Original Paper

Irisin Ameliorates Glucolipotoxicity-Associated β -Cell Dysfunction and Apoptosis via AMPK Signaling and Anti-Inflammatory Actions

Dan Zhang Ting Xie Po Sing Leung

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

Key Words

Ins-1E cells • Insulin • Islets • Lipid metabolism • Nuclear factor- κ B • Pancreas • Triglyceride • Diabetes mellitus

Abstract

Background/Aims: Islet metabolic disorder and inflammation contribute to the pathogenesis and progression of type 2 diabetes mellitus (T2DM). Irisin is a recently identified adipomyokine with protective effects on metabolic homeostasis and inflammation-suppressing effects in hepatic and vascular cells. The present study examined the effects of irisin on lipid metabolism and inflammation in β cells under glucolipotoxic conditions. **Methods:** Rat INS-1E β cells and islets isolated from C57BL/6 mice were incubated in glucolipotoxic conditions with or without irisin. Intracellular lipid contents and lipogenic gene expression were determined by enzymatic colorimetric assays and real-time PCR, respectively. Inflammatory status was evidenced by Western blot analysis for the phosphorylation of nuclear factor- κ B (NF- κ B) p65 and real-time PCR analysis for the expression of pro-inflammatory genes. **Results:** Irisin reversed glucolipotoxicity-induced intracellular non-esterified fatty acid (NEFA) and triglyceride accumulation, suppressed associated elevations in lipogenic gene expression, and phosphorylated acetyl-CoA-carboxylase (ACC) in INS-1E cells. These demonstrated effects were dependent on irisin-activated adenosine monophosphate-activated protein kinase (AMPK). Meanwhile, AMPK signaling mediated the protective effects of irisin on INS-1E cell insulin secretory ability and survival as well. Additionally, irisin inhibited phosphorylation of NF- κ B p65 while decreasing the expression of pro-inflammatory genes in INS-1E cells under glucolipotoxic conditions. Moreover, irisin also improved insulin secretion, inhibited apoptosis, and restored β -cell function-related gene expression in isolated mouse islets under glucolipotoxic conditions. **Conclusion:** Irisin attenuated excessive lipogenesis in INS-1E cells

under glucolipotoxic state through activation of AMPK. Irisin also suppressed overnutrition-induced inflammation in INS-1E cells. Our findings implicate irisin as a promising therapeutic target for the treatment of islet lipid metabolic disorder and islet inflammation in T2DM.

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Introduction

In obese subjects, hyperglycemia and hyperlipidemia contribute to the development and progression of type 2 diabetes mellitus (T2DM) through mechanisms that involve the phenomenon, namely islet β -cell failure, a condition characterized by islet β -cell dysfunction and loss of β -cell mass [1]. Accordingly, chronic exposure to high levels of glucose and palmitic acid (PA) has been shown to impair β -cell insulin secretion and promote β -cell apoptosis in *in vitro* and *ex vivo* studies [2, 3]. Overnutrition-related glucolipotoxicity perturbs normal lipid metabolism in β cells and islets by promoting lipogenesis and subsequent lipid deposition [2]. Meanwhile, glucolipotoxicity triggers intra-islet inflammation, especially in β cells, as well [4, 5]. The abnormalities in lipid metabolism and inflammation under glucolipotoxic conditions are thought to be the key players of β -cell failure and pathogenesis of T2DM.

Fundamentally, T2DM is a disorder of metabolism characterized by insulin resistance in peripheral tissues (primarily adipose, hepatic and skeletal muscle tissues) and hyperglycemia due to insufficient insulin secretion. Exercise-induced secretion of small protein molecules, including myokines, can have beneficial effects on metabolic syndromes. Of particular interest in this context is irisin, a newly identified adipomyokine derived from fibronectin type III domain containing 5 (FNDC5), which has gained attention for its potential in the prevention and treatment of obesity and T2DM [6, 7]. In this regard, the circulating levels of irisin were found to be altered in obese individuals and diabetic patients [8]; these levels were considered to be one biomarker for the prediction of the development of diabetes and mortality risk of cardiovascular diseases [9, 10]. We and others have demonstrated that, in an overnutrition state, irisin can restore metabolic homeostasis, thereby alleviating insulin resistance and protecting cell function as well as survival in hepatic tissues [11–13]. For instance, irisin attenuated PA-induced excessive lipid accumulation in hepatocytes [13], while deficiency of FNDC5 exacerbated hepatic lipogenesis and lipid accumulation in obese mice [11].

Activation of adenosine monophosphate-activated protein kinase (AMPK) is associated, at least partially, with the protective effects of irisin on lipid metabolism via regulation of the expression and activity of lipogenic factors, such as acetyl-CoA-carboxylase (ACC) and fatty acid synthase (FAS) [12, 13]. AMPK is a highly evolutionarily conserved cellular energy status sensor that transmits metabolic stress signals and restores metabolic homeostasis by integrating physiological signals [14]. Its activation is facilitated by phosphorylation at its threonine 172 residue [15]. As a master metabolic regulator, activated AMPK promotes energy production via glycolysis while repressing ATP-consuming processes, such as fatty acid and triglyceride (TG) synthesis [14, 16, 17]. For instance, AMPK activation can downregulate fatty acid synthesis by phosphorylating ACC in adipose and hepatic tissues [12, 18]. In β cells, AMPK activation is speculated to limit lipid accumulation under glucolipotoxic condition [3]. Despite these findings, it remains unknown whether irisin can restore metabolic homeostasis via AMPK signaling in β cells under T2DM-like conditions.

Irisin has been reported to alleviate syndromes in T2DM and non-diabetic obesity by inhibiting inflammation in hepatic and vascular as well as adipose tissues [13, 19, 20]. For example, irisin attenuated PA-induced expression of pro-inflammatory factors, including cyclooxygenase-2 (COX2), in hepatocytes [13] and alleviated T2DM-related vascular dysfunction through repression of nuclear factor- κ B (NF- κ B) p65 phosphorylation in endothelial cells [19]. Irisin has also been shown to exhibit anti-inflammatory actions in macrophages via inhibiting activation of NF- κ B p65 and release of cytokines, including chemokine (C-C motif) ligand 2 (CCL2) and chemokine (C-X-C motif) ligand 1 (CXCL1) [21].

It is well established that β -cell inflammation under hyperglycemic and hyperlipidemic milieu is attributed to the pathogenesis and development of T2DM [4, 5].

Regarding the regulatory actions on β cells, it has been reported that irisin administration attenuates β -cell apoptosis and restores β -cell function under glucotoxic condition [22]. In addition, a recent study has shown that irisin suppresses apoptosis in β cells and islets under lipotoxic condition via modulation of protein kinase B/B-cell lymphoma 2 (BCL2) signaling pathway [23]. However, it has yet to be determined whether irisin can alleviate lipid metabolic disorder and pro-inflammatory actions in β cells under T2DM-like conditions. Therefore, the present study aimed to investigate the effects of irisin on hyperlipogenesis in β cells under glucolipotoxic conditions and whether such effects were mediated by AMPK signaling. Meanwhile, the potential involvement of AMPK in the protective effects of irisin on β -cell insulin secretion and survival was assessed as well. Additionally, we examined the regulatory action of irisin on β -cell inflammation under glucolipotoxic conditions.

Materials and Methods

Cell and islet treatments

Rat INS-1E β cells were cultured in RPMI 1640 containing glucose (11.1 mM) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin, 100 μ g/ml streptomycin, 1 mM sodium pyruvate, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 50 μ M 2-mercaptoethanol (all from Life Technologies, Carlsbad, CA). Intact pancreatic islets isolated from 8-wk-old male C57BL/6 mice following intra-ductal injection of collagenase P (Roche, Kaiseraugst, Switzerland) were cultured in 5.6 mM glucose containing RPMI 1640 medium supplemented with 10% (v/v) FBS, 100 U/ml penicillin and 100 μ g/ml streptomycin. The cells and islets were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

To induce glucolipotoxicity, INS-1E cells and isolated mouse islets were exposed to a PA-high glucose (PAHG) condition consisting of 0.5 mM PA and 28 mM glucose (both from Sigma-Aldrich, St. Louis, MO). To determine the effects of irisin on phosphorylation of AMPK α and ACC, cells were incubated with 1 μ g/ml recombinant irisin (University of Hong Kong, China) for 5, 15, 30, 60, or 90 min, with or without 20-min pretreatment of 5 μ M compound C (Sigma-Aldrich), an AMPK inhibitor. For other assessments, cells and islets were treated with recombinant irisin for 48 h in the presence or absence of compound C.

Gene expression analysis

Total RNA extracted from INS-1E cells or isolated mouse islets with TRIzol reagent (Takara Bio., Shiga, Japan) was subjected to reverse transcription with a PrimeScript RT Master Mix kit (Takara Bio.), according to the manufacturer's instructions. Relative gene expression levels were quantified by real-time polymerase chain reaction (PCR) using Power SYBR Green PCR Master Mix (Applied Biosystems Life Technologies, UK). The primers used were listed in Table 1. Transcript levels were calculated by the 2^{- $\Delta\Delta$ CT} method [24] and normalized to β -actin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Western blot analysis

Total protein per standardized number of cells was extracted with CytoBuster protein extraction reagent (Novagen, Darmstadt, Germany),

Table 1. Sequences of primers used in real-time PCR experiments

Gene	Sequence
Rat Acc_F	5'-GACGTTCCGCATAACCAAGT-3'
Rat Acc_R	5'-CTGCAGGTTCTCAATGCAAA-3'
Rat Bcl2_F	5'-CTTTGAGTTCGGTGGGGTCA-3'
Rat Bcl2_R	5'-AGTTCCACAAAGGCATCCAG-3'
Rat β -actin_F	5'-TTTAATGTACGCACGATTTC-3'
Rat β -actin_R	5'-CCCATCTATGAGGGTTACGC-3'
Rat Ccl2_F	5'-GTAGAAGTGACCAGTATGACAGAGAAC-3'
Rat Ccl2_R	5'-TACATTGAAAGTGTGAACCAGGATTTC-3'
Rat COX-2_F	5'-CTCTACAACAACCTCCATCTCCTT-3'
Rat COX-2_R	5'-TTTCATCTCTCTGCTCTGGTCAAT-3'
Rat Cxcl1_F	5'-AATGTGTGAGAGGCTATGTTGTATGTT-3'
Rat Cxcl1_R	5'-ACGAGAAGGAGCATTGGTTAAAGAATA-3'
Rat Fas_F	5'-TCGACTTCAAAGGACCCAGC-3'
Rat Fas_R	5'-GGCAATACCCGTTCCCTGAA-3'
Rat Glk_F	5'-CAGGACTTGCACCTTCACGA-3'
Rat Glk_R	5'-GCAAGGTCCAGGAAGCATT-3'
Rat Glut2_F	5'-TGCTCTCATCATGTGCTGGGAAGAAG-3'
Rat Glut2_R	5'-CTGACTAATAAGAATGCCTGTGACAAGA-3'
Rat Pdx1_F	5'-GAAATCCACCAAAGCTCACG-3'
Rat Pdx1_R	5'-AATTCTCTCTCCAGCTCCAG-3'
Mouse Gapdh_F	5'-GCCTTCTCCATGGTGGTGA-3'
Mouse Gapdh_R	5'-GCACAGTCAAGGCCGAGAAT-3'
Mouse Glk_F	5'-AAGAGGAGAGGGGACTTTGA-3'
Mouse Glk_R	5'-GGTGCCCAATCATGCCGA-3'
Mouse Pdx1_F	5'-GAAATCCACCAAAGCTCACG-3'
Mouse Pdx1_R	5'-TTCAACATCACTGCCAGCTC-3'

fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and then transferred to polyvinylidene difluoride membranes (Millipore Corp., Billerica, MA). After blocking, membranes were probed with primary antibodies targetting the following proteins: AMPK α , phosphorylated (p)-AMPK α (Thr172), acetyl-CoA-carboxylase (ACC), p-ACC (Ser79), NF- κ B p65, and p-NF- κ B p65 (Ser536) (Cell Signaling, Danvers, MA). After washing with phosphate buffered saline with 0.1% Tween 20, the membranes were probed with corresponding horseradish peroxidase-conjugated secondary antibodies (Cell Signaling). Labeled protein bands were revealed with a chemiluminescence system (GE Healthcare, Piscataway, NJ, USA), exposed to Fuji medical film (FUJIFILM Corp., Tokyo, Japan), and quantitated with Image J software (NIH, USA).

Measurement of lipid constituents

Intracellular TG and non-esterified fatty acid (NEFA) contents in INS-1E cell lysates were measured by specific enzymatic colorimetric kits (Wako Pure Chemicals, Osaka, Japan), according to the manufacturer's instructions, and normalized to cellular protein concentration.

Glucose stimulated insulin secretion (GSIS)

Insulin secretory ability was assessed as described previously [25]. Briefly, INS-1E cells and isolated mouse islets were allowed to equilibrate in Krebs-Ringer bicarbonate buffer (KRBB, supplemented with 0.1% NEFA-free bovine serum albumin and 10 mM HEPES) with 1.7 mM glucose for 1 h. They were then incubated in KRBB containing 1.7 mM glucose for 1 h, and in KRBB with 16.7 mM glucose for one additional hour. Subsequently, samples from KRBB solution were subjected to insulin quantification with an enzyme-linked immunosorbent assay (ELISA) kit (University of Hong Kong, China).

Cell proliferation and apoptosis assays

INS-1E cells were seeded at a density of 1×10^4 per well in 96-well microplates and cultured under experimentally indicated conditions. Cell proliferation was determined by detecting bromodeoxyuridine (BrdU) incorporation. After cells were incubated with BrdU for 2 h, DNA synthesis was assessed with a BrdU cell proliferation ELISA kit (Roch Applied Science, Basel, Switzerland) in accordance with the manufacturer's instructions. Cell apoptosis was assessed with a cell death detection ELISA plus kit (Roche Applied Science) according to the manufacturer's instructions.

Statistical analysis

Data are expressed as means $\pm$ standard errors of means (SEMs). Comparisons between different groups were analyzed with one-way analyses of variance (ANOVAs) followed by Tukey's *post hoc* test. In all cases, $p < 0.05$ was considered statistically significant.

Results

Irisin reverses glucolipotoxicity-induced lipid hyperaccumulation in INS-1E cells

A 48-h treatment with PAHG condition led to dramatic accumulation of intracellular NEFA and TG molecules within INS-1E cells, in relation to the controls (Fig. 1A). However, co-treatment with irisin (1 μ g/ml) repressed these adverse effects of PAHG (Fig. 1A), as well as reduced the upregulated mRNA expression of the key lipogenesis-related enzymes, i.e. ACC and FAS, in relation to the respective controls (Fig. 1B).

Irisin activates AMPK-ACC signaling in INS-1E cells

As shown in Fig. 2A, acute incubation with irisin (1 μ g/ml) resulted in increased levels of p-AMPK α and p-ACC in INS-1E cells under normal condition. These stimulatory effects were detectable by 15 min and 30 min after irisin treatment, and returned to the baseline levels within 90 min (Fig. 2A). Following exposure to the glucolipotoxic condition for 48 h, an 30 min-incubation with irisin resulted in increases in p-AMPK α and p-ACC in INS-1E cells (Fig. 2B). In addition, the effect of irisin on ACC phosphorylation was repressed by the AMPK inhibitor, compound C (5 μ M), suggestive of its dependence on activation of AMPK α (Fig. 2B).

Fig. 1. Irisin alleviated glucolipotoxicity induced lipogenesis. Intracellular levels of NEFA and TG contents (A), and expression levels of ACC and FAS mRNAs (B) in INS-1E cells exposed to PAHG condition (500 μ M palmitic acid and 28 mM glucose) for 48 h with or without irisin treatment (1 μ g/ml). *** p <0.001 vs control (Ctrl); # p <0.05, ## p <0.01 vs PAHG group (n = 6 per group).

Fig. 2. Irisin stimulated phosphorylation of AMPKα and ACC in INS-1E cells under normal and glucolipotoxic conditions. (A) Phosphorylated and total forms of AMPKα and ACC in INS-1E cells treated with irisin (1 μ g/ml) for the indicated periods of time. * p <0.05, ** p <0.01 vs 0 min. (B) Phosphorylated and total forms of AMPKα and ACC in INS-1E cells after 48-h incubation in PAHG medium, with or without 20-min pretreatment with compound C (5 μ M) prior to 30-min irisin treatment (1 μ g/ml). * p <0.05 vs PAHG; # p <0.05 vs PAHG+irisin (n = 3 per group).

Irisin represses lipid hyperaccumulation and expression of lipogenic genes in INS-1E cells under glucolipotoxic conditions in an AMPK-dependent manner

As shown in Fig. 3A, co-treatment with compound C resulted in a significant attenuation of the inhibitory effects of irisin on accelerated intercellular NEFA and TG deposition within INS-1E cells under glucolipotoxic conditions. Consistently, compound C-induced alterations in NEFA and TG contents were associated with parallel changes in ACC and FAS expression; in fact, compound C diminished the repressive effects of irisin on ACC and FAS transcription in INS-1E cells under glucolipotoxic conditions as well (Fig. 3B).

Irisin restores GSIS of INS-1E cells under glucolipotoxic conditions via AMPK

The glucolipotoxic treatment reduced the insulin secretory response of INS-1E cells to 16.7 mM glucose challenge (normalized to insulin secretion at 1.7 mM glucose) by about 50%, and this reduction was partially reversed by co-treatment with irisin. Conversely, co-incubation with the AMPK inhibitor compound C attenuated this protective effect of irisin on insulin secretion (Fig. 4A). In addition, the levels of transcripts encoding for glucose transporter 2 (GLUT2) and glucokinase (GLK), the two key enzymes that facilitate GSIS, were altered in the same patterns. Irisin partially reversed the decreased mRNA expression of GLUT2 and GLK under glucolipotoxic condition, while compound C co-treatment repressed those protective effects (Fig. 4B).

Irisin improves INS-1E cell survival via AMPK under glucolipotoxic conditions

As shown in Fig. 5A, cell proliferation of INS-1E cells was impaired while cell apoptosis was increased dramatically upon the glucolipotoxic exposure, and these glucolipotoxicity-induced effects were attenuated by co-treatment with irisin. On the other hand, AMPK inhibition with compound C diminished the protective effects of irisin on INS-1E cell proliferation and apoptosis under the glucolipotoxic conditions (Fig. 5A). In corroboration with these findings, compound C inhibited irisin-mediated regulation of the mRNA expression of pancreas/duodenum homeobox protein 1 (*Pdx1*) and *Bcl2*, two master regulatory genes for β cell proliferation and apoptosis, in INS-1E cells chronically exposed to high levels of PA and glucose (Fig. 5B).

Fig. 3. Irisin inhibited lipogenesis in INS-1E cells under glucolipotoxic conditions via a mechanism that requires AMPK activation. Intracellular levels of NEFA and TG contents (A), and mRNA expression levels of ACC and FAS (B) in INS-1E cells exposed to PAHG medium for 48 h with or without irisin (1 μ g/ml) and compound C (5 μ M). *** p <0.001 vs Ctrl; # p <0.05, ## p <0.01 vs PAHG; \$ p <0.05, \$\$ p <0.01 vs PAHG+irisin group (n = 6 per group).

Fig. 4. Irisin protected GSIS of INS-1E cells under glucolipotoxic conditions in an AMPK-dependent manner. GSIS in response to 16.7 mM glucose in reference to 1.7 mM glucose (A), and mRNA expression of GLUT2 and GLK (B) in INS-1E cells exposed to PAHG condition for 48 h with or without irisin (1 µg/ml) and compound C (5 µM). ***p<0.001 vs Ctrl; #p<0.05 vs PAHG; \$p<0.05 vs PAHG+irisin group (n = 6 per group).

Fig. 5. Irisin countered glucolipotoxic effects on the cell proliferation and apoptosis in INS-1E cells through AMPK activation. (A) Cell proliferation (left) and apoptosis (right), and mRNA expression of PDX1 and BCL2 (B) in INS-1E cells exposed to PAHG medium for 48 h with or without irisin (1 µg/ml) and compound C (5 µM). ***p<0.001 vs Ctrl; #p<0.05, ##p<0.01, ###p<0.001 vs PAHG (n = 6 per group).

Fig. 6. Irisin attenuated glucolipotoxicity induced inflammation in INS-1E cells. (A) mRNA expression of COX2, CXCL1, and CCL2 ($n = 6$ per group), and (B) phosphorylated and total forms of p65 ($n = 3$ per group) in INS-1E cells exposed to PAHG condition for 48 h with or without irisin ($1 \mu\text{g/ml}$). * $p < 0.05$, *** $p < 0.001$ vs Ctrl; # $p < 0.05$, ## $p < 0.01$ vs PAHG.

Irisin exhibits anti-inflammatory effects in INS-1E cells under glucolipotoxic conditions

A 48-h PAHG incubation triggered pro-inflammatory responses in INS-1E cells, as indicated by elevated expression levels of mRNAs encoding COX2, CXCL1 and CCL2, as well as phosphorylation of p65 (Fig. 6). Co-treatment with irisin inhibited PAHG-induced expression of the above three pro-inflammatory factors (Fig. 6A), whilst repressing p65 phosphorylation in INS-1E cells under glucolipotoxic conditions (Fig. 6B).

Irisin improves insulin secretion, attenuates apoptosis, and modulates expression of related genes in isolated mouse islets under glucolipotoxic conditions

A 48-h incubation with PAHG conditions impaired GSIS and augmented apoptosis in islets isolated from mice (Fig. 7A). In line with our observations on INS-1E cells, co-treatment with irisin ($1 \mu\text{g/ml}$) restored GSIS and reduced PAHG-induced apoptosis in isolated mouse islets (Fig. 7A). Consistently, irisin restored the expression of genes that are positively associated with GSIS (*Glut2*) and β -cell function (*Pdx1*) and negatively correlated with apoptosis (*Bcl2*) in mouse islets under *ex vivo* glucolipotoxic conditions (Fig. 7B).

Fig. 7. Irisin protected GSIS and repressed apoptosis in isolated mouse islets under glucolipotoxic conditions. (A) GSIS in response to 16.7 mM glucose in reference to 1.7 mM glucose (left) and cell apoptosis (right), and (B) mRNA expression of GLK, PDX1, and BCL2 (B) in islets isolated from mice and exposed to PAHG for 48 h with or without irisin (1 μ g/ml). * $p < 0.05$, *** $p < 0.001$ vs Ctrl; # $p < 0.05$, ## $p < 0.01$ vs PAHG group (n = 6 per group).

Discussion

The present study is the first to have reported the regulatory actions of irisin on β -cell lipid metabolism and inflammation under glucolipotoxic conditions that mimic T2DM. Irisin administration reversed the elevation of intracellular NEFA and TG content, repressed the induced expression of lipogenic genes and inhibited ACC activity, as well as improved the GSIS and enhanced the survival of INS-1E cells under T2DM-like state. These beneficial effects of irisin on lipogenesis, insulin release and cell survival were found to be dependent on irisin-activated AMPK. Meanwhile, irisin suppressed the phosphorylation of NF- κ B p65 and expression of inflammatory genes in INS-1E cells under glucolipotoxic conditions. Fig. 8 is a schematic summary proposing irisin's protection against glucolipotoxicity-mediated islet β -cell dysfunction, apoptosis and inflammation.

In T2DM, hyperglycemia and hyperlipidemia contribute synergistically to ectopic lipid accumulation in β cells by promoting lipogenesis when glucose and lipid intake exceed cellular storage and oxidation capacity [2, 26, 27]. Mechanistically, lipogenesis and lipid deposition in β cells are positively associated with the activity and/or expression of ACC and FAS [28, 29]. The long-term exposure to high levels of glucose and lipid can lead to induction of ACC and FAS in β cells [2, 28, 30], while the activity of ACC can be suppressed by reversible phosphorylation [29, 31]. ACC can catalyze acetyl-CoA carboxylation to generate malonyl-CoA. Subsequently, FAS facilitates *de novo* biosynthesis of long-chain saturated fatty acids from malonyl-CoA; the resultant fatty acids are essential for TG synthesis via esterification [28, 32].

AMPK is a metabolic sensor and regulator that modulates glucose and lipid metabolism in diverse tissues and cells [17, 33], including β cells [27, 29]. Notably, it is the main kinase that mediates the activity-suppressing phosphorylation of ACC [29, 31]. Our present findings, showing that irisin treatment of INS-1 cells suppressed glucolipotoxicity-associated changes in ACC and FAS expression, as well as ACC phosphorylation and hyperlipogenesis via AMPK signaling, are consistent with and extend prior reports. Specifically, these findings are in line with our previous observations that AMPK mediates the inhibitory effects of irisin on ACC and FAS,

and the subsequent ectopic lipogenesis in hepatic cells under T2DM-like conditions [12]. The present findings are also consistent with previous report that irisin inhibited PA-induced ACC and FAS expression and lipid accumulation in hepatocytes [13].

Our results also implicate that AMPK signaling is involved in the protective effects of irisin on β -cell insulin secretion and survival, particularly under glucolipotoxic conditions. In this context, we found that AMPK was necessary for the beneficial effects of irisin on the expression of *Glut2*, *Glk*, *Pdx1* and *Bcl2*, which have vital roles in GSIS and/or β -cell function and survival [34-38]. AMPK activation in β cells has been shown to restore impaired GSIS and to inhibit apoptosis in nutrient oversupply states [3, 39]. In contrast, inactivation of AMPK in β cells triggers TG deposition and attenuates insulin secretory capacity [29]. It is thus plausible to speculate that the protective effects of irisin on β -cell function and viability observed here may be due, at least partly, to lipid metabolism-altering effects.

Alterations in lipid metabolism triggered by glucolipotoxicity are instrumental in the process of β -cell dysfunction and apoptosis [2]. Glucotoxicity/lipotoxicity-induced lipid hyperaccumulation correlates with GSIS defects in β cells [2, 40]. Mechanistically, the induction in ACC contributes, at least in part, to the upregulation of TG synthesis, and consequently to impaired β -cell function in the over nutritional states [2, 29]. Meanwhile, glucolipotoxicity-induced lipogenic signaling, such as ACC, causes β -cell apoptosis as well [2, 3]. We found that AMPK mediated the effects of irisin on both lipogenic homeostasis and β -cell function/survival. Given the detrimental effects of excessive lipogenesis on β -cell

Fig. 8. Schematic diagram summarizing the regulatory actions of irisin in lipid metabolism, cell survival, and inflammation in INS-1E cells and islets experiencing glucolipotoxicity. Irisin treatment leads to phosphorylation of AMPK α , thereby inhibiting ACC activity and repressing expression of lipogenic enzymes (ACC and FAS). Consequently, lipogenesis and the intracellular NEFA and TG accumulation are decreased, resulting in the protective effects on expression of genes related to β -cell survival (Pdx1 and Bcl2) and function (Glut2 and Glk). Ultimately, cellular proliferation and GSIS are restored, while apoptosis is inhibited. Additionally, irisin exhibits anti-inflammatory effects via attenuation of the induction of p65 phosphorylation and thus attenuation of expression of downstream pro-inflammatory factors ensues (COX2, CCL2 and CXCL1).

function and viability, we speculate that the protective effects of irisin on β -cell insulin secretion and survival are, at least in part, secondary to irisin-AMPK signaling-mediated inhibition of lipogenesis under glucolipotoxic conditions.

In addition to demonstrating the protective effects of irisin on β -cell lipid metabolic homeostasis, the present study also showed that irisin administration can inhibit glucolipotoxicity-induced inflammatory responses in INS-1E cells. Hyperglycemia and hyperlipidemia both cause pro-inflammatory responses in macrophages, pancreatic islets, vascular tissues and other peripheral tissues, leading to T2DM pathogenesis [13, 19, 41-43]. In this regard, inhibitory effects of irisin on pro-inflammatory actions in macrophages, endothelial cells and hepatocytes can alleviate diabetic syndromes [13, 19, 21]. Consistently, our findings indicate that irisin can moderate the induction in NF- κ B p65 phosphorylation under glucolipotoxic conditions. In fact, activation of NF- κ B p65 is able to promote the expression of a series of pro-inflammatory factors (e.g. COX2, CCL2 and CXCL1) in diverse tissues and cells, such as β cells [44-46].

Irisin is an exercise-induced adipomyokine and circulating irisin levels are significantly elevated after both acute and chronic exercise [6, 47-49]. Moreover, it has been reported that exercise-stimulated release of irisin is independent of age or fitness level [49], although there are differences in the basal circulating irisin levels [48, 49]. Accumulating evidence have shown that irisin exhibits protective effects on glucose homeostasis in obesity and T2DM, probably via the modulation of glucose and lipid metabolism in hepatic tissue and skeletal muscle [11, 12, 50, 51]. The present study extends the previous findings via demonstrating the protective action of irisin on β -cell lipid metabolism and inflammation under type 2 diabetic condition. Our findings, if confirmed, will provide extra evidence for the beneficial action of physical exercise-induced irisin in glucose homeostasis in obesity and obesity-related T2DM.

Conclusion

In summary, we found that irisin countered glucolipotoxicity-induced lipid metabolic dysfunction in INS-1E cells via a mechanism that involves AMPK signaling. We also found that irisin inhibited β -cell pro-inflammatory responses to glucolipotoxicity. These findings provide scientific basis for the notion that the adipomyokine irisin has preventative and therapeutic potential for pancreatic β -cell failure due to excessive lipogenesis and inflammation triggered by hyperglycemia and hyperlipidemia in T2DM via modulation of β -cell lipid metabolism and inflammation.

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Disclosure Statement

No conflict of interests exists.

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