Article

Discovery Medicine 2024; 36(186): 1370–1377
https://doi.org/10.24976/Discov.Med.202436186.127

Ghrelin Improves Glucolipotoxicity-Induced Pancreatic β-Cellular Dysfunction and Apoptosis by Inhibiting Endoplasmic Reticulum Stress-Induced IRE1/JNK Pathway

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Published: 20 July 2024

Background: Glucose and fatty acid overload-induced glucolipid toxicity of pancreatic β-cells is associated with the development of diabetes. Endoplasmic reticulum stress (ERS) plays an essential role in this process. Ghrelin, a peptide secreted by the pancreas, negatively correlates with oxidative stress. The study aimed to investigate ghrelin's role in glycolipid-induced β-cell dysfunction and its possible mechanism.

Methods: Mouse insulinoma β-cell, NIT-1 cells, were stimulated with high fat and high glucose to induce glucolipid toxicity. High fat and high glucose-induced NIT-1 cells were treated with acylated ghrelin (AG) or [d-Lys3]-growth hormone releasing peptide (GHRP)-6. Flow cytometry and Cell Counting Kit-8 (CCK-8) assay were performed to assess apoptosis and cell viability. The protein expression related to apoptosis, inositol-requiring kinase 1 (IRE1)/c-Jun N-terminal kinase (JNK) signaling, and ERS were investigated using western blot. Enzyme-linked immunosorbent assay (ELISA) was adopted to examine insulin's synthesis and secretion levels.

Results: Ghrelin treatment improved cell viability while inhibiting cell glucolipotoxicity-induced NIT-1 cell apoptosis. Ghrelin can promote the synthesis and secretion of insulin in NIT-1 cells. Mechanistically, ghrelin attenuates ERS and inhibits the IRE1/JNK signaling pathway in NIT-1 cells induced by glucolipotoxicity.

Conclusion: Ghrelin improves β-cellular dysfunction induced by glucolipotoxicity by inhibiting the IRE1/JNK pathway induced by ERS. It could be an effective treatment for β-cellular dysfunction.

Keywords: glucolipid; ghrelin; β-cellular dysfunction; IRE1/JNK signaling pathway

Introduction

Metabolic disorders, including obesity and diabetes mellitus, put a significant burden on the world health system [1]. The pancreatic β-cells belong to an essential group of pancreatic cells that coordinate insulin release in response to changes in blood glucose level, thereby regulating glucose homeostasis. In contrast, the loss and dysfunction of pancreatic β-cells result in insulin resistance and lead to diabetes [2–4]. In addition, metabolic disorders impair the function and survival of pancreatic β-cells in diabetes [5]. Therefore, further understanding of the mechanisms for regulating pancreatic β-cells function remains to be uncovered.

Studies have shown that the native structure of insulin is further processed in the secretory vesicles after its formation of the endoplasmic reticulum to become bioactive insulin [6]. The endoplasmic reticulum, a membrane-bound organelle widely distributed in the cytoplasm of pancreatic β-cells, is the primary site for the folding and quality control of secreted and transmembrane proteins. Since the insulin folding process mainly occurs in the endoplasmic reticulum, maintaining the stability of endoplasmic reticulum proteins is crucial [7–10]. Endoplasmic reticulum dysfunction can aggravate metabolic stress, including type 1 and type 2 diabetes, resulting in endoplasmic reticulum stress (ERS) that further exacerbates metabolic disorders [11–13]. Therefore, targeting the endoplasmic reticulum of pancreatic β-cells shows therapeutic opportunities for metabolic disorders.

The ERS-induced unfolded protein response is activated by various membrane transducers, including Protein kinase R-like endoplasmic reticulum kinase (PERK), inositol-requiring kinase 1 (IRE1), and activating transcription factor 6 (ATF6). PERK activation promotes the expression of pro-apoptotic CCAAT/enhancer-binding protein homologous protein (CHOP). C-Jun N-terminal kinase (JNK) could be activated by IRE1, which can induce cell apoptosis [14]. The 78-kDa glucose-regulated protein (GRP78), a critical regulator of endoplasmic reticu-

lum function, is crucial in regulating endoplasmic reticulum transmembrane transducers and facilitates protein folding [15,16]. The IRE1/JNK signaling pathway is an important pathway to regulate the ERS, and previous studies have revealed that inhibiting the IRE1/JNK/CHOP signaling pathway could suppress the ERS [17,18]. Recent studies have elucidated the mechanisms by which ERS induces the IRE1/JNK pathway. Prolonged exposure of β-cells to high glucose induces ERS and hyperactivates IRE1. Activated IRE1 splices X-box binding protein-1 (XBP1) mRNA. The phosphorylation of IRE1 induces a cascade of events that activate mitogen-activated protein kinase kinase kinase (MAP3K) and apoptosis signal-regulating kinase 1 (ASK1), ultimately leading to the phosphorylation and subsequent activation of JNK [19,20]. Flintoaca Alexandru *et al*. [21] demonstrated that ER degradation-enhancing alpha-mannosidase-like protein1 (EDEM1) regulates insulin mRNA levels by inhibiting the ERS-induced IRE1/JNK/c-Jun pathway.

Ghrelin, produced primarily by the pancreas and stomach, is a 28-amino acid peptide. Clinical trials have shown that ghrelin levels in type 2 diabetes had a negative correlation with oxidative stress [22,23]. Ghrelin has anabolic functions that stimulate growth hormone secretion, modulate hypothalamic circuits, and maintain β-cell viability and proliferation [24]. Studies have indicated that ghrelin has important obesogenic/diabetogenic properties, and ghrelin blockade might act as a method to prevent the diabetogenic effect [25]. The ghrelin system also regulates the progression of gastrointestinal tract cancer [26]. However, whether ghrelin could regulate insulin secretion and synthesis of pancreatic β-cells, as well as the underlying mechanisms, remain to be clarified. Herein, the effects of ghrelin on β-cells function and the ERS were investigated. This study is the first to demonstrate the protective effect of ghrelin against glucolipotoxicity in pancreatic β-cells, particularly in inhibiting the ERS-induced IRE1/JNK pathway. These findings advance our understanding of cellular stress responses and open new avenues for therapeutic strategies in diabetes and metabolic disorders.

Materials and Methods

Cell Culture and Treatment

The NIT-1 mouse insulinoma β-cell line (Procell, CL-0562, Wuhan, China) was used in this study. NIT-1 cells were cultivated in Low-glucose Dulbecco's Modified Eagle Medium (DMEM, Gibco, 11885084, Waltham, MA, USA) with the addition of 0.1 mg/mL streptomycin (15140-122, Gibco, Waltham, MA, USA), 10% fetal bovine serum (FBS, Hyclone, V30087.03, Logan, UT, USA), and 100 U/mL penicillin at 37 °C with 5% CO_2. The cells were identified by immunofluorescence with insulin antibody (ET1601-12, HUABIO, Hangzhou, China) (**Supplementary Fig. 1**) and tested as mycoplasma-free before experiments.

NIT-1 cells were separated into different groups with various treatments. In the control group, conventional culture medium [0.5% bovine serum albumin (BSA) + 5.6 mM glucose] was utilized to culture NIT-1 cells; NIT-1 cells were cultured with high fat and high glucose [0.5 mM palmitic acid (PA) + 0.5% BSA + 33.3 mM glucose (HG)] in the HG/PA group [27]; in the acylated ghrelin (AG) group, NIT-1 cells were incubated with high fat and high glucose, and treated with acylated Ghrelin (10 mM Acyl Ghrelin, HY-P1366, MedChemExpress, Monmouth Junction, NJ, USA); in the AG+[d-Lys3]-growth hormone releasing peptide (GHRP)-6 group, cells were treated with high glucose and high-fat culture, using acylated ghrelin (10 mM Acyl ghrelin) and ghrelin receptor antagonist [d-Lys3]-GHRP-6 (100 µM, HY-P4278, MedChemExpress, Monmouth Junction, NJ, USA) [28,29].

Cell Counting Kit-8 (CCK-8) Assay

After 12, 24, and 48 hours of treatments, the CCK-8 Kit (HY-K0301, MedChemExpress, Monmouth Junction, NJ, USA) was used for cell viability detection *in vitro*. A 96-well plate was used to inoculate NIT-1 cells (5.0×10^3 cells/well). Then, CCK-8 solution was applied to wells for incubation at 37 °C for 2 hours. Each well's optical density (OD) value was determined using the enzyme-linked immunosorbent assay at 450 nm. Cell viability% = [(Ae – Ab)/(Ac – Ab)] × 100%. Ac is the OD value of the control well; Ab is the OD value of the blank well; Ae is the OD value of the experimental well.

Flow Cytometry

After various interventions, 1% bovine serum albumin (BSA)/phosphate buffer saline (PBS) buffer was gently used to scrape NIT-1 cells. Then, a 5-min centrifugation (800 ×g) was performed with PBS/BSA buffers, and resuspended in PBS/BSA buffer after the supernatant was thrown away. After repeating the above steps twice, cells were diluted at about 1×10^6 cells/mL in PBS/BSA buffer. Then, cells were stained with Annexin V-fluorescein isothiocyanate (FITC, abs50001, absin, Shanghai, China) for 15 min in the dark and then stained with propidium iodide (PI, abs50001, absin, Shanghai, China). Cells without antibody staining were used as the negative control. Samples were analyzed using BD FACSCanto II (Becton Dickinson, Franklin Lakes, NJ, USA) instrument, and all data was detected by FlowJo 10.6.2 (Treestar Inc., Ashland, OR, USA) software.

Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA kit (Crystal Chem, CCM-90082-1, Downers Grove, IL, USA) determined the insulin level. Two types of samples in this study include insulin from cells and supernatant, respectively. Cell supernatant was collected to detect insulin in the supernatant and then cells were rinsed with PBS to detect insulin in cells following

lysis. Briefly, a 5 µL sample was added with 95 µL diluent before incubation at 4 °C for 2 h. After three rinses, 100 µL conjugated solution was supplemented and then subjected to 30 min of incubation at ambient temperature. Then, after rinsing the plate three times, 100 µL substrate solution was added. Followed by incubation for 40 min, 100 µL stop solution was mixed, and the OD value of each well was analyzed at 450/630 nm by Multiskan Spectrum Microplate Spectrophotometer (Multiskan SkyHigh, thermofisher, Waltham, MA, USA) [30].

Western Blot

After the diverse treatments described above, Cell Lysis (C2978, Sigma-Aldrich, St. Louis, MO, USA) with the protease inhibitor cocktail (CW2200S, CWBIO, Jiangsu, China) was adopted to obtain total protein from cells. Western blot was adopted based on the standard protocol, and β-actin was considered the loading control. Briefly, the equivalent amounts of proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto PVDF membranes at 0.45 µm. Then, 5% BSA buffer $\times$ Tris Buffered Saline with Tween (TBST) diluted with one was performed to block the membranes at ambient temperature for 1 h, followed by incubating with corresponding primary antibodies overnight at 4 °C. The primary antibodies were purchased from Cell Signaling Technology (CST, Danvers, MA, USA), including B-cell lymphoma-2 (BCL-2; 15071, 1:1000), BCL-2 associated X protein (BAX; 41162, 1:1000), β-actin (4967, 1:1000), CHOP (2895, 1:1000), PERK (3192, 1:1000), ATF6 (65880, 1:1000), IRE1 (3294, 1:1000), p-JNK (9251, 1:1000), and JNK (9252, 1:1000). The primary antibodies GRP78 (ab21685, 1:1000) and p-IRE1 (ab124945, 1:1000) were obtained from Abcam (Cambridge, MA, USA). After three rinses with TBST, a Horseradish Peroxidase (HRP)-conjugated secondary antibody (7074, 1:5000; CST, Danvers, MA, USA) was utilized to incubate the membranes at ambient temperature for 1 h. Finally, a luminescent imaging instrument (Amersham Imager 680, GE Healthcare, Chicago, IL, USA) and enhanced chemiluminescence (WBKLS0500, Millipore, San Diego, CA, USA) were used to expose the membranes. The Gray values were detected through ImageJ software (V1.8.0, National Institutes of Health, NIH, Bethesda, MD, USA).

Statistical Analysis

GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA) was used to conduct the statistical analysis, and the mean $\pm$ standard deviation (SD) was used to express all data. The normality was evaluated using the Shapiro-Wilk test after the variance test. One-way analysis of variance (ANOVA) was used to compare the differences among multiple groups. Statistical differences were considered if the value was $p < 0.05$.

Results

Ghrelin Improves the Viability of NIT-1 Cells Induced by Glucolipotoxicity

The function of ghrelin on pancreatic β-cell viability was studied by CCK-8. As shown in the result, in the comparison of the control group, the cell viability in the HG/PA group was markedly reduced ($p < 0.01$, Fig. 1); relative to the HG/PA group, the AG group displayed a noticeable increase in cell viability ($p < 0.01$, Fig. 1); as opposed to the AG group, the cells viability in the AG+[d-Lys3]-GHRP-6 group was remarkably lower ($p < 0.01$, Fig. 1). Furthermore, the trend of cell viability in all groups at different culture times (12 h, 24 h, and 48 h) was similar. Still, the cell viability of the HG/PA group, the AG group, and the AG+[d-Lys3]-GHRP-6 group gradually decreased during intervention time, while the cell viability of the control group decreased slowly without significant difference (Fig. 1). These results demonstrated that ghrelin could improve the viability of NIT-1 cells induced by glucolipotoxicity.

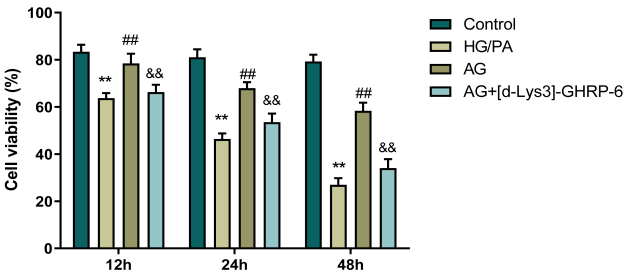

Fig. 1. Ghrelin improves the viability of NIT-1 cells induced by glucolipotoxicity. The cell viability of the acylated ghrelin (AG)+[d-Lys3]-growth hormone releasing peptide (GHRP)-6 group, the AG group, the high glucose (HG)/palmitic acid (PA) group, the AG group, and the control group was detected by the Cell Counting Kit-8 (CCK-8) test at 12, 24, and 48 h after intervention, respectively. N = 3, $^{\#\#}p < 0.01$ *vs.* HG/PA group; $^{\&\&}p < 0.01$ *vs.* AG group; $^{**}p < 0.01$ *vs.* Control group.

Ghrelin Inhibits the Apoptosis of NIT-1 Cells Induced by Glucolipotoxicity

The role of ghrelin on cell apoptosis was investigated using flow cytometry. It was found that, as opposed to the control group, the apoptosis level of HG/PA group cells markedly increased ($p < 0.01$, Fig. 2A). In contrast, the apoptosis level of AG group cells was significantly decreased compared to the HG/PA group. Besides, the apoptosis level of AG+[d-Lys3]-GHRP-6 group cells was markedly increased relative to the AG group ($p < 0.01$, Fig. 2A), which revealed that ghrelin could inhibit cell apoptosis while this ability was impaired by [d-Lys3]-GHRP-6, the ghrelin antagonists. Moreover, the western

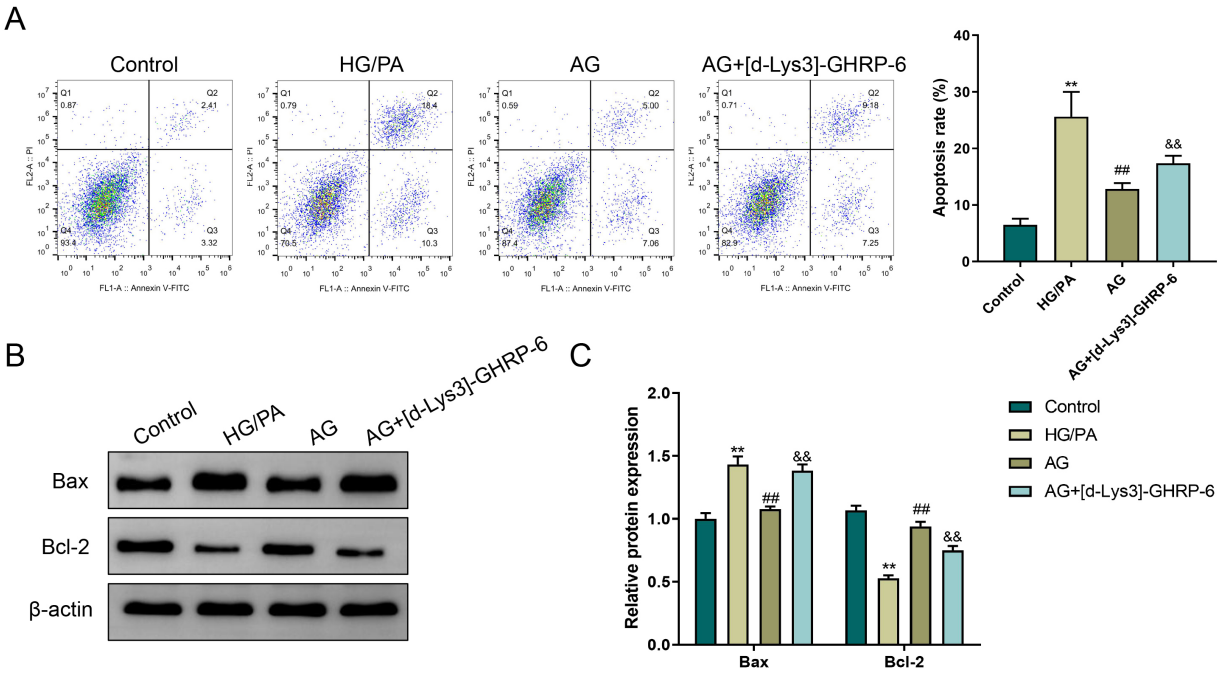

Fig. 2. Ghrelin inhibits the apoptosis of NIT-1 cells induced by glucolipotoxicity. (A) The apoptosis levels of cells were examined by flow cytometry after intervention for 48 h. (B,C) Western blot was adopted to measure the levels of BCL-2 and BAX proteins. β-actin was chosen to be the loading control. N = 3, $^{\#\#}p < 0.01$ *vs.* HG/PA group; $^{\&\&}p < 0.01$ *vs.* AG group; $^{**}p < 0.01$ *vs.* Control group. BCL-2, B-cell lymphoma-2; BAX, BCL-2 associated X protein; AG, acylated ghrelin; HG/PA, high glucose/palmitic acid.

blot demonstrated that in comparison to the control group, the BAX protein level in the HG/PA group markedly increased, while the BCL-2 protein level was significantly lower ($p < 0.01$, Fig. 2B,C). However, after the ghrelin treatment, the BAX protein level in the AG group cells was remarkably reduced, with the BCL-2 protein level increasing relative to the HG/PA group. Furthermore, in the comparison of the AG group, the BAX protein level in the AG+[d-Lys3]-GHRP-6 group was higher, while the protein level of BCL-2 was lower ($p < 0.01$, Fig. 2B,C).

Ghrelin Promotes the Synthesis and Secretion of Insulin from NIT-1 Cells

As insulin synthesis and secretion are essential roles of pancreatic β-cells, the effects of ghrelin on synthesizing and secreting insulin from NIT-1 cells were examined. The insulin level of both cells and cell culture medium was detected using ELISA. As the results showed, compared with the control group, the insulin levels in cells and culture medium from the HG/PA group markedly decreased. In contrast, after the ghrelin treatment, the insulin levels in cells and culture medium increased significantly compared to the HG/PA group in the AG group ($p < 0.01$, Fig. 3A,B). However, compared to the AG group, the insulin levels in cells and cell culture medium from the AG+[d-Lys3]-GHRP-6 group were significantly reduced ($p < 0.01$, Fig. 3A,B). These results revealed that the promotion of ghrelin promotes the synthesizing and secreting of insulin from NIT-1 cells.

Ghrelin Attenuates Endoplasmic Reticulum Stress in Glucolipotoxicity-Induced NIT-1 Cells

The relation between the synthesis and secretion of insulin and the ERS signaling pathway is close. As pancreatic β-cells are affected easily by ERS, a western blot was adopted to analyze the ERS-related protein levels. As the results suggested, in the HG/PA group, the protein levels of GRP78, CHOP, PERK, and ATF6 were remarkably increased compared to the control group ($p < 0.01$, Fig. 4A,B). In contrast, after the intervention of ghrelin, these protein levels were decreased. Besides, the treatment of [d-Lys3]-GHRP-6 increased the expression of these proteins ($p < 0.01$, Fig. 4A,B). The above conclusions demonstrated that ghrelin attenuates ERS in glucolipotoxicity-induced NIT-1 cells.

Ghrelin Inhibits the Activation of IRE1/JNK Signaling Pathway in NIT-1 Cells Induced by Glucolipotoxicity

The IRE1/JNK signaling pathway is an essential pathway regulating ERS [18]. Therefore, the phosphorylation level of the IRE1/JNK signaling pathway was measured using a western blot. Relative to the control group, the ratio of p-JNK/JNK and p-IRE1/IRE1 was significantly higher in HG/PA group cells ($p < 0.01$, Fig. 5A,B). Besides, in contrast to the HG/PA group, the ratio of p-IRE1/IRE1 and p-JNK/JNK in the AG group was markedly reduced ($p < 0.01$, Fig. 5A,B). However, the ratio of p-IRE1/IRE1 and

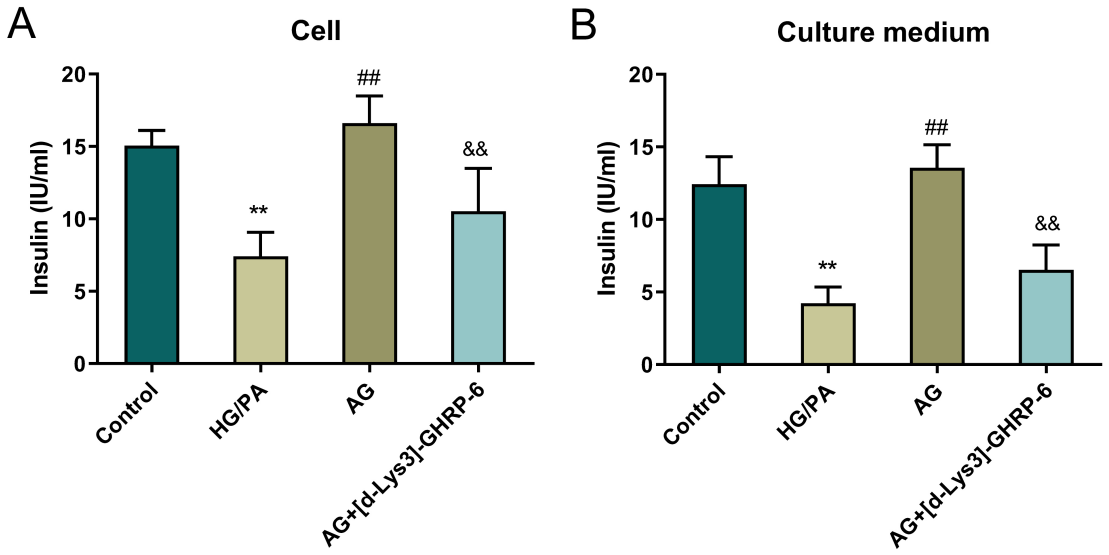

Fig. 3. Ghrelin promotes the synthesis and secretion of insulin from NIT-1 cells. (A,B) The insulin levels in NIT-1 cells (A) and cell culture media (B) of the AG group, the AG+[d-Lys3]-GHRP-6 group, the HG/PA group, and the control group were examined by enzyme-linked immunosorbent assay (ELISA). N = 3, $^{\&\&}p < 0.01$ *vs*. AG group; $^{**}p < 0.01$ *vs*. Control group; $^{\#\#}p < 0.01$ *vs*. HG/PA group.

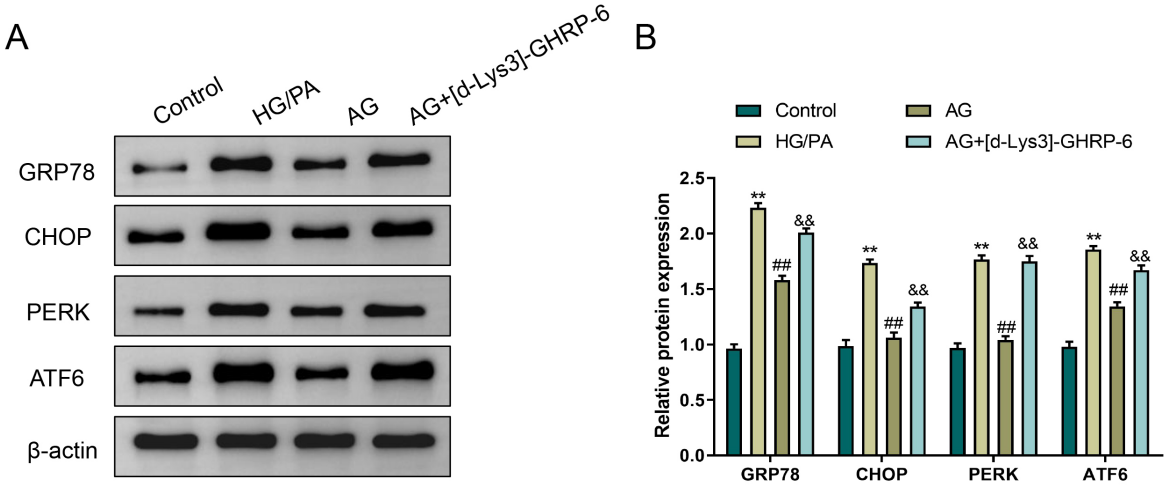

Fig. 4. Ghrelin attenuates endoplasmic reticulum stress in glucolipotoxicity-induced NIT-1 cells. (A,B) The endoplasmic reticulum stress-related protein (GRP78, CHOP, PERK, and ATF6) in the AG+[d-Lys3]-GHRP-6 group, the HG/PA group, the AG group, and the control group were analyzed based on western blot. β-actin was considered to be a loading control. N = 3, $^{\#\#}p < 0.01$ *vs*. HG/PA group; $^{**}p < 0.01$ *vs*. Control group; $^{\&\&}p < 0.01$ *vs*. AG group. GRP78, 78-kDa glucose-regulated protein; CHOP, CCAAT/enhancer-binding protein homologous protein; PERK, Protein kinase R-like endoplasmic reticulum kinase; ATF6, activating transcription factor 6.

p-JNK/JNK was upregulated after the intervention of [d-Lys3]-GHRP-6 ($p < 0.01$, Fig. 5A,B). Therefore, Ghrelin could inhibit the activation of the IRE1/JNK signaling pathway in NIT-1 cells induced by glucolipotoxicity.

Discussion

Metabolic homeostasis in mammals is mainly regulated by hormones, including insulin. Insulin is secreted from the pancreatic β-cells and is essential for controlling blood glucose levels [31]. Pancreatic β-cells release insulin following changes in blood glucose, a process central to the type 2 diabetes etiology [32]. Therefore, maintaining pancreatic β-cells normal function is essential, and targeting pancreatic β-cells has direct implications for metabolic disorder therapies [33]. In this study, we used NIT-1 cells as pancreatic β-cells to evaluate the effects of ghrelin treatment. The HG + PA treatment is commonly used to simulate diabetes for a mechanistic approach, which was also adopted in our study [34].

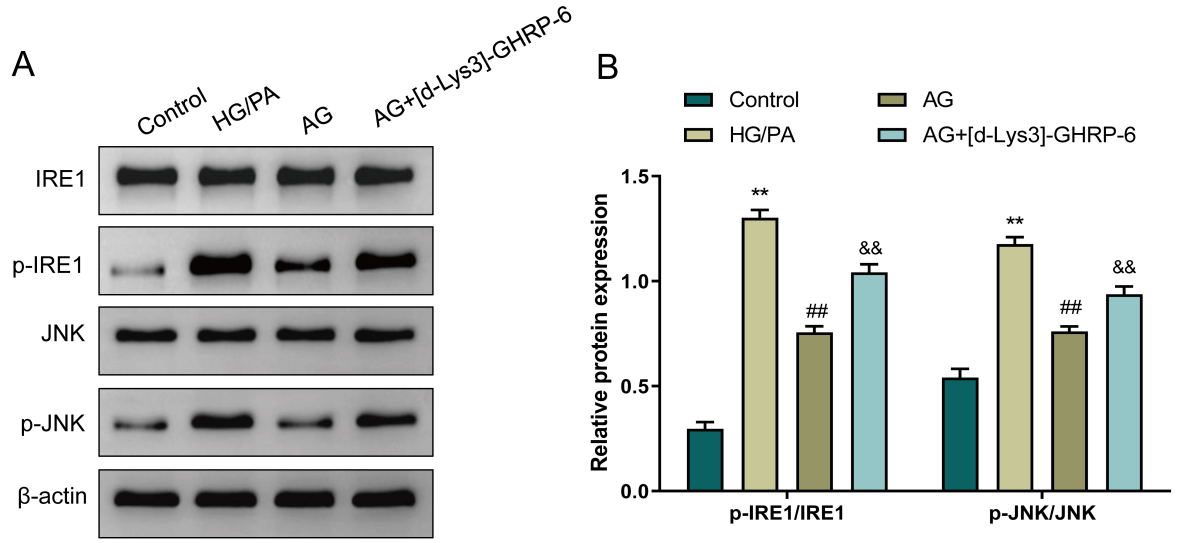

Fig. 5. Ghrelin inhibits the activation of the inositol-requiring kinase 1 (IRE1)/c-Jun N-terminal kinase (JNK) signaling pathway in NIT-1 cells induced by glucolipotoxicity. (A,B) The protein levels of JNK/IRE1 pathway-associated proteins (p-JNK and JNK, IRE1, p-IRE1) in the HG/PA group, the AG+[d-Lys3]-GHRP-6 group, the AG group, and the control group were measured by western blot. β-actin was considered to be the loading control. N = 3, $^{\#\#}p < 0.01$ *vs*. HG/PA group; $^{**}p < 0.01$ *vs*. Control group; $^{\&\&}p < 0.01$ *vs*. AG group.

In previous studies, pancreatic β-cell apoptosis resulted in diminished β-cell mass, reduced insulin secretion, and aggravated hyperglycemia onset [35–37]. Herein, we discovered that ghrelin treatment improved the viability of glucolipotoxicity-induced NIT-1 cells while inhibiting apoptosis. These results suggest that β-cells could be protected by ghrelin. As revealed in a recent study, ghrelin altered islet size and β cell mass, and several studies have reported that cell survival could be promoted by ghrelin treatment and the apoptosis of HIT-T15 pancreatic β-cells was inhibited [38–40]. Similar results were observed in the present study but by different mechanisms.

The ERS has been implicated in the initiation and progression of diabetes mellitus [41,42]. The complications of metabolic disorders are closely related to the dysfunction of ERS. Cell apoptosis results from chronic ERS in the context of persistent hyperglycemia, with the change of expression of various ERS mediators in diabetics [10,43,44]. Therefore, we detected ATF6, GRP78, CHOP, and PERK protein levels, showing that ghrelin treatment attenuated ERS in glucolipotoxicity-induced NIT-1 cells. In accordance with a previous report, ghrelin treatment alleviated ERS and suppressed the activation of ERS markers, including ATF6, CHOP, and GRP78, related to the Toll-like receptor 4 (TLR4)/nuclear factor kappa-B (NF-κB) signaling pathway [23]. Besides, as activating the IRE1/JNK pathway could trigger ERS-induced apoptosis, we examined the phosphorylation level of the IRE1/JNK signaling pathway. For the first time, we revealed that the activation of the IRE1/JNK signaling pathway in NIT-1 cells was induced by glucolipotoxicity and inhibited using ghrelin treatment, indicating a new mechanism by which ghrelin regulates the ERS. In addition, the relationship between cell viability, apoptosis, and ERS is complex. Prolonged or severe ER stress can trigger apoptosis through various pathways, including CHOP, BCL-2, and BAX [14]. Ghrelin's protective role against glucolipotoxicity-induced apoptosis in NIT-1 cells may involve the IRE1/JNK and other ERS-related pathways, such as PERK-eIF2α and ATF6 [45]. However, this needs to be verified by further experiments.

The interplay between BCL-2 and BAX is a critical determinant of cell fate, particularly in the context of apoptosis [46,47]. In our study, ghrelin's role in modulating the balance between these proteins under glucolipotoxic conditions highlights its potential in protecting pancreatic β-cells. BCL-2, an anti-apoptotic protein, and BAX, a pro-apoptotic counterpart, play pivotal roles in the mitochondrial apoptosis pathway, and our results indicate that ghrelin positively influences this balance, promoting cell survival. This study illustrated that ghrelin promoted NIT-1 cells to synthesize and secrete insulin by regulating the ERS through the IRE1/JNK pathway, which indicated that ghrelin played a β-cell-protective role. However, future experiments *in vivo* still need to be performed to reveal the effect of ghrelin treatment on glucose homeostasis. Additionally, we need to block the IRE1/JNK signaling pathway to see whether the IRE1/JNK signaling pathway mediates the effect of ghrelin on the ERS.

Several limitations in this study merit further investigation. While this study focused on protein expression levels to directly assess the impact on cellular functions, we acknowledge the potential relevance of gene expression

analysis in elucidating the underlying mechanisms. This study's absence of gene expression analysis was due to our emphasis on protein-mediated changes, particularly in the context of ghrelin's modulatory effects. Future research could integrate protein and gene expression analyses to understand better the molecular pathways involved. Future studies would also benefit from including immunofluorescence techniques to visualize the distribution of critical proteins within cells. This approach would provide valuable insights into the cellular localization and dynamics of proteins such as IRE1, JNK, BCL-2, and BAX, especially under conditions of ER stress and in the presence of ghrelin.

Conclusion

In summary, ghrelin treatment increased cell viability and insulin levels and reduced cell apoptosis in glucolipotoxicity-induced β-cells, thus improving β-cellular dysfunction. These effects may be ascribed to reveal the underlying mechanism by downregulating the ERS-induced IRE1/JNK pathway. Besides, [d-Lys3]-GHRP-6 treatment could reverse the role of ghrelin on glucolipotoxicity-induced β-cell injury. Ghrelin treatment can be considered an effective therapy for treating patients with diabetes mellitus. Therefore, this issue deserves further investigation.

Availability of Data and Materials

Data involved in the present work are available from the corresponding author upon request.

Author Contributions

XYL and JMR designed the research study. XYL and JMR performed the research. CRZ, JCW and CHY provided help and advice on experiments. CRZ, JCW and CHY analyzed the data. All authors were involved in drafting and critical revision of the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202436186.127.

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