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Glucose fluctuations aggravate cardiomyocyte apoptosis by enhancing the interaction between Txnip and Akt

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Abstract

Background Glucose fluctuations may be involved in the pathophysiological process of cardiomyocyte apoptosis, but the exact mechanism remains elusive. This study focused on exploring the mechanisms related to glucose fluctuation-induced cardiomyocyte apoptosis.

Methods Diabetic rats established via an injection of streptozotocin were randomized to five groups: the controlled diabetic (CD) group, the uncontrolled diabetic (UD) group, the glucose fluctuated diabetic (GFD) group, the GFD group rats with the injection of 0.9% sodium chloride (NaCl) (GFD + NaCl) and the GFD group rats with the injection of N-acetyl-L-cysteine (NAC) (GFD + NAC). Twelve weeks later, cardiac function and apoptosis related protein expressions were tested. Proteomic analysis was performed to further analyze the differential protein expression pattern of CD and GFD.

Results The left ventricular ejection fraction levels and fractional shortening levels were decreased in the GFD group, compared with those in the CD and UD groups. Positive cells tested by DAB-TUNEL were increased in the GFD group, compared with those in the CD group. The expression of Bcl-2 was decreased, but the expressions of Bax, cleaved caspase-3 and cleaved caspase-9 were increased in response to glucose fluctuations. Compared with CD, there were 527 upregulated and 152 downregulated proteins in GFD group. Txnip was one of the differentially expressed proteins related to oxidative stress response. The Txnip expression was increased in the GFD group, while the Akt phosphorylation level was decreased. The interaction between Txnip and Akt was enhanced when blood glucose fluctuated. Moreover, the application of NAC partially reversed glucose fluctuations-induced cardiomyocyte apoptosis.

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Conclusions Glucose fluctuations lead to cardiomyocyte apoptosis by up-regulating Txnip expression and enhancing Txnip-Akt interaction.

Keywords Glucose fluctuation, Cardiomyocyte apoptosis, Thioredoxin-interacting protein, Protein kinase B/Akt

Introduction

Diabetes mellitus (DM) has been recognized as one of the major health issues, posing a serious threat to human health worldwide [1, 2]. An accumulating body of studies has shown that blood glucose fluctuations may be a greater risk of the development of cardiovascular diseases than persistent hyperglycemia [3, 4]. Glucose fluctuation has been confirmed in several recent studies to promote cardiomyocyte apoptosis more significantly compared with persistent hyperglycemia [5, 6]. However, the underlying mechanisms remain unknown.

Recently, several studies have reported that glucose fluctuations could lead to excessive oxidative stress [7]. Under normal physiological condition, there is a balance between oxidants and antioxidants. While in various pathological processes, overproduction of reactive oxygen species (ROS) contribute to excessive oxidative stress, resulting in cellular dysfunction, such as cell apoptosis [8]. Thioredoxin is an antioxidant protein and involved in maintaining the balance of oxidative stress [9, 10]. Thioredoxin-interacting protein (Txnip) negatively regulates thioredoxin, which functions as pro-oxidant and sensitizes myocytes to ROS-associated apoptosis [11]. The increase level of Txnip and excessive oxidative stress contribute to myocardium apoptosis in diabetes [12]. Moreover, several studies have reported that the level of Txnip is upregulated significantly in state of glucose fluctuations [3, 13].

Protein kinase B (PKB)/Akt is a serine–threonine kinase, and the Akt signaling plays important roles in cell growth, proliferation and apoptosis [14, 15]. The Akt signaling participates in regulating apoptosis through multiple mechanisms, including directly phosphorylating several anti-apoptotic factors and activating the transcriptional genes supporting cell survival [16]. The activation of Akt alleviates myocardium apoptosis in streptozotocin (STZ)-induced diabetes [17]. A previous study reported that Txnip might regulate the activity of Akt via direct interaction, leading to glucose-mediated metabolic stress [18]. However, whether Txnip regulates Akt activity after exposure to glucose fluctuations and the mechanism remains unknown.

In the present study, we explored the influences of glucose fluctuations on the process of cardiomyocyte apoptosis and the underlying mechanisms. We found that glucose fluctuations upregulated Txnip and enhanced the interaction between Txnip and Akt, which play a vital role in the promotion of cardiomyocyte apoptosis.

Materials and methods

Experimental animal models

Male Sprague–Dawley (SD) rats (6–8 weeks) were purchased from Changzhou Cavens Laboratory Animal Company in China. The rats were raised at a standard of care specific pathogen-free (SPF) conditions (temperature 22–24℃ and humidity 55–65%) in a light/dark cycle adapted to 12 h. After acclimatization, intraperitoneal injection of streptozotocin (STZ, 60 mg/kg; S0130, Sigma-Aldrich) was performed to establish type 1 diabetic rat model, as previously reported [19]. Blood glucose was measured in the tail vein of rats after one week. If the blood glucose was >300.6 mg/dL, the rat was enrolled and then randomized to five groups. Rats in the controlled diabetic (CD) group (n=8) were performed with a subcutaneous injection of long-acting insulin (20 IU/kg; Sanofi-Aventis) at 8 a.m. and 8 p.m. every day. Rats in the uncontrolled diabetic (UD) group (n=8) were raised without any treatments. Rats in the glucose fluctuated diabetic (GFD) group (n=8) were treated with a 24 h of starvation, and would receive regular insulin (0.5 IU/kg; Novo Nordisk) if their blood glucose levels were >99 mg/dL. Then, rats were treated with a 24 h of consumption with adequate food. Rats in the GFD group rats with the injection of 0.9% sodium chloride (NaCl, GFD+NaCl, n=3) were injected with 0.9% NaCl daily, while rats in the GFD group rats with the injection of N-acetyl-L-cysteine (NAC, GFD+NAC, n=3) were intraperitoneally injected with NAC (150 mg/kg; A7250, Sigma-Aldrich). Twelve weeks later, after being exposed to adequate food for the last two days, rats were sacrificed with an intraperitoneally injection of sodium pentobarbital (60 mg/kg). Hearts were harvested and stored at -80℃. The ratios of heart weight/body weight (HW/BW) were calculated. Animal care and experimental protocol were approved by the Institutional Animal Care of the Wuxi People's Hospital Affiliated to Nanjing Medical University (Approval number: IACUC-1712028). The study was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and this study was carried out in compliance with the ARRIVE guidelines.

Tandem Mass Tags (TMT) labeled quantitative proteomics analysis

The quantitative proteomics analysis of rat cardiac ventricle tissues was performed in Luming Biotechnology Co., Ltd (Shanghai, China). Briefly, proteins were extracted from rat cardiac ventricle tissues and quantified

with bicinchoninic acid kit (BCA). Then, 100 μg proteins were hydrolyzed using trypsin and labeled with TMT reagent. Then, separation of the labeled peptides was done on a reverse phase chromatography (Agilent Zorbax Extend-C18) with an Agilent 1100 HPLC and the identification of proteins was carried out with a Q-Exactive HF MS (ThermoFisher, U.S.A). Proteome Discoverer software (v2.4, Thermo Fisher Scientific) was used to search all of the Q Exactive raw data thoroughly against the sample protein database. The global FDR was set to 0.01, and for protein groups to be considered for quantification at least 2 peptides were required. The identity of proteins in a set was verified on the basis of the following two values: a Sequest HT score > 0 and unique peptide > 1. Differentially expressed or secreted proteins were identified on the basis of a FC > 1.2 or < 1/1.2 and p-value < 0.05. A GO enrichment analysis was carried out online (https://david.ncifcrf.gov/). Figures of cluster heatmap and GO enrichment analysis were made using bioinformatics (https://www.bioinformatics.com.cn/).

Echocardiography

Rats were anesthetized with isoflurane (2%) before the echocardiography (ie33, Philip) was performed. Images were obtained using M-mode echocardiography to calculate the left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), left ventricular internal diameter at end-diastole (LVIDd), and left ventricular internal diameter at end-systole (LVIDs). All measurements were made by a sonologist who was blinded to the identity of the tracings.

ELISA of malondialdehyde (MDA)

Rat blood samples were collected from the inferior vena cava and the levels of MDA in rat plasma were measured using a MDA ELISA kit (ER1878, Wuhan Fine Biotech Co.).

Cell culture and treatment

H9C2 cells were purchased from Chinese Academy of Science, and were cultured in DMEM (11885-084, Gibco) which contains 10% fetal bovine serum (FBS) (12664025, Gibco). Primary cultures of neonatal rat ventricular cardiaomyocytes (NRVCMs) were obtained from 1 to 3 days old SD rats according to a procedure published previously [20, 21]. In brief, the hearts of neonatal rats were cut off atrial tissue and cut into small pieces, and then were digested with cardiaomyocyte isolation enzyme 1 and enzyme 2 (88281, Thermo). Cardiaomyocytes were collected and resuspended with DMEM (88287, Thermo) which contains 10% FBS (12664025, Gibco). H9C2 cells and NRVCMs were cultured in 37℃ with 5% CO_2 incubator. Then, the cells were divided into three groups: the normal glucose (NG) group which was cultured with 5.5 mmol/L glucose, the high glucose (HG) group which was cultured with 25 mmol/L glucose, and the glucose fluctuation (GF) group which was cultured with glucose alternating between 5.5 and 25 mmol/L every 12 h for 72 h.

Glucose monitoring

The blood glucose levels of all diabetic rats were measured with a blood glucose meter (Roche) daily.

DAB-TUNLE

Hearts were fixed in 4% paraformaldehyde, and then dehydrated in graded ethanol. After soaking xylene and paraffin imbedding, heart issues were cut into 5 μm slices, followed by dewaxing and rehydration. Firstly, the paraffin slides were deparaffinized and rehydrated, and then endogenous peroxidase was blocked. After equilibrium at room temperature, the slides were treated with the TUNEL kit (G1507, Servicebio). DAB chromogenic reagent to marked tissue followed by counterstaining with hematoxylin. The images were captured under a light microscope (Leica Microsystems). The positive apoptosis cells developed by DAB reagent have brown nucleus.

Confocal microscopy

H9C2 cells and NRVCMs were respectively incubated over night at 4℃ with the mixture of anti-Txnip antibody (ab210826, Abcam, raised in mouse) and anti-AKT antibody (4691, Cell Signaling Technology, raised in rabbit). Then, cells were incubated with the mixture of Alexa Fluor 594 AffiniPure Donkey Anti-Mouse IgG (H + L) (34112ES60, Yeasen Biotechnology), Alexa Fluor 488 AffiniPure Donkey Anti-Rabbit IgG (H + L) (34206ES60, Yeasen Biotechnology) and together with DAPI (P0131, Beyotime).

Western blot

Heart tissues were homogenized and lysed in lysis buffer (89900, Pierce), which contains protease and phosphatase inhibitors (04693159001, Roche). After being added with protein SDS-PAGE loading buffer (9173, Takara), sample proteins were transferred onto polyvinylidene difluoride membranes (IPVH00010, Immobilon). Then, polyvinylidene difluoride membranes were incubated with the specific primary antibodies to α-tubulin (2144, Cell Signaling Technology), β-actin (4970, Cell Signaling Technology), Bax (2772, Cell Signaling Technology), Bcl-2 (sc-7382, Santa Cruz Biotechnology), cleaved caspase-3 (9661, Cell Signaling Technology), caspase-9 (10380-1-AP, Proteintech), NADPH oxidase 4 (Nox4, ab154244, Abcam), Txnip (14715, Cell Signaling Technology), Akt (4691, Cell Signaling Technology), p-AKT (4060, Cell Signaling Technology). The optical density of immunoblot bands were analyzed using ImageJ software. Densities

were normalized to control treatment and relative folds were normalized to α-tubulin or β-actin.

Co-immunoprecipitation

H9C2 cells were lysed by NP-40 Lysis Buffer (P0013F, Beyotime). Primary antibodies and protein A/G magnetic beads (HY-K0202, MCE) were mixed together with lysates for 12 h in 4℃. Magnetic beads were washed by lysis buffer for 3 times, followed by boiled with loading buffer (9173, Takara). The next procedure was western blot as described above. Then, polyvinylidene difluoride membranes were incubated with the specific primary antibodies to Txnip (ab210826, Abcam), Akt (4691, Cell Signaling Technology) and β-actin (4970, Cell Signaling Technology).

Statistical analysis

Data were presented as the mean ± SEM. Student's t-test was used to compare data between two groups. One-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison tests was used to compare data from multiple groups. SPSS 22.0 was used for analyzing statistical data. Statistical significance was defined as a p value of <0.05.

Results

Glucose fluctuations aggravate the LVEF in diabetic rats

Figure 1A shows the blood glucose levels in the CD group (around 90 mg/dL), UD group (higher than 450 mg/dL) and GFD group (between 90 mg/dL and 450 mg/dL). Figure 1B shows the body weights of rats in the CD, UD and GFD groups. The fluctuated weight in the GFD group was resulted from repeated starvation. Figure 1C shows the representative echocardiographic images. After twelve weeks, heart-to-body weight ratios among the three groups show no significant differences (Fig. 1D). As shown in Fig. 1E-H, the LVEF level in the UD group (86.74 ± 1.25%) was lower than that in the CD group (91.74 ± 0.94%), and was to a greater extent in the GFD group (81.93 ± 2.00%). While the FS level in the UD group (51.20 ± 1.55%) decreased compared with the CD group (58.81 ± 1.81%), which was more pronounced than that in the GFD group (45.90 ± 2.12%). The LVIDd data showed no significant differences among the three groups. However, the LVIDs data was increased in the UD group compared with the CD group, which was more pronounced in the GFD group. These results suggested that left ventricular systolic function was impaired in the GFD group rats.

Glucose fluctuations promote cardiomyocyte apoptosis

As shown in Fig. 2A, compared with the CD group, the positive apoptosis cells tested by DAB-TUNEL were increased in the GFD group. There was a decreasing trend of Bcl-2 protein expression, while there was an increasing trend of Bax, cleaved caspase-3 and cleaved caspase-9 protein expressions in the UD group, and these trends showed more pronounced in the GFD group (Fig. 2B). Figure 2C-E show that the protein level of Bcl-2 was downregulated, while the protein levels of Bax and cleaved caspase-3 were upregulated in the GF group, compared with these changes in the NG and HG groups. These results indicated that glucose fluctuations could lead to cardiomyocyte apoptosis.

Glucose fluctuations upregulate the expression of Txnip

Proteomic analysis was done to further analyze the differential protein expression pattern of the CD and GFD groups. Compared with CD, there were 527 upregulated and 152 downregulated proteins in GFD group. Gene Ontology analysis showed that differentially expressed proteins were involved in several biological processes such as RNA splicing, metabolism, response to oxidative stress and so on (Fig. 3A). Oxidative stress was associated with cell death. The differentially expressed proteins related to oxidative stress response were presented in Fig. 3B. Moreover, the MDA level was significantly increased in the GFD group than that in the CD group (Fig. 3C). The protein expressions of Nox4 and Txnip were higher in the GFD group than that in the CD and UD groups, and the Akt phosphorylation level was decreased due to glucose fluctuations (Fig. 3D). Moreover, the protein expression of Txnip was increased and the Akt phosphorylation level was decreased in H9C2 cells which exposed to the fluctuated glucose concentrations (Fig. 3E-F).

Glucose fluctuations could enhance the interaction between Txnip and Akt

To identify the interaction between Txnip and Akt, we performed co-immunoprecipitation (IP) experiments in H9C2 cells. As shown in Fig. 4A, glucose fluctuations enhanced the Txnip-Akt interaction. Immunofluorescence results show that Txnip and Akt were distributed in the cytoplasm and overlapped, the Txnip-Akt interaction was observed both in the H9C2 cells and NRVCMs of the GF group (Fig. 4B-C).

NAC treatment reversed myocardium apoptosis in glucose fluctuation

The LVEF and FS levels were increased in the GFD + NAC group, compared with those in the GFD + NaCl group (Fig. 5A-C). As shown in Fig. 5D, the protein expression of Bcl-2 was increased, while the protein expressions of Bax and cleaved caspase 3 were decreased in the GFD + NAC group. Additionally, Txnip expression was significantly decreased, and the AKT phosphorylation level was increased in the GFD + NAC group, compared

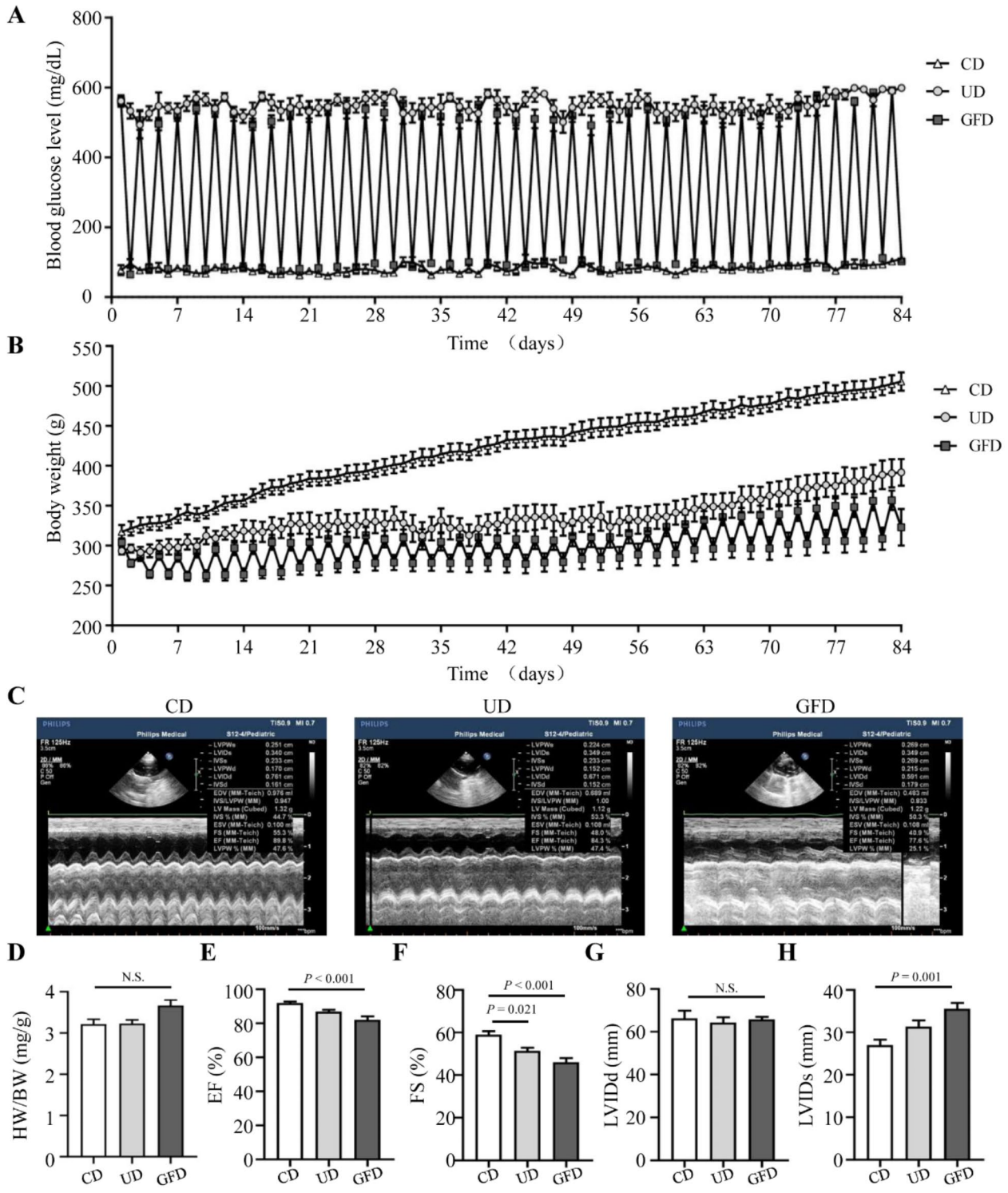

Fig. 1 Effects of glucose fluctuations on cardiac function (**A**) Blood glucose levels in the CD, UD and GFD groups ($n = 8$ per group). (**B**) Body weights in the CD, UD and GFD groups ($n = 8$ per group). (**C**-**H**) Representative echocardiographic images, heart weight/body weight (HW/BW), left ventricular ejection fraction (% EF), left ventricular fractional shortening (% FS), left ventricular internal diameter at end-diastole (LVIDd), and left ventricular internal diameter at end-systole (LVIDs) examined by echocardiography in the CD, UD and GFD groups ($n = 8$ per group). N.S. No significant difference

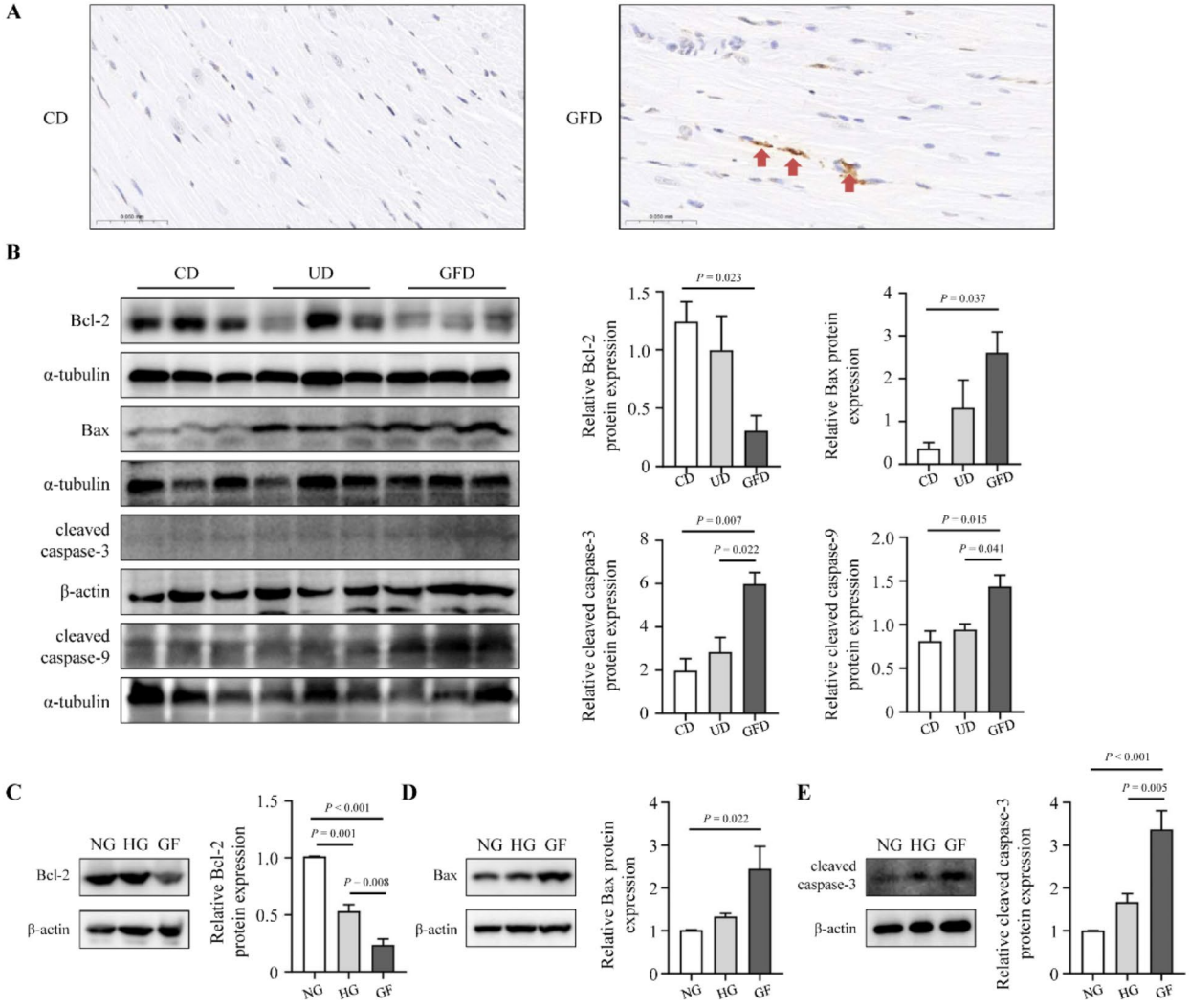

Fig. 2 Glucose fluctuations promote cardiomyocyte apoptosis in diabetic rats (**A**) Representative DAB-TUNEL staining images of heart tissues in the CD and GFD groups. (**B**) The protein expressions of Bcl-2, Bax, cleaved caspase-3 and cleaved caspase-9 in rat hearts of the three groups ($n=3$ per group). (**C**-**E**) The protein expressions of Bcl-2 ($n=3$), Bax ($n=4$) and cleaved caspase-3 ($n=4$) in H9C2 cells of the NG, HG and GF groups

with those in the GFD+NaCl group (Fig. 5E-F). These results suggested that NAC treatment partially reversed glucose fluctuations-induced left ventricular systolic dysfunction and cardiomyocyte apoptosis.

Discussion

Recently, an accumulating number of studies have reported that glucose fluctuation is an important contributor to cardiomyocyte damage in DM [6, 22–24]. Our study was focused on the influence and underlying mechanisms of glucose fluctuations in development of myocardium apoptosis. The most important findings in this study are: (1) Glucose fluctuations could result in the upregulation of Txnip and the enhanced the interaction between Txnip and Akt, leading to cardiomyocyte apoptosis; (2) The application of NAC partially reversed cardiomyocyte apoptosis in diabetic rats with fluctuated blood glucose.

DM is regarded as a vital contributor to the development of cardiac dysfunction, including left ventricle diastolic and systolic dysfunction [25]. Moreover, glucose fluctuation has been reported to have more deleterious effects on cardiac dysfunction than persistent hyperglycemia. We found that the LVEF and LVFS levels were reduced to a greater extent in the GFD rats than those in the CD and UD rats, indicating that glucose fluctuations promoted the development of left ventricle systolic dysfunction. The diabetes-associated cardiac remolding is responsible for cardiac dysfunction, including myocardium hypertrophy, myocardium apoptosis and interstitial fibrosis [26, 27]. Hyperglycemia has been considered as a pathophysiological trigger of myocardium apoptosis

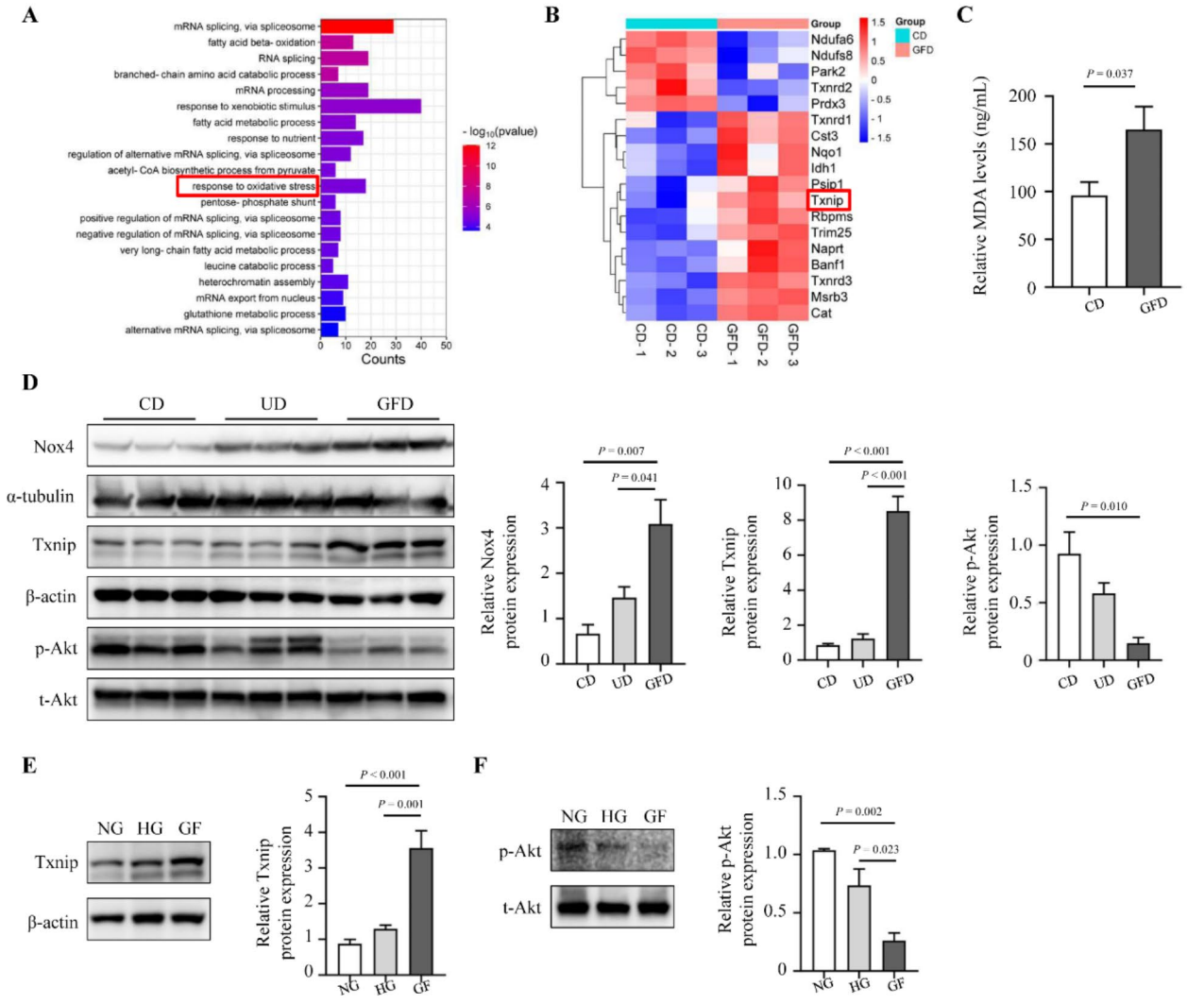

Fig. 3 Glucose fluctuations lead to the upregulation of Txnip (**A**) Gene Ontology analysis-biological process of differentially expressed proteins ($n=3$ per group). (**B**) The heatmap of differentially expressed proteins involved in response to oxidative stress between CD and GFD ($n=3$ per group). (**C**) The MDA levels in rat plasma of the CD and GFD groups ($n=5$ per group). (**D**)The protein expressions of Nox4, Txnip and the Akt phosphorylation level in rat hearts of the three groups ($n=3$ per group). (**E**-**F**) The protein expression of Txnip ($n=3$) and the Akt phosphorylation level ($n=4$) in H9C2 cells of the NG, HG and GF groups

in the diabetic heart. Several studies have reported that glucose fluctuations aggravate myocardium apoptosis than persistent hyperglycemia [5, 6]. Caspase-3 is a key enzyme in the execution of apoptosis, which belongs to endoprotease and mediates apoptosis by cleaved caspase-3. Bcl-2 and Bax regulate apoptosis together to keep cell homeostasis [28]. In our study, we found that the protein expressions of Bax, cleaved caspase-3 and cleaved caspase-9 were increased, while the protein expression of Bcl-2 was decreased in the GFD group, compared with the CD and UD groups.

Proteomic analysis results showed that Compared with CD, there were 527 upregulated and 152 downregulated proteins in GFD group. Gene Ontology analysis showed that differentially expressed proteins were involved in several biological processes such as RNA splicing, metabolism, response to oxidative stress and so on. Oxidative stress in diabetes is involved in the process of myocardium apoptosis [29, 30]. MDA has been confirmed as a biomarker of lipid peroxidation caused by ROS overproduction, thus we tested the MDA level in rat plasma and MDA level was increased in GFD group. Moreover, NADPH oxidases are the major sources of ROS production, Nox4 protein expression was higher in the diabetic rats with glucose fluctuations, indicating that glucose fluctuations could contribute to excessive oxidative stress. Oxidative stress is the imbalance between oxidation and anti-oxidation and tends to oxidize. Txnip and

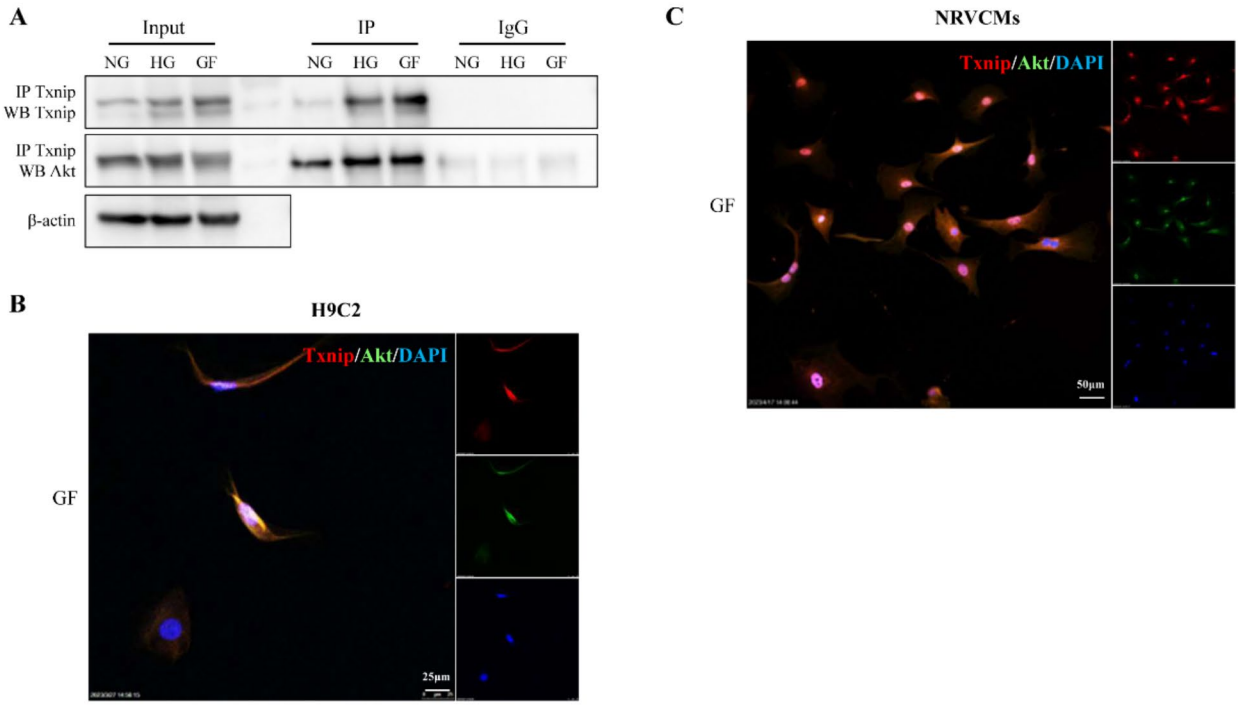

Fig. 4 Glucose fluctuations enhance the Txnip-Akt interaction (**A**) Co-immunoprecipitation (Co-IP) of Txnip and Akt in H9C2 cells of the NG, HG and GF groups ($n=3$ per group). IgG represents a control antibody used for IPs. (**B**) Immunofluorescence staining of intracellular Txnip–Akt interaction examined by confocal microscopy in H9C2 cells of the GF groups. The cells were labeled with anti-Txnip (red), anti-Akt (green), and DAPI (blue) ($n=3$ per group). (**C**) Immunofluorescence staining of intracellular Txnip–Akt interaction examined by confocal microscopy in NRVCMs of the GF groups. The cells were labeled with anti-Txnip (red), anti-Akt (green), and DAPI (blue) ($n=3$ per group)

thioredoxin interact with each other directly and maintain the oxidative balance in cells to a certain extent. Txnip mediates redox homeostasis by increasing ROS production, besides ROS promotes the activation of Txnip pathway [31]. Our proteomic analysis results showed that Txnip was one of the differentially expressed proteins related to oxidative stress response. In this study, we found that the Txnip level was increased significantly in the GFD group.

Akt is involved in various kinds of cellular processes including cell survival, growth and metabolism. Akt signaling regulates cell apoptosis in multiple ways, such as directly phosphorylating several anti-apoptotic factors (caspase and Bcl2 associated agonist of cell death) [32]. In diabetes, Akt activation is reduced due to hyperglycemia, leading to the increase of cardiomyocyte apoptosis [17]. Our data showed that the Akt phosphorylation level declined significantly in the GFD group, indicating that Akt is involved in glucose fluctuation-induced cardiomyocyte apoptosis. The structural of Akt comprises of an N-terminal pleckstrin homology (PH) domain, a kinase domain (KD), and a C-terminal hydrophobic regulatory region. In an inactive state, PH domain binds with KD through an intramolecular interaction. Phosphoinositide 3-kinase activates Akt through interacting with the PH domain, thereby dislodging the PH domain from the KD. Moreover, PH domain can interact with other proteins, modulating the structure of Akt to allow for its activation [14]. It has been reported that Txnip negatively regulates the activity of Akt under glucose stress and oxidative stress through directly interacting with the PH domain [18]. In our study, we found that the Txnip-Akt interaction was enhanced in response to glucose fluctuations. We suppose that the increase level of Txnip induced by glucose fluctuations negatively regulated Akt activity through their direct interaction.

NAC, acting as an inhibitor of ROS, can eliminate ROS in oxidative stress and inhibit the activation of Txnip pathway [33, 34]. In this study, our results showed that NAC treatment could partially reversed glucose fluctuations-induced left ventricle systolic dysfunction and cardiomyocyte apoptosis.

Conclusions

In sum, our study demonstrated that glucose fluctuations up-regulate the Txnip expression and enhance the Txnip-Akt interaction, aggravating cardiomyocyte apoptosis. While the NAC treatment can reverse these changes in

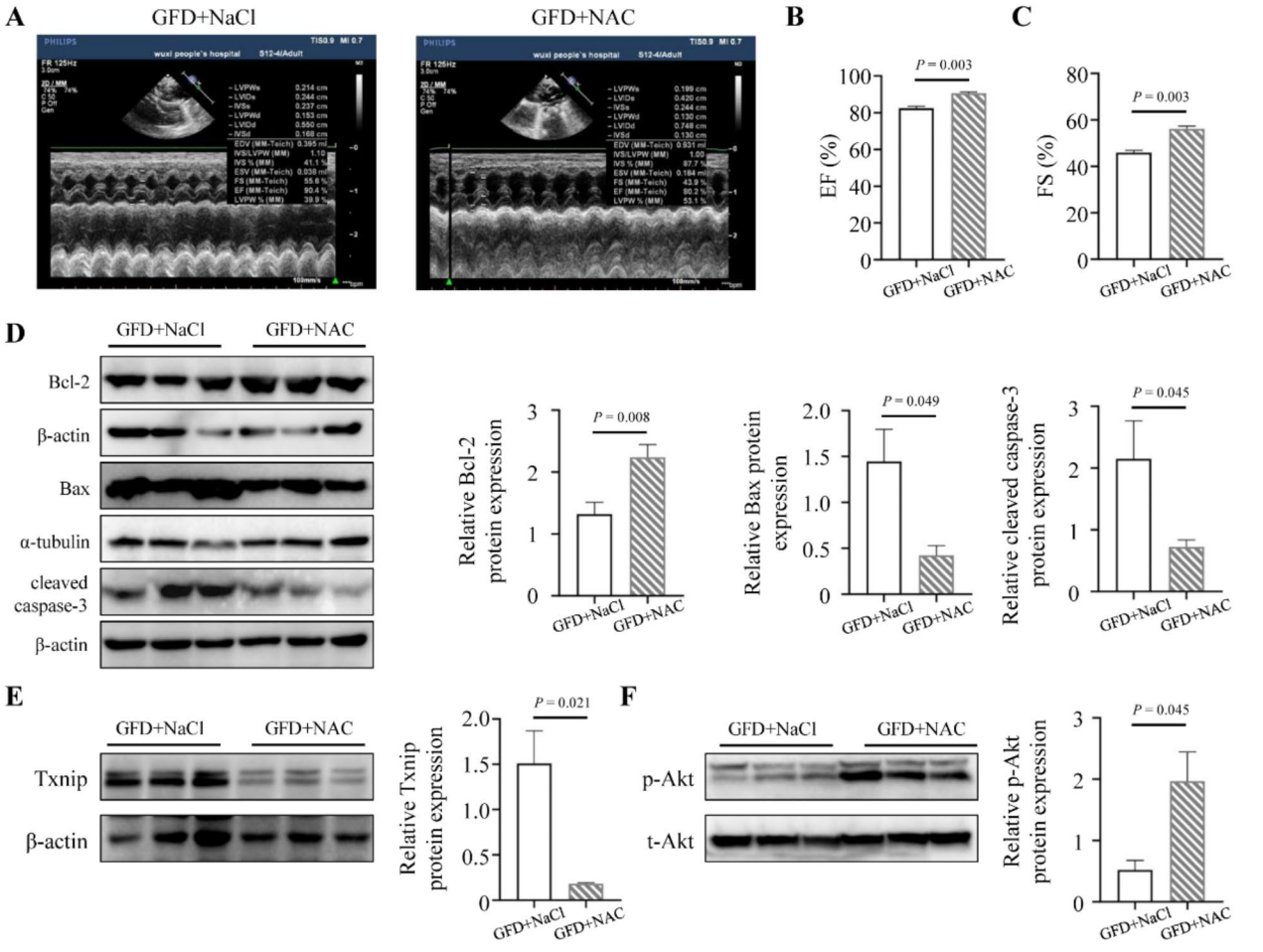

Fig. 5 NAC treatment declined glucose fluctuation-induced cardiomyocyte apoptosis (**A**-**C**) Representative echocardiographic images, the EF and FS of rats in the GFD + NaCl and GFD + NAC groups ($n = 3$ per group). (**D**) The protein expressions of Bcl-2 ($n = 6$), Bax ($n = 3$) and cleaved caspase-3 ($n = 6$) in rat hearts of the two groups. (**E**-**F**) The protein expression of Txnip and the Akt phosphorylation level in rat hearts of the two groups ($n = 3$ per group)

response to glucose fluctuation and function as a potential therapeutic option, suggesting a new therapy target to slow or reverse the cardiovascular complications of diabetes in future.

Abbreviations

BCA	bicinchoninic acid
BW	body weight
CD	controlled diabetic
DAB	diaminobenzidine
DM	diabetes mellitus
FBS	fetal bovine serum
GF	glucose fluctuation
GFD	glucose fluctuated diabetic
HG	high glucose
HW	heart weight
IACUC	Institutional Animal Care and Use Committee
IOD	integrated optical density
IP	immunoprecipitation
KD	kinase domain
LVEF	left ventricular ejection fraction
LVFS	left ventricular fractional shortening
LVIDd	left ventricular internal diameter at end-diastole
LVIDs	left ventricular internal diameter at end-systole
NAC	N-acetyl-L-cysteine
NaCl	sodium chloride
NG	normal glucose
NRVCMs	neonatal rat ventricular cardiaomyocytes
PH	pleckstrin homology
PKB	protein kinase B
ROS	reactive oxygen species
SD	Sprague–Dawley
SPF	specific pathogen-free
STZ	streptozotocin
TMT	Tandem Mass Tags
Txnip	thioredoxin-interacting protein
UD	uncontrolled diabetic

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12872-024-04134-0.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
Z-YZ, LP and R-XW were involved in the experiment design. Z-YZ, LP, S-PD, NW, S-YZ, F-L, L-DW, LZ, H-HL and NZ performed the experiments. Y-JY and L-LQ analyzed the data. Z-YZ wrote the manuscript. L-LQ, TL and R-XW edited the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets generated and analyzed during the current study are not publicly available due the principle of funding confidentiality but are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Animal care and experimental protocol were approved by the Institutional Animal Care of the Wuxi People's Hospital Affiliated to Nanjing Medical University (Approval number: IACUC-1712028). The study was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and this study was carried out in compliance with the ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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