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ORIGINAL RESEARCH

EGCG Upregulates UCP_3 Levels to Protect MIN_6 Pancreatic Islet Cells from Interleukin-1β-Induced Apoptosis

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Objective: The protective effects of epigallocatechin gallate (EGCG) on interleukin-1β (IL-1β)-induced apoptosis were investigated in murine MIN_6 pancreatic β-cells. The role of uncoupling protein-3 (UCP_3) signaling in this process was also explored.

Methods: After treatment with IL-1β and EGCG, cells were collected and analyzed. Cell viability was measured using the CCK_8 assay and the function of β-cells was evaluated by analyzing insulin secretion. Detection of mitochondrial function in cells was performed by measuring mitochondrial membrane potential, the concentration of ATP and activity of ROS. Apoptosis was analyzed by Hochest33258 staining and flow cytometry. Expression levels of UCP_3 were interrogated using immunohistochemistry, RT-PCR and Western blotting.

Results: Compared with the control group, IL-1β treatment (20nM) for 24 h significantly decreased cell viability and insulin secretion, damaged mitochondrial function and increased ROS activity. Results also showed increased apoptosis and a decrease in UCP_3 expression levels ($p<0.01$). However, treatment with low (1mM) or high (5mM) concentrations of EGCG significantly decreased IL-1β-induced apoptosis ($p<0.01$), restored mitochondrial function and subsequently increased UCP_3 levels in IL-1β-induced β-cells ($p<0.01$).

Conclusion: These results suggest that EGCG protects against IL-1β-induced mitochondrial injury and apoptosis in β-cells through the up-regulation of UCP_3.

Keywords: EGCG, pancreatic β-cells, apoptosis, UCP_3

Introduction

Diabetes is a worldwide public health issue. According to the International Diabetes Federation, the number of type 2 diabetes (T2DM) cases in adults ranging from 18 to 99 years of age was 451 million. If this trend continues, this number will rise to 693 million in 2045.[1]

Insulin resistance and increased β-cell apoptosis are the main mechanisms of pathogenesis behind T2DM.[2] High glucose levels, high fat levels and inflammation can result in apoptosis. Studies suggest that reactive oxygen species (ROS) and oxidative stress (OS) are the main culprits of diabetes.[3] ROS is mainly produced by the mitochondria, which is generally considered as toxic byproducts of aerobic metabolism and includes superoxide anion radicals (O_2^-), hydroxyl-free radicals (OH^-) and hydrogen peroxides (H_2O_2). Apoptosis pathways include the endogenous mitochondrial pathway, endogenous endoplasmic reticulum pathway and exogenous death receptor pathway. In the endogenous mitochondrial pathway, B-cell lymphoma-2 (Bcl-2) family proteins increase mitochondrial permeability by

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reducing membrane potential. Reduced membrane potential leads to the destruction of mitochondrial function and structure, an increased production of ROS and reduces antioxidant enzyme activity. Excessive ROS damages the nucleus and mitochondrial DNA. As a consequence, the pro-apoptotic factor cytochrome C (cyt-C) from the mitochondria is released into the cytoplasm and binds with an apoptotic protein activating factor to activate pro-caspase -9 and further activate downstream caspase-3, which mediates apoptosis.[4,5] Uncoupling proteins (UCPs) are a class of mitochondrial transporters located on the mitochondrial membrane. These transporters include UCP_1, UCP_2, UCP_3, UCP_4, and UCP_5, which can regulate the synthesis of ATP and ROS.[6] UCPs can affect the survival of β-cells by regulating ATP synthesis, fatty acid release and glucose oxidation, and is closely related to mitochondrial dysfunction mediated by mitochondrial oxidation. Uncoupling protein 3 (UCP_3), a member of the uncoupling protein family, can induce proton leakage across the inner mitochondrial membrane and decreases the electrochemical proton gradient (Δp). In this process, mitochondrial uncoupling generates heat instead of ATP and thereby protects against mitochondrial oxidative damage by reducing the production of ROS.[7,8] UCP_3 mRNA expression is ubiquitous in vertebrates and can be detected in skeletal muscle, fat, the heart, thymus, spleen and skin cells.[9] However, in 2008, it was first uncovered that the UCP_3 gene is expressed on the pancreatic islet, which can promote insulin secretion.[10] There is strong evidence that UCP_3 is decreased in the skeletal muscles of T2DM patients.[11] Early research identified that fasting blood glucose and insulin levels were reduced in mice overexpressing UCP_3, suggesting that glucose homeostasis may be improved.[12] Diabetes mediates an increase in ROS levels that can lead to impaired endothelial function, decreased smooth muscle in the diabetic corpus cavernosum and increased apoptosis.[13] Additionally, previous studies have shown that UCP_3 can promote fatty acid oxidation and limit ROS generation.[14] These studies suggested that decreased UCP_3 may be a potential mechanism for T2DM.[15] However, whether UCP_3 affects the onset and development of diabetes is still unknown.

IL-1β is considered an important endogenous cytokine that can induce β-cell apoptosis and lead to the onset of diabetes by various pathways such as NO,[16] $Ca2^+$,[17] mitochondrial membrane potential, ROS[18] and endoplasmic reticulum stress (ERS).[19] The pro-apoptotic effect of IL-1β was observed in MIN_6 islet cells, which are mouse tumor cells expressing SV40 large T antigen under the control of an insulin promoter. Therefore, IL-1β-induced apoptosis has been used to evaluate disease mechanisms and potential treatment strategies for diabetes. EGCG is the most abundant tea-specific catechin and shows evidence in preventing cancer, obesity, diabetes and cardiovascular diseases. More specifically, the treatment of diabetes with EGCG has garnered attention.[20] EGCG has an insulin-like effect, where it can regulate blood glucose by improving insulin sensitivity, affecting liver gluconeogenesis and restricting intestinal glucose transport. This mechanism may be attributed to the inhibition of the NF-kB pathway, limiting the release of pro-inflammatory cytokines and reducing lipid peroxidation in the heart of diabetic animals.[19] In addition, EGCG has strong antioxidant activity, which can prevent or T2DM and complications by scavenging oxygen-free radicals and improving OS. Myung-Kwan Han's study revealed that EGCG can combat IL-1β-induced RINm5F cell apoptosis. This study also found that this anti-apoptotic mechanism is related to inhibition of NF-kB and NO.[21] Whether EGCG protects pancreatic islet cells from interleukin 1β-induced apoptosis by regulating UCP_3 levels remains to be further studied.

Here, IL-1β-processed MIN_6 cells were incubated with low and high doses of EGCG to observe the effects of EGCG on β-cell secretion, mitochondrial function and apoptosis. Simultaneously, our study found that UCP_3 was expressed in MIN_6 cells and down-regulated when pro-processed by IL-1β. Thus, the goal of this work was to reveal whether the protection of EGCG on IL-1β-induced apoptosis may be related to UCP_3.

Materials and Methods

Cell Culture

MIN_6 cells were purchased from the Baili Company (China) and cultured in High Glucose Dulbecco's minimal essential medium (DMEM) (Hyclone) supplemented with 15% fetal bovine serum (nzk0677, Hyclone), 10000U/mL penicillin (Hyclone) and 1000ug/mL streptomycin (Hyclone) in a 37°C, humidified atmosphere containing 5% CO_2. Culture medium was replaced every 2–3 days and cells were passaged every 5–6 days. Experiments were performed when 28–35th generation cells reached 80% confluency. Briefly, MIN_6 cells (5×10^5 cells/mL) were seeded in 96-well plates overnight and randomly divided into 4 groups (n=6). Then, IL-1β (20 nM, 792008D, Invitrogen) was used to pre-treat cells for 24h during

experiments except for the blank group. Finally, cells were treated with EGCG (A-0159, YaJi biological) at low (1mM) or high (5mM) concentrations for 24 hours and cultured in a 37°C incubator.

CCK-8 Assay

The viability of MIN_6 cells was evaluated using a Cell Counting Kit-8 (CCK-8) (AR1160-100, Boster). After administration, 96-well cell culture plates were centrifuged (500 r/min,5min) and the supernatant was removed. Next, cells were resuspended in 200μL serum-free cell culture supplemented with 20ul CCK-8 solution and cultured for 1h at 37°C. A Microplate Reader (Bio-Rad, USA) was used to detect the absorbance of the culture at 450 nm.

Glucose-Stimulated Insulin Secretion (GSIS) Assay

MIN_6 cells were treated with or without high glucose (16.7 mM) culture medium for 2h. Cell culture supernatants were collected to determine the insulin concentration using an ELISA kit (H203, Nanjing Jian Cheng).

Mitochondrial Activity

Mitochondrial membrane potential in MIN_6 cells was measured using the mitochondrial membrane potential assay kit with JC-1 (C2006, Beyotime). JC-1, a fluorochrome, can be accumulated in the mitochondrial matrix to form polymers, which produce a strong red fluorescence (Ex = 585 nm, Em = 590 nm). JC-1 exists in the form of a monomer in the cytoplasm of unhealthy cells and produces green fluorescence (Ex = 514 nm, Em = 529 nm). Quantitative characterization of the mitochondrial membrane potential is R = red fluorescence/green fluorescence. Briefly, 1×10^4 cells were resuspended in 0.5 mL culture medium, and 0.5 mL of JC-1 staining working solution was added before incubating cells at 37°C for 20 minutes. After centrifugation (4°C, 600r/min,4min) and discarding the supernatant, JC-1 staining buffer was used to wash the cells two times. Finally, cells were resuspended with buffer and detected by flow cytometry. The ATP assay kit (A095, Nanjing Jian Cheng) and ROS Assay Kit (A087, Nanjing Jian Cheng) were performed according to manufacturer's instructions to further test mitochondrial function.

Apoptosis Assay

To investigate apoptosis of MIN_6 cells, Hoechst 33258 staining (C1017, Beyotime) solution was added to the cells for a final concentration of 10ug/mL and incubated at 37°C for 15min. Cells were collected and chromatin morphologic changes were observed using fluorescence microscopy (Leica, Germany). Reduced nuclear size, chromatin condensation, intense fluorescence and nuclear fragmentation were indicators of apoptotic cells. Then, we further detected apoptosis using flow cytometry. MIN_6 cells (5×10^5) were plated in a 1.5 mL centrifuge tube and washed twice with cold PBS, suspended in binding buffer and stained by Annexin V-FITC/propidium iodide (PI) (C1056, Beyotime). Cells were incubated for 20 min in the dark (37°C) and the FACSCalibur flow cytometer (Acuuri, USA) was used to observe apoptotic cells within 1 hour. Annexin V-$FITC^+PI^-$: represented early apoptotic cells, Annexin V-$FITC^-PI^+$: represented necrotic cells, Annexin V-$FITC^+PI^+$: represented late apoptotic cells necrotic cells and Annexin V-$FITC^-PI^-$: represented living cells.

Immunohistochemistry

For immunostaining, MIN_6 cells growing on cover glass slides were fixed with 4% paraformaldehyde for 30 min at room temperature, washed 3 times with PBS and then permeabilized for 15 min using 0.5% Triton X-100 in PBS. Cells were washed 3 times with PBS.

Endogenous peroxidase activity was inhibited by immersing sections in methanol with 0.6% H_2O_2 for 30 min at room temperature. After washing with distilled water 3 times and blocking for 30 min at room temperature, diluted primary antibody UCP_3 was added to the cells and incubated at 4°C overnight. After washing twice with PBS, secondary antibody was added and incubated in the darkroom for 30min. At this time, strep avidin-biotin complex (SABC) was added at a proper dilution for 30min (RT) and subsequently washed 4 times with PBS. Colorimetric development of the reaction was performed by incubation in DAB (diaminobenzidine) solution and then washed with water several times. Finally, all specimens were counterstained with hematoxylin solution and mounted with neutral gum. Cells were subjected to microscopy. Brown-yellow staining was considered as positive staining in the cytoplasm as previously described.[22] The immunohistochemical score (IHS) was calculated by combining an estimate of the percentage of immunoreactive cells (quantity score=A) with an estimate of the staining intensity (staining intensity score=B). This was scored as follows: For A: no staining was scored as 0, 1~10% of cells stained scored as 1, 11~50% scored as 2, 51~80% scored as 3 and 81~100% scored as 4. B was scored on

a scale of 0 to 3, where 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). IHS=A×B.

Quantitative Real-Time PCR

Total RNA was extracted using the Ultra-pure RNA extraction kit (CW0581, CWbio) according to the manufacturer's instructions. Reverse transcription was performed using the HiFi-MMLV cDNA Kit (CW0744, CWbio) as follows: the mixture was incubated at 37°C for 50 min, then kept at 70°C for 10 minutes and cDNA was stored at −20°C. For qPCR, SYBR green (CW0956, CWbio) was used along with the Real-Time PCR System (ABI7500, USA). Thermal cycles included 10 min at 95°C, 45 cycles of 15s at 95°C, 60 s at 60°C. Primers included: UCP-3-F: 5′-ACTCCAGCGTCGCCATCAGG-3′; R:5′-CTGTAGGCATCCATAGTCCC-3′. GAPDH-F: 5′ -TGGAGTCTACTGGCGTCTT-3′; R: 5′ -TGTCATATTTCTCGTGGTTCA-3′. Gene expression levels were normalized to Gapdh and relative quantitative calculation analysis was based on the $2^{-\Delta\Delta ct}$ method.

Western Blotting

To examine the protein expression levels of UCP_3 between different experimental groups, MIN_6 cells were collected after washing twice with PBS and lysed with 200 μL of lysis buffer containing Phenylmethylsulfonyl fluoride (PMSF) (ST505, Beyotime). Protein concentrations were measured using Coomassie blue staining (P0017B, Beyotime). An equal amount of protein for each sample was denatured at 95°C for 5 minutes in protein-loading buffer, separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE) (80V) and subsequently semi-dry-transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, USA). After PVDF membranes were blocked for 1 hour at room temperature, they were incubated with the following primary antibodies overnight at 4°C: Anti-UCP_3 antibody (ab10985, abcam) (1:300) and mouse mAb-actin (AA128, Beyotime) (1:1000). Finally, membranes were washed with TBST and incubated with an HRP-conjugated goat anti-rabbit (ZB-2301, ZSGB-BIO) or goat anti-mouse (ZB-2305, ZSGB-BIO) secondary antibody (1:3000) for 1 hours at room temperature. Finally, blots were developed with a chemiluminescent HRP substrate (Merk Millipore, USA) and visualized using the Western Lighting-ECL (Fusion solos, China). The absorbance values of target proteins were analyzed using Image J 180.

Statistical Analysis

Data were expressed as mean ± standard deviation and analyzed using SPSS 15.0 statistical software for all groups following normal distribution. The *F*-test was used to compare the mean of multiple samples. The Dunnet *t* test and SNK-q test were used for multiple comparisons between multiple sample means. A significant difference was considered when $p < 0.05$.

Results

EGCG Treatment Significantly Improved the Activity and Function of IL-1β-Stimulated MIN_6 Cells

Consistent with previous reports, we observed that IL-1β-stimulation reduced cell activity in MIN_6 cells, indicated by significantly decreased OD_{450} values. Co-cultivation with EGCG for another 24 h improved MIN_6 cells activity (Figure 1A). Moreover, IL-1β pretreatment impaired GSIS (basal and high glucose stimulated) in MIN_6 cells when compared with control group. However, the low and high doses of EGCG treatment significantly increased insulin secretion of IL-1β-stimulated MIN_6 cells and showed a dose-dependent trend (Figure 1B and C).

EGCG Treatment Recovered Mitochondrial Function and Reduced Oxidative Stress Response of IL-1β-Stimulated MIN_6 Cells

To further evaluate the effect of EGCG on mitochondrial damage response induced by IL-1β, we assessed mitochondrial membrane potential and ATP activity. In the IL-1β-induced MIN_6 cells, a significant decrease in mitochondria membrane potential and ATP activity was observed (Figure 2A–C). Simultaneously, levels of ROS, an oxidative stress response indicator, in IL-1β-induced MIN_6 cells increased (Figure 2D). EGCG reversed these alterations, dose-dependently increased cell mitochondrial membrane potential, inhibited oxidative stress response and enhanced the activity of ATP (Figure 2D).

EGCG Treatment Decreased Cell Apoptosis in IL-1β-Stimulated MIN_6 Cells

To confirm the effects of EGCG on IL-1β-induced apoptosis in MIN_6 cells, Hoechst 33258 staining and flow cytometry were used to examine apoptosis. Microscope analysis revealed condensed and bright apoptotic nuclei were readily observed in cells treated with IL-1β. However, when treated with EGCG, obvious reduced nuclear condensation in MIN_6 cells was observed (Figure 3), suggesting that

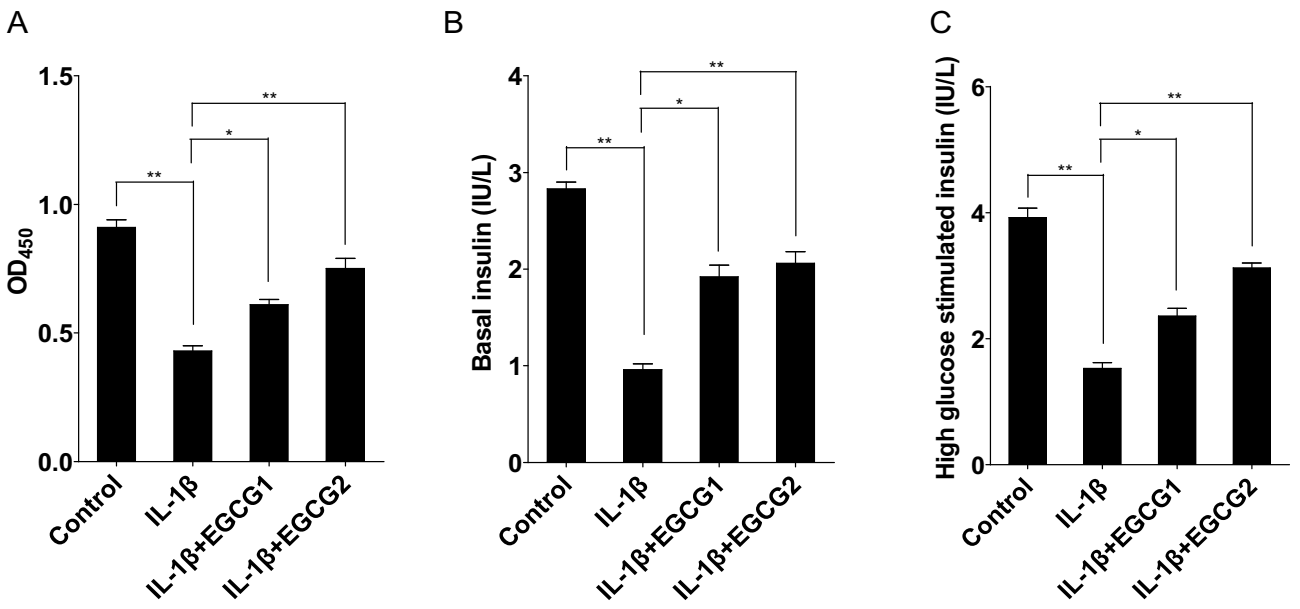

Figure 1 EGCG treatment significantly improved the activity and function of IL-1β-stimulated MIN_6 cells. MIN_6 cells were stimulated with IL-1β followed by co-cultivation with low (1mM) or high (5mM) EGCG for 24 h. (**A**) Cell viability was assessed in each group using the CCK-8 assay. Mitochondrial membrane potential of MIN_6 cells in each group was determined using flow cytometry. Basal insulin (**B**) and high glucose stimulated insulin (**C**) levels for group in the cell supernatant were determined using an ELISA kit. Data are representative of 2 independent experiments and presented as mean ±SD; *p < 0.05, **p < 0.01. Significant differences were evaluated using one-way ANOVA with a post hoc test (Fisher's least significant difference).

EGCG had a protective effect on IL-1β-stimulated MIN_6 cells. To quantitively characterize this observation, we examined apoptotic changes using flow cytometry. Compared with the control, culturing MIN_6 cells in IL-1β conditions for 24 h markedly increased the fraction of apoptotic cells (Figure 4). As expected, EGCG treatment significantly reduced IL-1β-induced apoptosis in a dose-dependent manner. These results were consistent with those observed using the Hoechst 33258 assay.

Effect of EGCG on UCP_3 Expression Levels in IL-1β-Stimulated MIN_6 Cells

UCP_3 is a member of the mitochondrial uncoupling protein family. UCP_3-deficient mice show increased levels of ROS.[23] To assess the potential mediatory role of UCP_3 in IL-1β-induced apoptosis in MIN_6 cells, UCP_3 protein expression levels were analyzed in cells using immunohistochemistry. UCP_3 protein stained brown and was mainly expressed in the cytoplasm of MIN_6 cells (Figures 5 and 6). EGCG significantly increased UCP_3 protein in MIN_6 cells. Next, both gene and protein expression levels of UCP_3 were detected for each group of MIN_6 cells using RT-PCR and Western blotting, respectively. As expected, when compared to the control group, UCP_3 gene and protein levels were decreased in the IL-1β-stimulated MIN_6 cells. In contrast, EGCG treatment significantly increased UCP_3 gene and protein levels (Figure 6). Thus, we speculated that decreased UCP_3 levels under IL-1β-stimulated conditions could disrupt normal mitochondrial function, thereby resulting in excessive production of ROS and apoptosis, and EGCG reverses this damage through upregulation of UCP_3.

Discussion

Studies have shown that apoptosis is the main method for β-cell death in diabetic patients. IL-1β can damage β-cell function and mediate apoptosis. Our previous work confirmed that IL-1β causes β-cell apoptosis in neonatal rats, and this mechanism is related to the NO,[24] $Ca2^+$ and HO-1/CO pathways.[25] A recent study suggested that IL-1β upregulates ROS, destroys mitochondrial function and causes apoptosis. John Zeqi Luo et al[26] confirmed that mitochondrial membrane potential of INS-1 cells weakens, ATP synthesis decreases and apoptosis increases after IL-1β treatment. After stimulating with glucose, insulin secretion ability of MIN_6 returned to levels observed in normal islet β-cells. Here, we found that treatment with IL-1β dramatically increased the apoptosis rate of MIN_6 cells and inhibited cell activity. In addition, insulin secretion function decreased after stimulating with or without glucose. Therefore, we speculated that IL-1β affected the activity

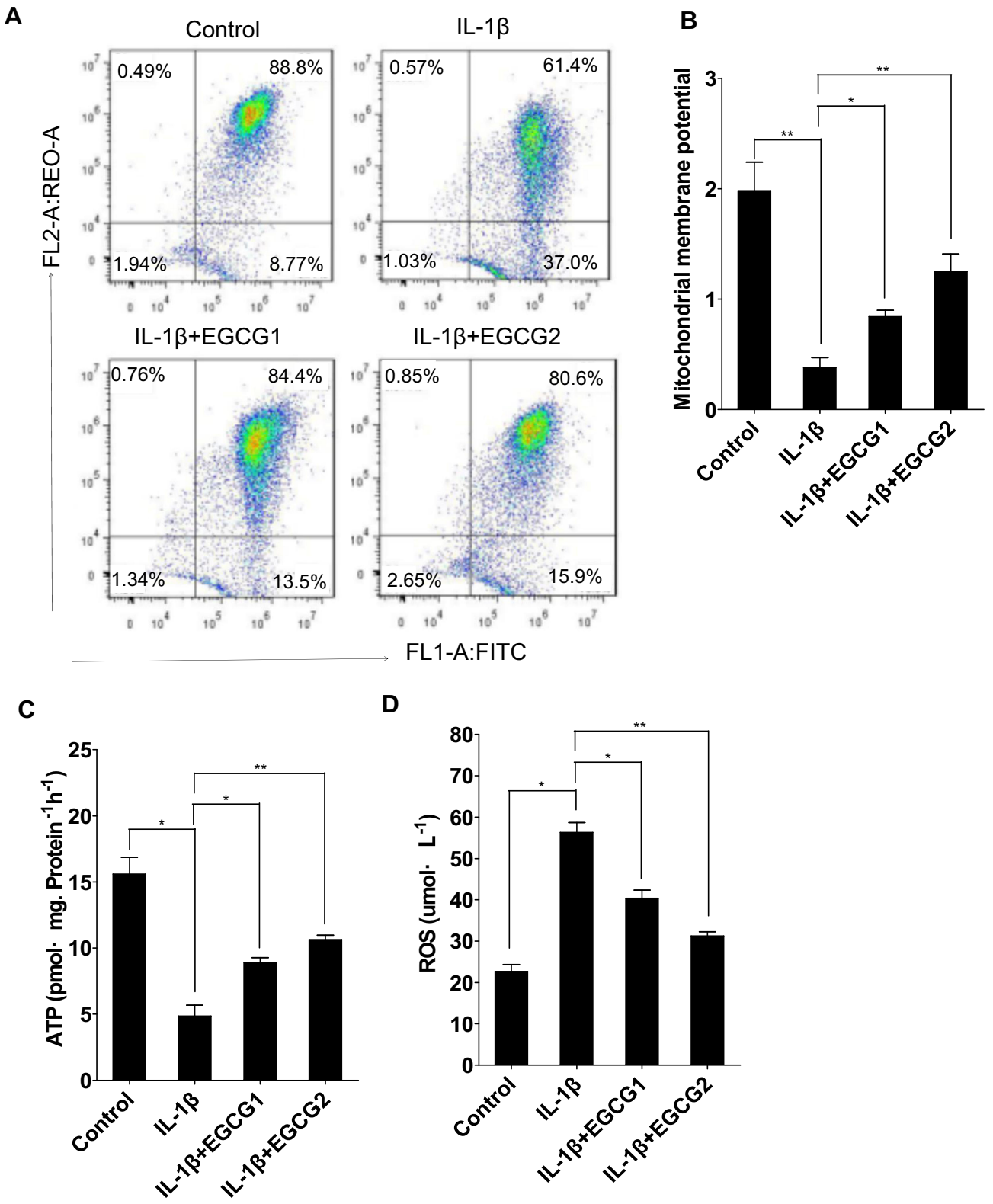

Figure 2 EGCG treatment recovered mitochondrial function and reduced oxidative stress response in IL-1β-stimulated MIN_6 cells. MIN_6 cells were stimulated with IL-1β followed by co-cultivation with low (1mM) or high (5mM) EGCG for 24 h. (**A**) Mitochondrial membrane potential of MIN_6 cells in was determined for each group using flow cytometry. (**B**) The bar chart show a summary of statistical results in (**A**). The activity of ATP (**C**) and content of ROS (**D**) in MIN_6 cells for each group. Data are representative of 2 independent experiments and presented as mean ±SD; *p < 0.05, **p < 0.01. Significant differences were evaluated using one-way ANOVA with a post hoc test (Fisher's least significant difference).

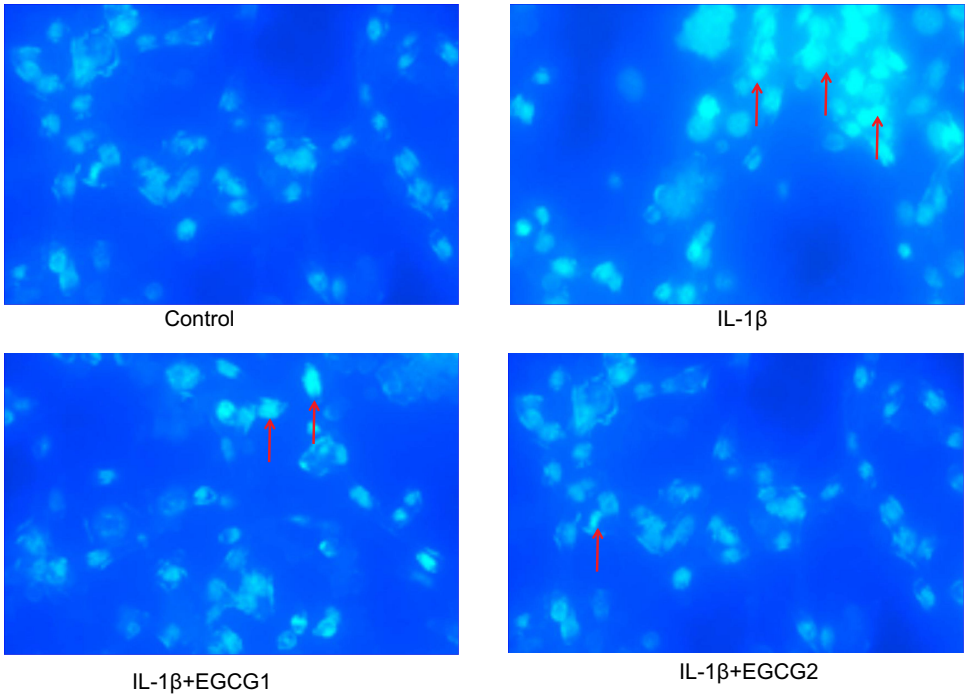

Figure 3 EGCG treatment decreased cell apoptosis in IL-1β-stimulated MIN_6 cells. MIN_6 cells were stimulated with IL-1β followed by co-cultivation with low (1mM) or high (5mM) EGCG for 24 h. Morphological analysis of the nuclei were stained using Hoechst 33258. Red arrows indicate apoptotic MIN_6 cells. Representative images for each group (n=6 per group) are shown (magnification×400).

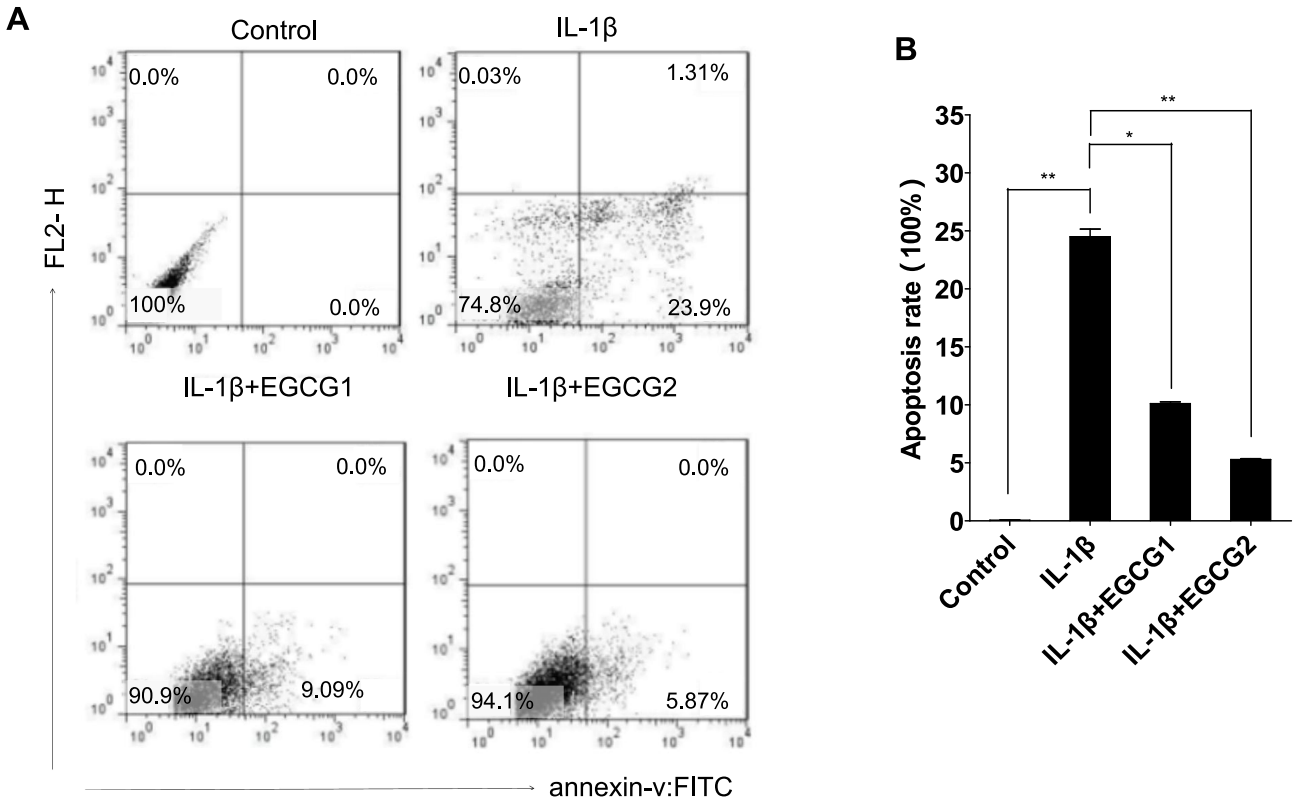

Figure 4 EGCG treatment decreased cell apoptosis in IL-1β-stimulated MIN_6 cells. (**A**) Representative flow cytometry plots show percentages of early apoptotic cells (Annexin V-$FITC^+PI^-$), necrotic cells (Annexin V-$FITC^-PI^+$), late apoptotic cells and necrotic cells (Annexin V-$FITC^+PI^{+)}$ and living cells (Annexin V-$FITC^-PI^-$) for each group of MIN_6 cells receiving specified treatment (n ≥ 6 per group). (**B**) The bar charts show a summary of the statistical results in (**A**). Data are representative of 2 independent experiments and presented as mean ±SD; *p < 0.05, **p < 0.01. Significant differences were evaluated using one-way ANOVA with a post hoc test (Fisher's least significant difference).

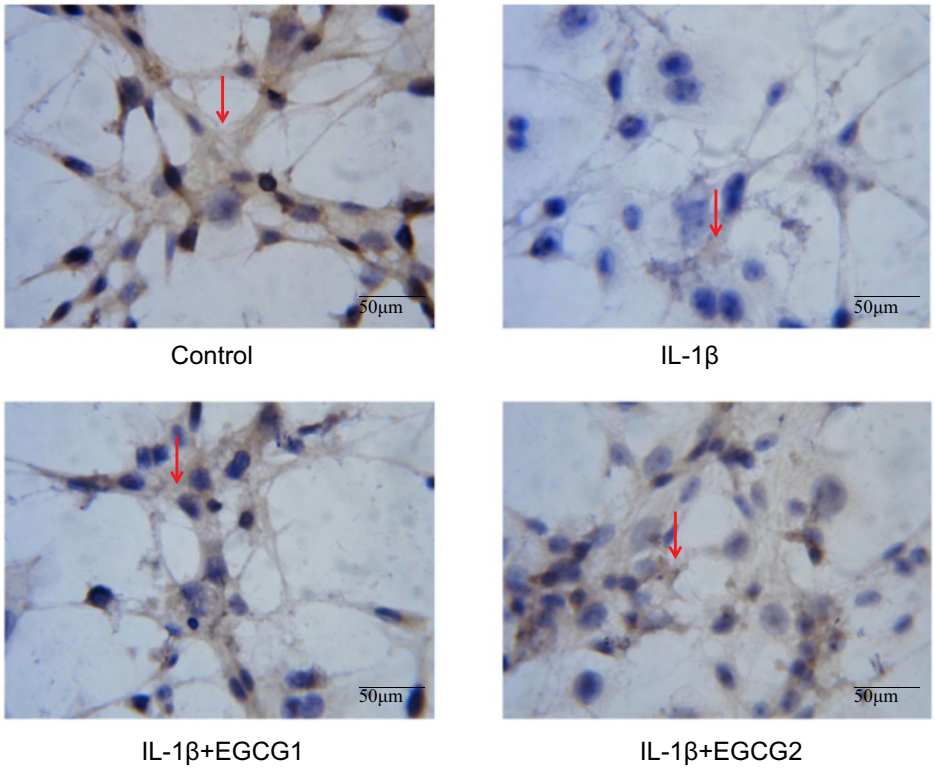

Figure 5 UCP_3 was mainly expressed in the cytoplasm of MIN_6 cells. MIN_6 cells were stimulated with IL-1β followed by co-cultivation with low (1mM) or high (5mM) EGCG for 24 h. UCP_3 protein levels were examined using immunohistochemistry and were indicated by red arrows. Representative histology images for each group (n=6 per group) are shown (magnification×400).

and secretory function of MIN_6 cells and had the ability to induce apoptosis.

Mitochondria are not the only main site of ROS production, but also the first target attacked by ROS. Maladjusted mitochondria causes an accumulation of ROS and reduces antioxidant activity, which further aggravates mitochondrial damage and eventually leads to apoptosis. There is evidence suggesting that UCP_3 inhibits the production of ROS and reduces $\Delta\Psi$, increases proton leaking, affects ATP generation and has an influence on apoptosis.[27] Recent studies showed that UCP_3 deficiency impairs myocardial fatty acid oxidation and contractile recovery following ischemia/reperfusion[28]. In prediabetic subjects and T2DM patients, skeletal muscle UCP_3 content is decreased by almost 50%.[29] Our results confirmed that UCP_3 is expressed in MIN_6 cells, however, its expression is significantly reduced after treatment with IL-1β. Importantly, we observed that ATP synthesis and mitochondrial membrane potential are significantly reduced and increases the synthesis of ROS. Thus, we assumed that IL-1β-mediated mitochondria disorder is attributed to the down-regulation of UCP_3.

Preventing pancreatic β-cells apoptosis can significantly reduce the incidence of diabetes. Therefore, selecting drugs that inhibit apoptosis is very important. EGCG is a natural catechin distributed in green tea that possesses anti-inflammatory, anticarcinogenic, antioxidative, free-radical scavenging in different cell types and animal models.[30–32] It has been suggested that the protective effects of green tea may result from antioxidant ingredients, which are molecules containing easily oxidizable phenolic hydroxyl groups. Studies have shown that EGCG protects cell function by reducing oxidative damage and preserving mitochondrial integrity.[33,34] Animal studies suggested that EGCG can reduce blood glucose in STZ, tetrazolopyrimidine-induced diabetic animals and animal models of spontaneous diabetes.[35] Moreover, EGCG can prevent the occurrence of diabetes.[36] Liu et al[37] emphasized that EGCG has an attenuated effect on myocardial mitochondrial dysfunction in diabetic Goto-Kakizaki rats. Nevertheless, EGCG has distinct effects on different types of cells. For example, H9C2 cells show more stable mitochondrial membrane potential after pre-treatment with EGCG, thereby reducing hypoxia/reoxygenation-induced apoptosis.[38] Experiments have confirmed

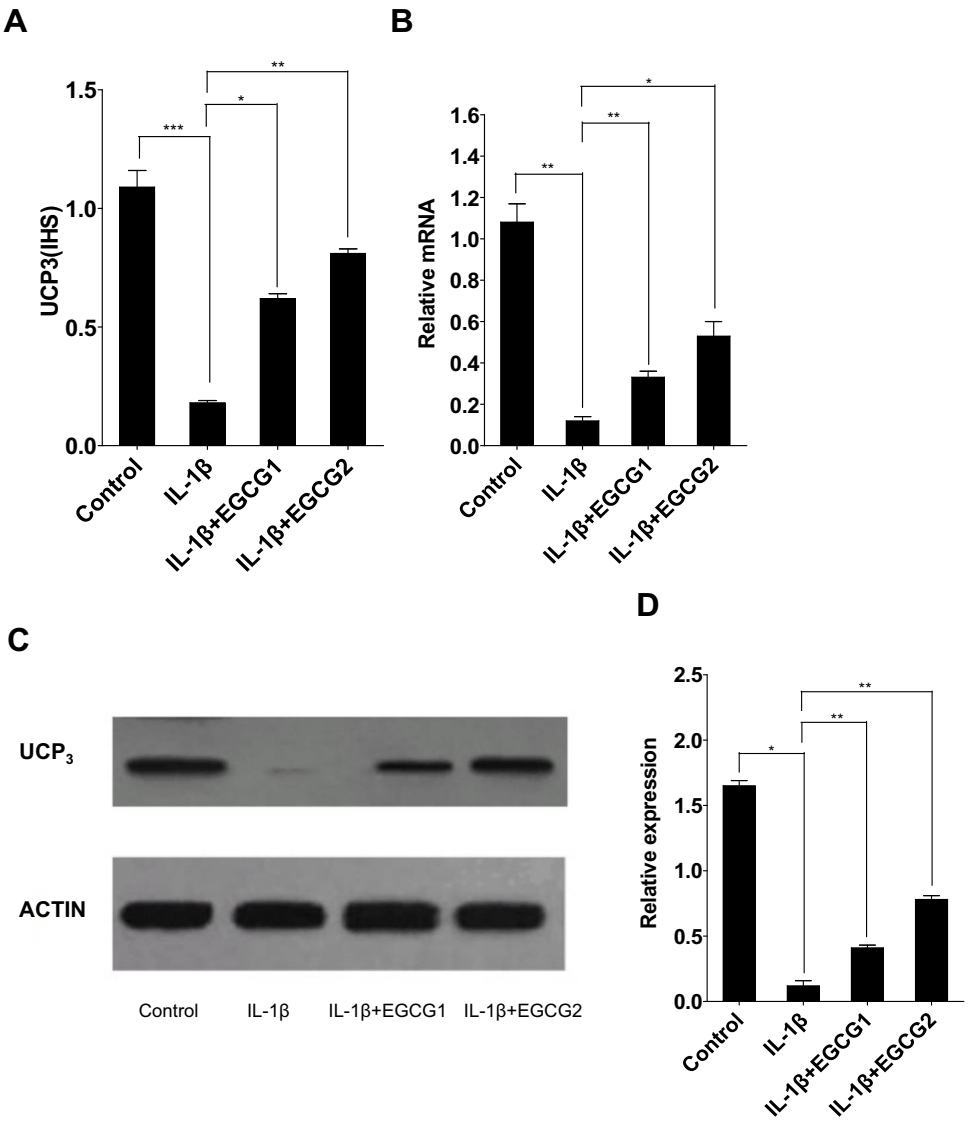

Figure 6 EGCG significantly increased the gene and protein expression levels of UCP_3 in IL-1β-stimulated MIN_6 cells. MIN_6 cells were stimulated with IL-1β followed by co-cultivation with low (1mM) or high (5mM) EGCG for 24 h. (**A**) Summary of the immunohistochemical scores (IHS) for UCP_3 protein levels for each group. (**B**) The gene expression levels of UCP_3 in each group measured by qRT-PCR. (**C**) The protein levels of UCP_3 in each group assessed by Western blotting and normalized to actin. (**D**) Bar chart showing a summary statistical results in (**C**). Data are representative of 2 independent experiments and presented as mean ±SD; *p < 0.05, **p < 0.01, ***p < 0.001. Significant differences were evaluated using one-way ANOVA with a post hoc test (Fisher's least significant difference).

that EGCG protected SH-SY5Y cells against the cytotoxicity induced by lead (Pb) and beta-amyloid peptides (APs) by decreasing OS and preventing apoptosis. This mechanism is related to improving the expression of Bax and BCL_2 and inhibiting annexin V and caspase-3.[39] However, Lee et al[40] found that EGCG promotes 3T3-L1 adipocyte differentiation by increasing ROS production. Our results reflect that EGCG can reduce the apoptosis of MIN_6 cells induced by IL-1β, enhance cell activity and increase insulin secretion function. These results suggest that EGCG has a protective effect on the function of pancreatic β-cells exposed to IL-1β. In our study, treatment of EGCG increases UCP_3 and subsequently increases ATP synthesis and mitochondrial membrane potential. As a result, there are decreased ROS levels observed in IL-1β-induced MIN_6 cells. These results indicate that EGCG can increase the activity of MIN_6 cells by increasing UCP_3 expression, which stabilizes mitochondrial function by maintaining ATP synthesis and mitochondrial membrane potential.

Conclusion

This study clearly demonstrated that the proinflammatory cytokine IL-1β significantly decreased the protein and gene expression levels of UCP_3, ATP synthesis and mitochondrial

membrane potential in MIN_6 cells. This work also provided evidence for the first time that EGCG markedly increased protein and gene expression of UCP_3 in IL-1β-stimulated MIN_6 cells. In conclusion, EGCG has the potential to ameliorate IL-1β-stimulated cell apoptosis in MIN_6 cells by up-regulating UCP_3 expression and may serve as a therapeutic intervention for metabolic diseases such as diabetes.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors do not have potential financial conflicts of interest related to this manuscript. The authors report no conflicts of interest for this work.

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