduction

Subclinical hypothyroidism has been defined as an elevated serum thyroid-stimulating hormone (TSH) with normal free thyroxine levels (1). Despite maintaining normal thyroid hormone levels, patients with subclinical hypothyroidism have a high risk for insulin resistance-associated disorders, such as metabolic syndrome and cardiovascular disease (1, 2). Moreover, independent of other variables, a significant positive association exists between TSH levels and insulin resistance (3, 4). As the primary peripheral tissue cells of insulin action, adipocytes express TSH receptors and have been recognized as extrathyroidal targets of TSH (5). However, the mechanism underlying high TSH level-induced insulin resistance in adipocytes has not been elucidated.

The endoplasmic reticulum (ER) is the main organelle responsible for the synthesis, processing, and secretion of proteins, as well as the metabolism of lipids and glucose. Extensive studies have shown that ER stress is involved in the development of insulin resistance (6, 7). c-Jun N-terminal kinase (JNK), a kinase that is activated in the early ER stress response, is involved in insulin resistance, which suppresses insulin signaling via the serine phosphorylation of insulin receptor substrate-1 (IRS-1) (8, 9, 10). In addition, studies have shown that the oral administration of chemical chaperones, such as 4-phenyl butyric acid (4-PBA), which alleviates ER stress, improved insulin signaling in ob/ob mice (11).

The present study investigates the effect of high TSH levels on insulin signal transduction in adipose tissue, and the role of ER stress in this process. We found that TSH can induce insulin resistance in adipose tissue, and the mechanism through which TSH disrupts insulin signal transduction appears to involve the ER stress–JNK pathway.

Materials and methods

Materials

Thyrotropic hormone from the human pituitary was purchased from ProSpec (AmyJet Scientific, Wuhan, China). The thyrotropic hormone was prepared by dissolving 10 μg of TSH in double distilled water to a final concentration of 5 mIU/L. Phenylbutyric acid (4-PBA) was obtained from MERCK (Beijing, China). Western blot reagents were obtained from Cell Signaling Technology, and cell culture materials were obtained from Thermos.

Animal experiment

C57BL/6 mice (7 weeks old) were purchased from the Hunan SJA Laboratory Animal (Hunan, China). After 1 week of acclimation, the mice were randomly divided into four groups: the control group ($n=6$), the SCH group ($n=6$), the 4-PBA-treated control mouse group (4-PBA group, $n=6$), and the 4-PBA-treated SCH mouse group (SCH + 4-PBA group, $n=6$). The SCH group mice were given 0.08 mg/kg/day methimazole (MMI, MERCK Pharmaceutical Jiangsu Company, Nantong, China) in their drinking water for 12 weeks, as previously described (12). The control group mice were given the same

amount of vehicle. The 4-PBA group mice were intraperitoneally injected with 4-PBA (100 mg/kg/day) for 4 weeks. The mice in the SCH+4-PBA group were given methimazole alone for the first 8 weeks, followed by simultaneous administration of methimazole in drinking water and intraperitoneal injections of 4-PBA for the subsequent 4 weeks. SCH mice exhibited elevated serum TSH levels but maintained normal thyroid hormone levels compared to control mouse. To normalize TSH levels, mice with subclinical hypothyroidism were provided with levothyroxine in their drinking water for 2 weeks. Levothyroxine (5 μg/mL) was added to drinking water in the treatment of hypothyroid mice, as previously described (13). In our study, levothyroxine (2.5 μg/mL) was used to treat SCH mice according to our preliminary experiment.

All mice were housed at the Animal Care Facility of Fujian Medical University according to the institutional guidelines for laboratory animals, and all the protocols were approved by the Fujian Medical University Institutional Animal Care and Use Committee.

Tolerance test and tissue sampling

At the end of the 12-week modeling period, an intraperitoneal glucose tolerance test (GTT) was performed in mice, as described previously (14). In brief, mice were intraperitoneally injected with glucose (1 g/kg of body weight) after a 14-h fast, and the blood glucose concentrations were measured from tail blood samples at 0, 15, 30, 60, and 120 min after glucose injection using a glucometer (OneTouch Ultra, Johnson). On the day of the GTT experiment, after fasting overnight (12 h), blood samples were collected, centrifuged at 1400 ***g*** for 10 min, and stored at −80°C. At the same time, adipose tissues were surgically removed and frozen in liquid nitrogen until analysis.

3T3-L1 adipocyte cultures and differentiation

3T3-L1 preadipocytes were purchased from the Institute of Basic Medicine, Chinese Academy of Medical Sciences (Beijing, China) and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin–streptomycin at 37°C with 5% CO_2. In the test, the cells were seeded in a six-well plate; when cells were confluent, they were differentiated with DMEM containing dexamethasone (1.0 μmol/L), IBMX (0.5 mmol/L), and insulin (10 μg/mL) with 10% FBS for 2 days. Then, dexamethasone and IBMX were withdrawn, and the treatment with insulin continued for an extra 2 days. Then, insulin was withdrawn, the cells were treated with DMEM containing 10% FBS for another 4–6 days, and more than 90% of the cells were differentiated into adipocytes, as previously reported (15).

Cell Counting Kit-8 assay

3T3-L1 adipocytes were treated with different concentrations of TSH (0.1, 1, 5, 10 mIU/L) for an additional 6, 24, or 48 h, and then cell viability was measured by Cell Counting Kit-8 (Beyotime, Shangai, China) according to the manufacturer's instructions.

Cell treatment

The experimental subjects were divided into a control group (vehicle only), TSH treatment group (i.e. treated with TSH at a final concentration of 5 mIU/L for 24 h), 4-PBA treatment group (i.e. treated with 4-PBA, an inhibitor of ER stress, at a final concentration of 2.5 mmol/L for 24 h), TM (a molecule that specifically aggravates ER stress) treatment group (i.e. treated with TM at a final concentration of 5 μg/mL for 5 h), TSH inhibitor group (TSH+4-PBA, treated with TSH and 4-PBA simultaneously for 24 h), and TM inhibitor group (TM+4-PBA, treated with 4-PBA for 24 h and TM for 5 h). Prior to protein extraction for downstream examination, cells were treated with 100 nM insulin for 10 min.

Glucose uptake measurement

For glucose uptake measurement, the medium of cells was replaced with DMEM (low glucose, 1 g/L) for 1 h followed by DMEM (high glucose, 4.5 g/L) containing 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxy-D-glucose (2-NBDG, a fluorescent glucose analog) (Sigma) (100 μM; final concentration) and 100 nM insulin for 30 min. After washing twice with PBS to remove free 2-NBDG, cells were collected and 2-NBDG uptake activity was measured with a FACS flow cytometer (BD Biosciences) and analyzed using CellQuest software, as previously reported (14).

Enzyme-linked immunosorbent assay

For 3T3-L1 adipocytes, the culture supernatant was collected after treatment, and TNF-α and interleukin-6 (IL-6) concentrations were measured using an ELISA kit (R&D Systems) according to the manufacturer's instructions. For the *in vivo* experiment, serum TSH, FT3, and FT4 levels of mice were determined using an ELISA kit (Jiangsu Meimian, Yancheng, China) following the product manual.

Quantitative real-time PCR

Total RNA from 3T3-L1 adipocytes and adipose tissue was extracted using TRIzol reagent (Takara Biotechnology) according to the manufacturer's instructions. The mRNA levels were determined by quantitative RT-PCR, as previously described (12). The relative mRNA levels of the target gene were normalized to β-actin,

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and the differences in mRNA levels were calculated using the $2^{-\triangle\triangle Ct}$ method. The primer sequences for GLUT4 are forward 5'-CTTCCTTCTATTTGCCGTCCTC-3' and reverse 5'-GTTTTGCCCCTCAGTCATTCTC-3', the primer sequences for IRE1 are forward 5'-ACGAAGGCCTGACGAAACTT-3' and reverse 5'-ATCTGAACTTCGGCATGGGG-3', and the pairs for β-actin are forward 5'-CTGTGCCCATCTACGAGGGCTAT-3' and reverse 5'-TTTGATGTCACGCACGATTTCC-3'.

Western blot

Proteins were extracted using a Protein Extraction Kit (Beyotime) according to the manufacturer's instructions. The protein concentration was determined using the BCA method. Approximately 80 μg protein were separated using 8–10% SDS-PAGE, transferred to a PVDF membrane, and probed with primary antibodies at 4°C overnight, followed by incubation in IRDye secondary antibodies (LI-COR) for 1 h at room temperature. The immunoreactive signals were visualized with an Odyssey Infrared Imaging System (LI-COR). Antibodies against P-AKT, AKT, and GLUT4 were all purchased from Cell Signaling Technology. Antibodies against P-IRS-1, IRS-1, P-PERK, PERK, ATF6, IRE1, P-IRE1, JNK, and P-JNK were all obtained from Abcam. Antibodies against β-Actin and GAPDH were purchased from Santa Cruz Biotechnology. β-Actin and GAPDH were used as the internal standard for the total protein.

Statistical analysis

Numerical data are expressed as mean ± S.E.M. Student's *t*-test and the Mann–Whitney *U* test were used to compare groups according to the parametric values. One-way or two-way ANOVA and Dunnett's multiple comparison tests were used to determine statistical significance, and $P < 0.05$ was considered statistically significant.

Results

The impact of different concentrations of TSH on the viability of 3T3-L1 adipocytes

We quantified the cell viability of 3T3-L1 adipocytes treated with different concentrations of TSH (0.1, 1, 5, 10 mIU/L) for different times (6, 24, 48 h) by cell counting kit-8 assays to exclude the cytotoxicity of TSH. In the 6-h group, cell viability significantly increased with different concentrations of TSH, while it significantly decreased in the 48-h group. In the 24-h group, cell viability was dose-dependently reduced by treatment with TSH. No cytotoxicity was observed by exposure of 3T3-L1 adipocytes to 5 mIU/L TSH for 24 h (Fig. 1). Therefore, in the following experiments, 3T3-L1 adipocytes were treated with 5 mIU/L TSH for 24 h.

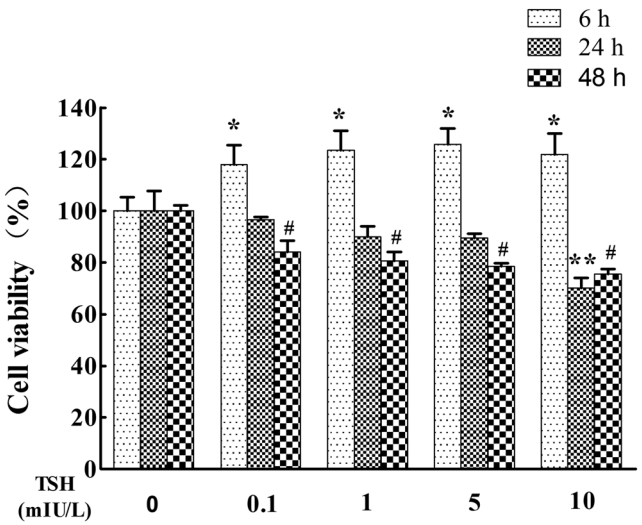

Figure 1

The effect of different concentrations of TSH on the viability of 3T3-L1 adipocytes. Cell viability was measured by Cell Counting Kit-8. Data are expressed as mean ± S.E.M. ($n = 8$). *$P < 0.05$ versus control in the 6-h group. **$P < 0.05$ versus control in the 24-h group. #$P < 0.05$ versus control in the 48-h group.

TSH induces insulin resistance in 3T3-L1 adipocytes

To explore the impacts of TSH on glucose metabolism, we assessed glucose uptake in 3T3-L1 adipocytes. Compared to the control group, the TSH group exhibited impaired 2-NBDG uptake, whereas treatment with the ER stress inhibitor 4-PBA restored the reduction in 2-NBDG uptake induced by TSH in 3T3-L1 adipocytes (Fig. 2A). Additionally, Western blot analysis revealed that TSH significantly reduced IRS-1 tyrosine phosphorylation at position Tyr632, decreased AKT phosphorylation at position Ser473, and downregulated GLUT4 protein and mRNA expression, all of which could be reversed by 4-PBA (Fig. 2B and C). These results underscored the involvement of the IRS-1/AKT/GLUT4 pathway in TSH-induced insulin resistance *in vitro* and highlighted that the ER stress inhibitor, 4-PBA, can attenuate TSH-induced insulin resistance by restoring the IRS-1/AKT/GLUT4 pathway in 3T3-L1 adipocytes.

TSH induces insulin resistance via ER stress in 3T3-L1 adipocytes

We next explored the involvement of the ER stress pathway in TSH-induced insulin resistance in 3T3-L1 adipocytes. The results showed that compared to the control group, the expression of PERK, ATF6, and IRE1, core proteins of the ER stress signaling pathway was significantly induced by TSH and TM (a molecule that specifically aggravates ER stress). In addition, as a key mediator of ER stress that leads to insulin resistance,

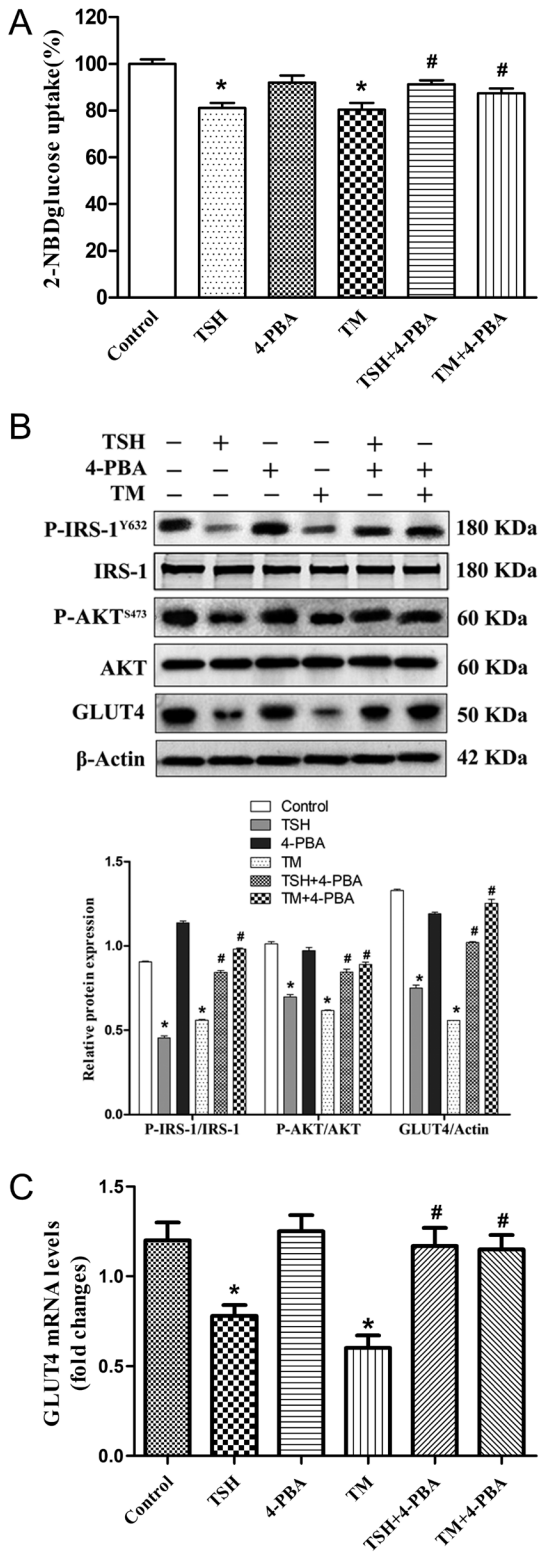

Figure 2

TSH induces insulin resistance in 3T3-L1 adipocytes. (A) 3T3-L1 adipocytes were treated with 2-NBDG (100 μM final concentration) and insulin (100 nM final concentration) for 30 min, and then 2-NBD glucose uptake was measured by flow cytometry ($n = 5$). (B) 3T3-L1 adipocytes were treated with 4-PBA (2.5 mmol/L, 24 h) before the cells were treated with or without TSH (5 mIU/mL, 24 h) or TM (5 μg/mL, 5 h). To check insulin signaling molecules, cells were treated with 100 nM insulin for 10 min before the protein was collected. The protein expression and phosphorylation of IRS-1, AKT, and GLUT4 were determined by Western blotting ($n = 3$). (C) The relative mRNA level of the GLUT4 gene was measured by qRT-PCR ($n = 5$). Data are expressed as mean ± S.E.M. *$P < 0.05$ versus the control group. #$P < 0.05$ versus TSH or TM group.

the phosphorylation level of JNK was also increased in the TSH and TM groups. Moreover, TSH-mediated activation of the ER stress signaling pathway can also be reversed by the ER stress inhibitor 4-PBA in 3T3-L1 adipocytes (Fig. 3). These data demonstrated that TSH induces insulin resistance in 3T3-L1 adipocytes by activating the ER stress pathway.

TSH increases TNF-α and IL-6 release in 3T3-L1 adipocytes

Chronic low-grade inflammation plays a major role in the development of insulin resistance. Our previous study found that TNF-α induced insulin resistance in HepG2 cells through activating the IRS-1/AKT/GSK3β pathway (14). Adipose tissue is also known to secrete a variety of cytokines, including TNF-α and IL-6, which are associated with a pro-inflammatory state that is related to insulin resistance. More importantly, TNF-α and IL-6 are critical downstream target genes of the JNK pathway (16), which is activated by TSH in our insulin resistance model. To examine the effect of TSH on TNF-α and IL-6 release in 3T3-L1 adipocytes, we determined the concentrations of TNF-α and IL-6 in the medium. Our results showed that compared to the control group, both the TM group and TSH group exhibited a strong increase in TNF-α and IL-6 secretion. However, the ER stress inhibitor 4-PBA significantly decreased TNF-α and IL-6 secretion (Fig. 4). These results indicated that TSH might increase TNF-α and IL-6 release in 3T3-L1 adipocytes by regulating the ER stress–JNK pathway.

Subclinical hypothyroidism induces insulin resistance via ER stress in mice

To determine the effects of subclinical hypothyroidism on glucose metabolism and the potential mechanisms *in vivo*, we established a subclinical hypothyroidism mouse model. There were no significant differences in the body weight of SCH mice compared with the control mice, suggesting that SCH did not affect the growth of mice. Compared to controls, mice with subclinical hypothyroidism displayed increased TSH and normal FT4 levels (Fig. 5A). First, we observed an increase in plasma glucose levels in the SCH group compared to the control group, which could be decreased by using 4-PBA (Fig. 5B). The difference was indicated by the area under the glucose curve (AUC) of the glucose tolerance test (GTT) (Fig. 5C). Meanwhile, Western blot analysis showed that the phosphorylation of IRS-1

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https://doi.org/10.1530/EC-23-0302

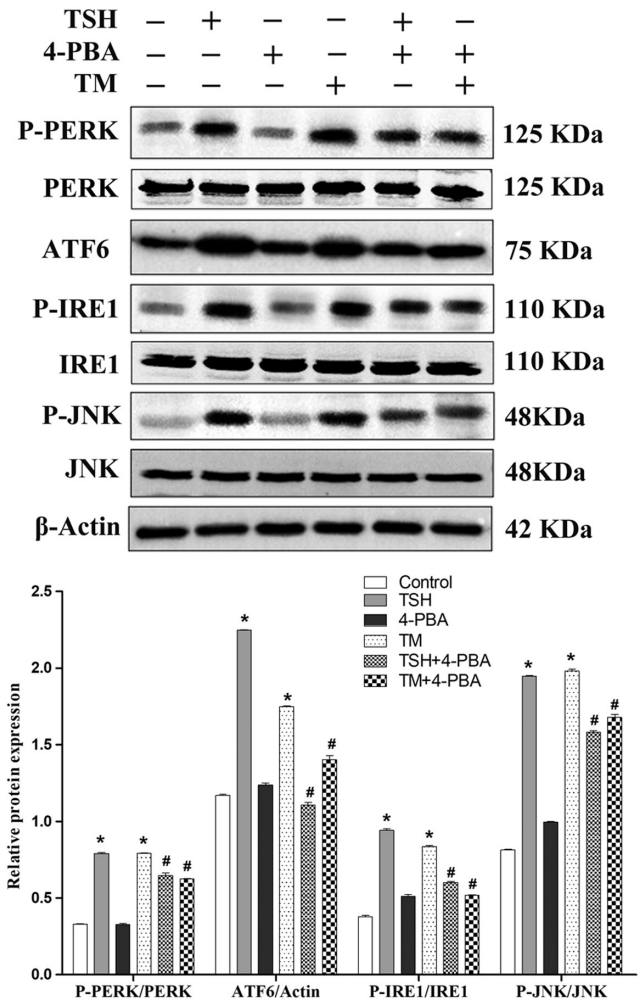

Figure 3

TSH induces insulin resistance via ER stress in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with 4-PBA (2.5 mmol/L, 24 h) before the cells were treated with or without TSH (5 mIU/L, 24 h) or TM (5 μg/mL, 5 h). The protein expression and phosphorylation of PERK, ATF6, IRE1, and JNK were determined by Western blotting. Data are expressed as mean ± S.E.M. ($n = 3$). *$P < 0.05$ versus control group. #$P < 0.05$ versus TSH or TM group.

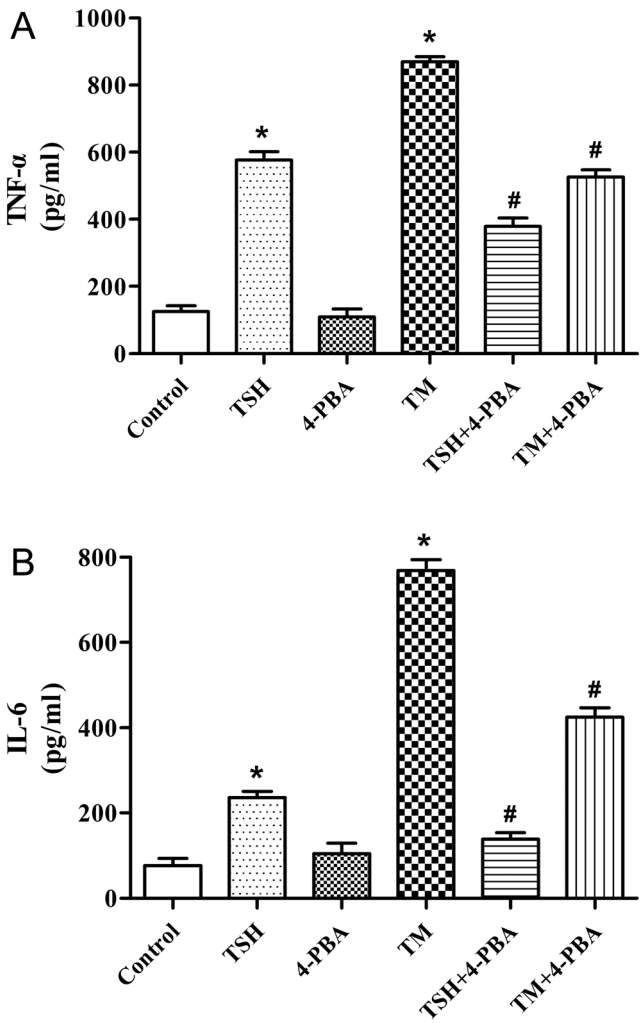

Figure 4

TSH increased TNF-α and IL-6 secretion in 3T3-L1 adipocytes. TNF-α and IL-6 concentrations in the medium were measured by ELISA. Data are expressed as mean ± S.E.M. ($n = 5$). *$P < 0.05$ versus control group. #$P < 0.05$ versus TSH or TM group.

and AKT decreased in adipose tissue in the SCH group but significantly increased in the SCH+4-PBA group. In contrast, the phosphorylation of IRE1 and JNK increased in mice in the SCH group but significantly decreased in mice in the SCH+4-PBA group (Fig. 5D). These *in vivo* data suggest that subclinical hypothyroidism impairs glucose metabolism by inactivating the IRS-1/AKT pathway in the adipose tissue of mice, which might involve the ER stress–JNK pathway.

Supplementation with thyroid hormone reverses ER stress and insulin resistance in mice

To further clarify whether the normalization of TSH can alleviate ER stress and insulin resistance in mice with subclinical hypothyroidism, the mice with subclinical hypothyroidism were supplemented with levothyroxine in drinking water for 2 weeks. Results showed that supplementation with levothyroxine restored the TSH to normal in subclinical hypothyroid mice but had no significant impacts on FT4 level (Fig. 6A). Meanwhile, the plasma glucose levels of mice treated with levothyroxine were significantly downregulated compared to those in the SCH group (Fig. 6B). The difference was indicated by the area under the glucose curve (AUC) of the GTT (Fig. 6C). The mRNA expression of IRE1 and the phosphorylation of JNK in adipose tissue of mice treated with levothyroxine were decreased compared to those in the SCH group (Fig. 6D). These data suggest that the normalization of TSH can alleviate ER stress and insulin resistance in mice with subclinical hypothyroidism.

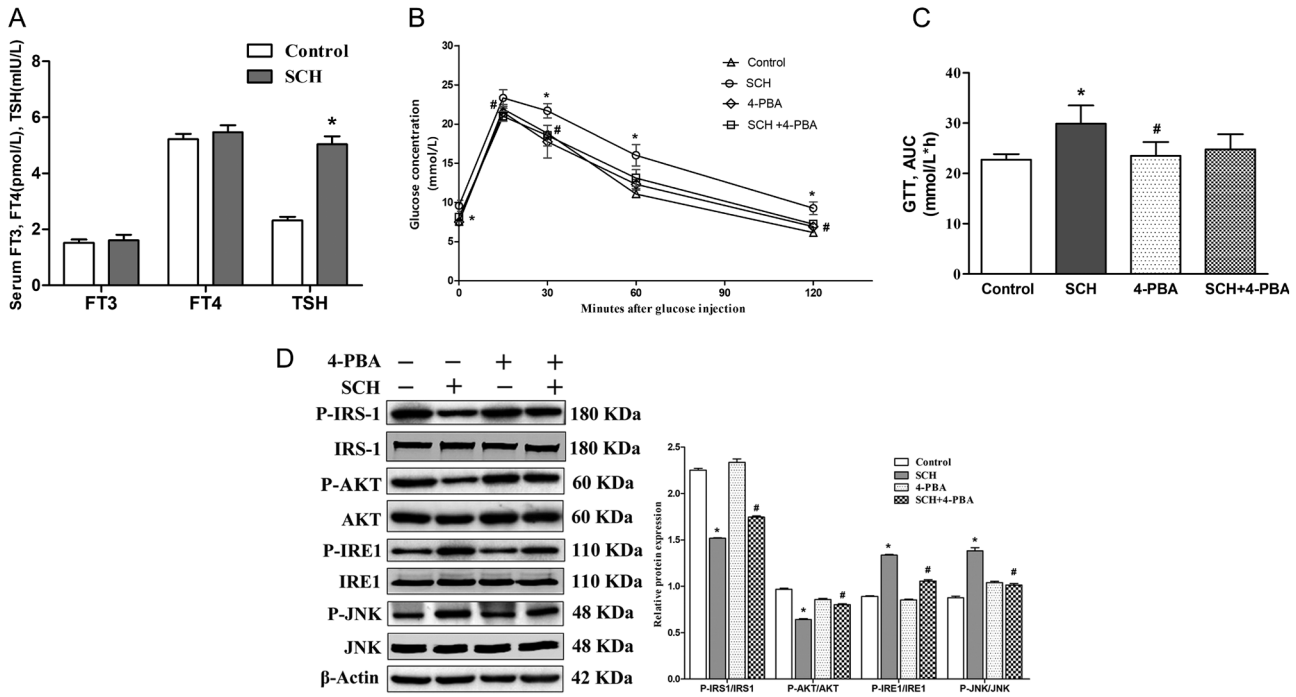

Figure 5

Subclinical hypothyroidism induces insulin resistance via ER stress in mice. The subclinical hypothyroidism (SCH) mouse model was established by giving methimazole (0.08 mg/kg/day) in their drinking water for 12 weeks. Serum levels of thyroid-stimulating hormone (TSH), FT3, and FT4 were measured by ELISA ($n = 6$). (B) A glucose tolerance test (GTT) was performed to detect glucose tolerance at the end of the 12-week modeling period ($n = 6$). (C) Blood glucose values for the area under the curve (AUC) were calculated during GTT. (D) Adipose tissue lysates from each group were prepared, and the protein expression and phosphorylation of IRS-1, AKT, IRE1, and JNK were determined by Western blotting ($n = 3$). Data are expressed as mean ± S.E.M.. *$P < 0.05$ versus control group. #$P < 0.05$ versus the SCH group.

Discussion

Studies have shown that patients with clinical hypothyroidism and subclinical hypothyroidism have an increased risk for insulin resistance (1, 17, 18). The common ground between clinical hypothyroidism and subclinical hypothyroidism is elevated TSH levels. Recent evidence has demonstrated that serum TSH levels are independently and positively correlated with insulin resistance in patients with normal thyroid function (3, 4, 19). TSH action is initiated by its binding to the TSH receptor, which is located on the surface of thyrocytes and adipose tissue (20). However, studies on the direct association between TSH and insulin resistance in adipocytes are limited.

Insulin resistance is characterized by decreased insulin sensitivity in three key insulin target tissues: skeletal muscle, liver, and adipose tissue (21). Recent studies have reached the unifying concept that adipose tissues are central regulators of systemic glucose homeostasis by controlling liver and skeletal muscle metabolism (22). In adipose tissues, insulin resistance is reflected as decreased insulin-stimulated glucose uptake and utilization, which is critically dependent on the IRS-1/AKT/GLUT4 signaling pathway (21). Consistent with these findings, our study found that TSH impaired glucose uptake in 3T3-L1 adipocytes, accompanied by a decrease in the expression of GLUT4 and the phosphorylation level of IRS-1 and AKT protein. Moreover, *in vivo* studies have also shown that subclinical hypothyroidism impairs glucose metabolism and inhibits activation of the IRS-1/AKT pathway, and normalization of the TSH by levothyroxine can sufficiently alleviate ER stress and insulin resistance. These results imply that TSH could inhibit insulin signal transduction in adipose tissue via the IRS-1/AKT/GLUT4 pathway, resulting in insulin resistance.

Adipose tissue is known to be an endocrine organ that secretes many cytokines, including TNF-α and IL-6. In a previous study, we found that TNF-α could induce insulin resistance in HepG2 cells (14), suggesting that chronic inflammation plays an important role in developing insulin resistance. Birsen *et al.* reported significantly elevated serum TNF-α levels in overt and subclinical hypothyroid patients (23), which suggests that an association exists between high TSH levels and chronic low-grade inflammation. Likewise, studies have shown that TSH can stimulate the production of adipokines, such as TNF-α and IL-6, in adipocytes (24, 25), and these adipokines have been considered a pro-inflammatory stimulus of adipocytes (26). Therefore, cytokine-related

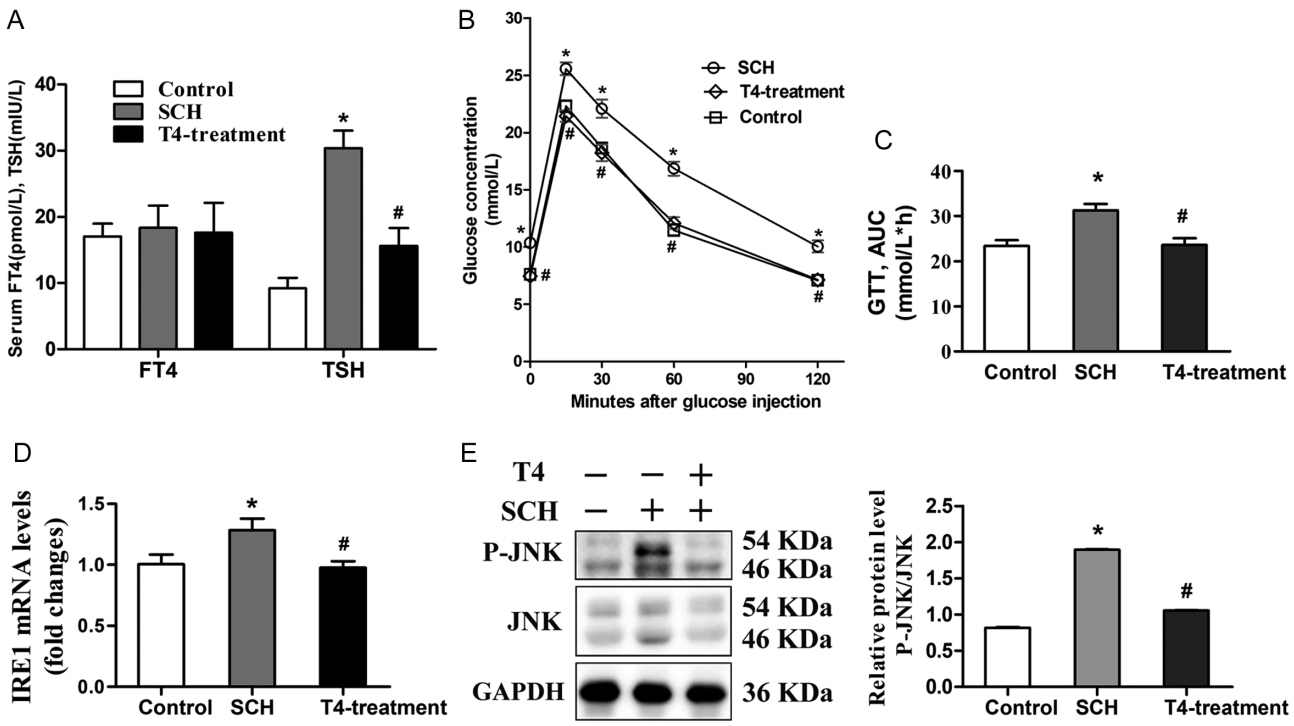

Figure 6

Supplementation with thyroid hormone reverses ER stress and insulin resistance in mice. (A) Mice with subclinical hypothyroidism were supplemented with levothyroxine in drinking water for 2 weeks, and serum levels of thyroid-stimulating hormone (TSH), and FT4 were measured by ELISA ($n = 5$). (B) A glucose tolerance test (GTT) was performed to detect glucose tolerance at the end of the 2-week levothyroxine supplementation ($n = 5$). (C) Blood glucose values for the area under the curve (AUC) were calculated during GTT. (D) The relative mRNA level of the IRE1 gene was measured by qRT-PCR ($n = 3$) (E) Adipose tissue lysates from each group were prepared, and the protein expression and phosphorylation of JNK were determined by Western blotting ($n = 3$). Data are expressed as mean ± S.E.M. $^{*}P < 0.05$ versus control group. $^{\#}P < 0.05$ versus the SCH group.

chronic inflammation may be considered the underlying mechanism for insulin resistance in hypothyroidism. In agreement with our previous results, in the present study, we found that TSH induced TNF-α and IL-6 secretion in 3T3-L1 adipocytes, and these effects were alleviated by 4-PBA, an ER stress inhibitor, suggesting that ER stress might be involved in the process of TSH-induced insulin resistance. We found that TSH significantly increased the protein levels of ER stress-related markers, including RNA-dependent protein kinase (PKR)-like ER eukaryotic translation initiation factor 2 alpha kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring protein enzyme (IRE)-1, which were similar to the increase in response to TM, an ER stress agonist. Moreover, in the current study, the addition of the ER stress inhibitor 4-PBA blocked the expression of PERK, ATF6, and IRE1 induced by TSH, suggesting that TSH could induce ER stress in adipocytes.

ER stress has emerged as a critical regulator of systemic metabolic homeostasis (27). Numerous studies have indicated that ER stress plays a crucial role in insulin resistance (7, 28). Under normal conditions, insulin receptor-mediated tyrosine phosphorylation of IRS-1 induces the activation of the PI3K–AKT pathway, while serine phosphorylation of IRS-1 at specific serine residues inhibits insulin signaling (29). It has been suggested that ER stress disrupts insulin signaling in adipocytes by activating the IRE1-JNK signaling pathway, which increases IRS-1 serine phosphorylation and decreases IRS-1 tyrosine phosphorylation, leading to insulin resistance (9, 30). Consistent with these findings, our study found that TSH significantly increased the phosphorylation level of JNK, which was blocked after the application of 4-PBA. Meanwhile, we observed an increase in IRS-1 tyrosine phosphorylation and AKT phosphorylation, as well as increased GLUT4 expression after the application of 4-PBA. These effects have also been confirmed *in vivo*, showing that inactivation of the IRS-1/AKT pathway and activation of the IRE1/JNK pathway occurred in the adipose tissue of mice with subclinical hypothyroidism, which was alleviated by 4-PBA. These findings suggested that TSH can induce insulin resistance in adipose tissue via ER stress.

In conclusion, our study suggests that TSH can induce insulin resistance in adipose tissue, and the mechanism through which TSH disrupts insulin signal transduction appears to involve the ER stress–NK pathway. Elevated TSH in subclinical hypothyroidism leads to ER stress in adipose tissue, possibly via impaired lipid metabolism,

expanded visceral adiposity, and systemic chronic inflammation. On the one hand, ER stress upregulates inflammatory gene expression and increases pro-inflammatory cytokine production. On the other hand, as a key mediator of ER stress that leads to insulin resistance, JNK blocks insulin signaling via the serine phosphorylation of IRS1 and downregulation of GLUT4 expression, leading to decreased glucose uptake. Our study's novelty lies in identifying ER stress in the development of TSH-induced insulin resistance, which may enlarge our understanding of insulin resistance in subclinical hypothyroidism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

Funding

This work was supported by the Fujian Provincial Natural Science Foundation of China (2020J05273) and the Startup Fund for Science and Technology Innovation, Fujian Children's Hospital (YCXX202102).

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