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Plasma microRNA expression profiles associated with zinc exposure and type 2 diabetes mellitus: Exploring potential role of miR-144-3p in zinc-induced insulin resistance

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ABSTRACT

Zinc exposure has been linked with disordered glucose metabolism and type 2 diabetes mellitus (T2DM) development. However, the underlying mechanism remains unclear. We conducted population-based studies and in vitro experiments to explore potential role of microRNAs (miRNAs) in zinc-related hyperglycemia and T2DM. In the discovery stage, we identified plasma miRNAs expression profile for zinc exposure based on 87 community residents from the Wuhan-Zhuhai cohort through next-generation sequencing. MiRNAs profiling for T2DM was also performed among 9 pairs newly diagnosed T2DM-healthy controls. In the validating stage, plasma miRNA related to both of zinc exposure and T2DM among the discovery population was measured by qRT-PCR in 161 general individuals derived from the same cohort. Furthermore, zinc treated HepG2 cells with mimic or inhibitor were used to verify the regulating role of miR-144-3p. Based on the discovery and validating populations, we observed that miR-144-3p was positively associated with urinary zinc, hyperglycemia, and risk of T2DM. In vitro experiments confirmed that zinc-induced increase in miR-144-3p expression suppressed the target gene Nrf2 and downstream antioxidant enzymes, and aggravated insulin resistance. Our findings provided a novel clue for mechanism underlying zinc-induced glucose dysmetabolism and T2DM development, emphasizing the important role of miR-144-3p dysregulation.

1. Introduction

Over the past decades, the worldwide prevalence of diabetes has been expanding and a pandemic has spread all over the world (Mayer-Davis et al., 2017; Safiri et al., 2022). According to the report of International Diabetes Federation, global diabetes prevalence in 2021 was 10.5% (i.e., over half a billion people), which would rise to 12.2% (i.e., 783.2 million people) in 2045 (Sun et al., 2022). Of note, more than 90% patients with diabetes were type 2 diabetes mellitus (T2DM). Accumulating evidence has emerged indicating that the increasing global T2DM prevalence is closely linked with environmental risk factors (Eze et al., 2015; Li et al., 2021; Renzi et al., 2018). Zinc is an essential nutrition element for human in growth, development, immune function, and metabolic regulation, etc., as well as a heavy metal which would be toxic for human health at high-dose exposure (Plum et al., 2010; Skalny et al., 2021). Based on a large-scale prospective cohort of urban community residents, we observed the adverse effects of zinc exposure on glucose homeostasis and T2DM risk in previous study (Ye et al., 2022). Similarly, the positive associations of zinc exposure with glycometabolic dysfunction and the development of T2DM have also been reported in several other epidemiological studies (Fernández-Cao et al., 2019; Yary et al., 2016). However, the underlying mechanisms are not well understood.

MicroRNAs (miRNAs) are small noncoding RNAs, negatively regulating target genes post-transcriptionally by binding 3′-untranslated region (3′-UTR) of mRNAs. MiRNAs have attracted much attention for

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their role in identification and diagnosis of T2DM in recent years (Nie et al., 2022; Willeit et al., 2017; Zampetaki et al., 2010). Emerging evidence from in vivo and in vitro studies have confirmed that miRNAs regulated key genes involved in glycometabolism, affecting the occurrence and development of T2DM (Belgardt et al., 2015; Tattikota et al., 2014; Xu et al., 2018). Xu et al. found that miR-125a-5p interacted with STAT3 mRNA and further downregulated its protein levels, thereby ameliorating glycolipid metabolic disorder (Xu et al., 2018). In addition, another study based on MIN6 cells and diabetic mice indicated that ablation of miR-200 family protected beta cells from apoptosis induced by oxidative stress and further alleviated T2DM (Belgardt et al., 2015). However, the specific role of miRNAs in zinc-related glucose metabolic dysfunction and T2DM is unclear.

In the current study, we aimed to profile zinc exposure- and T2DM-related circulating miRNAs expression by small RNAs next-generation sequencing. MiRNA related to both of zinc exposure and T2DM was selected and further validated by quantitative reverse-transcription polymerase chain reaction (qRT-PCR). The associations of candidate miRNA with urinary zinc, fasting plasma glucose (FPG) and risk of T2DM were evaluated. And potential role of candidate miRNA in the relationships of zinc exposure with FPG and T2D risk was assessed. Additionally, we used zinc-induced HepG2 cells to confirm the adverse effects of zinc on insulin resistance, a fundamental pathologic mechanism of T2DM, as well as on the candidate miRNA expression. We further interfered miRNA expression with mimic or inhibitor to verify the function of candidate miRNA and explored potential signaling pathway involved.

2. Materials and methods

2.1. Study population

The present study participants were derived from the Wuhan-Zhuhai cohort, a community-based prospective cohort established in 2011–2012 and followed up every 3 years. Detailed information has been fully described elsewhere (Song et al., 2014). All subjects fulfilled structured questionnaire and physical examination and provided morning urine and fasting blood samples. This study was based on data from the second follow-up (2017–2018) and contained two-stage population-based research.

Participants meeting the following criteria were included in the discovery stage: 1) free from chronic diseases such as cancers, severe cardiopulmonary diseases, chronic inflammation, and autoimmune diseases and 2) without history of antibiotics use in the past half a month. After excluding those without data on urinary zinc or plasma miRNA sequencing, there were 87 subjects in urinary zinc level-related miRNA screening. Additional 9 pairs of newly diagnosed T2DM patients and matched controls were included performing sequencing to conduct T2DM-related miRNA screening. Newly T2DM was identified if FPG $\geq$ 7.0 mmol/L or haemoglobin A1c (HbA1c) $\geq$ 6.5% (American Diabetes Association, 2021) but without antidiabetic drug use and without T2DM at baseline period to reduce the effects on miRNA expression. Healthy controls were with FPG $<$ 5.6 mmol/L and without antidiabetic drug use at baseline and follow-up periods and 1:1 matched on age ($\pm$5 years), sex, and city to the newly diagnosed cases.

In the validating stage, 180 subjects were randomly selected from the residents without severe cardiopulmonary diseases and cancers to perform qRT-PCR for miRNA related to both of zinc exposure and T2DM. After excluding 19 individuals with low RNA quality, 161 subjects were included in statistical analyses.

All participants gave a written informed consent. This work was approved by the Ethics and Human Subject Committee of Tongji Medical College, Huazhong University of Science and Technology.

2.2. Determination of urinary zinc

Urinary zinc was measured using Agilent 7700X series inductively coupled plasma-mass spectrometry (ICP-MS, Agilent, CA, USA), as previously reported (Xiao et al., 2018). Detailed protocol is supplied in supplementary materials. The limit of quantification (LOQ) for urinary zinc was 0.0003 μg/L with detection rate of 100%.

2.3. Determination of plasma miRNAs

Plasma total RNA was extracted by using the miRNeasy Serum/Plasma kit (Qiagen, NRW, Germany) according to the manufacturer's protocol. The quality of isolated RNA was detected with the Agilent Bioanalyzer 2100, followed by sequencing libraries preparation using the QIAseq miRNA library kit (Qiagen, NRW, Germany) and sequencing with a HiSeq 2500 (Illumina, CA, USA) in accordance with the manufacturer's instruction. All procedures for next-generation sequencing were performed by CapitalBio Technology (Beijing, China).

In the validating stage, extracted RNA was reverse transcribed using miScript II RT Kit (Qiagen, NRW, Germany) and then subjected to real-time PCR in duplicate using the miScript SYBR Green PCR Kit (Qiagen, NRW, Germany) and a QuantStudio™ 7 (Applied Biosystems, CA, USA). The relative expressions of miRNAs were normalized to cel-miR-39 and calculated by the $2^{-\Delta Ct}$ method.

2.4. Bioinformatic analysis

Predicted target genes of miR-144-3p were derived from the database of *miRDB* (https://mirdb.org/) and *TargetScan* (https://www.targetscan.org/). Gene Ontology (GO) analysis was conducted to explore the roles of miR-144-3p targeting genes in terms of cellular components, biological processes, and molecular functions. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed to identify potential pathways in relation to miR-144-3p targeting genes.

2.5. Cell culture and treatment

HepG2 cells (Shanghai Institutes for Biological Sciences, Shanghai, China) were cultured in DMEM (Gibco, CA, USA) containing 10% fetal bovine serum (Gibco, CA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Solarbio, Beijing, China) at 37 °C in a humidified 5% CO_2 atmosphere.

For the treatment, cells were incubated with various concentrations of zinc sulfate (Sigma-Aldrich, MO, USA) from 0 to 140 μM for 24 h. The range of concentration and incubation time were selected based on preliminary experiments and published literatures (Tsou et al., 2011; Wang et al., 2013). And the doses of 0, 60, 80, 100, 120, and 140 μM were finally selected in the formal experiments according to the cell viability and insulin resistance levels of HepG2 cells. For measurements of insulin resistance indicators (insulin stimulated glucose consumption; phosphoenolpyruvate carboxy kinase (PEPCK), a key enzyme in gluconeogenesis; and glycogen synthase kinase 3 beta (GSK3β), a key enzyme in glycogen synthesis) (Yan et al., 2018), cells were incubated with 100 nM insulin (Sigma-Aldrich, MO, USA) for 30 min at the end of treatment.

MiRNA mimic, inhibitor, and corresponding negative controls were synthesized by GenePharma (Shang, China) and the sequences are presented in Supplementary materials. Cells were transiently transfected with 25 nM miR-144-3p mimic, 200 nM miR-144-3p inhibitor, or respective negative controls using Lipofectamine 3000 (Invitrogen, CA, USA) for 6 h, and then incubated in DMEM for 18 h. Successful transfection was confirmed by the measurements of miR-144-3p expression with qRT-PCR. Transfected cells were treated with different concentrations of zinc sulfate for 24 h.

2.6. Cell viability measurement

Cell Counting Kit-8 (CCK-8) assay (Dojindo, Kumamoto, Japan) was used to measure the cell viability of HepG2 cells. After treatment, 10 μL of CCK-8 solution was added in each well followed by incubation for 1 h at 37 °C. The absorbance at 450 nm was detected using a microplate reader (BioTek, VT, USA).

2.7. Glucose consumption assay

Cellular glucose consumption was assayed in the medium by the glucose oxidase–peroxidase assay kit (Nanjing Jiancheng, Jiangsu, China). After treatment of zinc sulfate for 24 h and insulin for 30 min, the supernatants were collected and the glucose concentrations of the wells with cells were subtracted from that of blank wells to calculate the glucose consumption levels.

2.8. qRT-PCR assay

RNA of HepG2 cells was extracted by Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's protocol. The quality and quantity were evaluated with a NanoDrop ND-1000 (Thermo Fisher Scientific, MA, USA) and the integrality was measured with agarose gel electrophoresis. Isolated RNA was reverse transcribed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA) and then subjected to real-time PCR in triplicate using the PowerUp™ SYBR® Green Master Mix and a QuantStudio™ 7 (Applied Biosystems, CA, USA). The reverse transcription of miR-144-3p was performed with stem-loop RT primers synthesized by Ribobio (Guangdong, China) and RevertAid First Strand cDNA Synthesis Kit. And the quantitative processes were the same as other mRNAs. The sequences of specific primers are listed in supplementary materials. Relative genes expressions were normalized to β-actin or U6 (only for miR-144-3p) and calculated by the $2^{-\Delta\Delta Ct}$ method.

Table 1
General characteristics of study participants.

	Discovery stage				Validating stage
	Zinc-miRNAs discovery set	T2DM-miRNAs discovery set			Validating set
		T2DM	Control	*P* value *	
No. subjects	87	9	9		161
Age, years, mean ± SD	57.57 ± 6.39	57.22 ± 8.67	56.38 ± 8.14	0.834	56.35 ± 5.72
Male, n (%)	27 (31.03)	3 (33.33)	3 (33.33)	1.000	44 (27.33)
BMI, kg/m^2, mean ± SD	24.51 ± 3.16	26.06 ± 2.56	24.65 ± 2.33	0.237	24.59 ± 3.32
Current smoker, n (%)	22 (25.29)	1 (11.11)	1 (11.11)	1.000	17 (10.56)
Current drinker, n (%)	24 (27.59)	2 (22.22)	1 (11.11)	0.527	22 (13.67)
Physical activity, active, n (%)	54 (62.07)	4 (44.44)	4 (44.44)	1.000	88 (54.66)
Urinary zinc, μg/L, median (IQR)	391.03 (276.49, 599.75)	432.39 (218.26, 719.58)	409.89 (222.55, 707.54)	0.971	350.45 (225.26, 537.50)
FPG, mmol/L, mean ± SD	5.50 ± 1.72	7.98 ± 0.86	5.08 ± 0.37	<0.001	5.49 ± 1.27

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; T2DM, type 2 diabetes mellitus; SD, standard deviation; IQR, interquartile range.

* *P* values were estimated by paired Student's *t*-test or Wilcoxon signed-rank test for continuous variables and Chi-square test for categorical variables.

2.9. Western blot

Protein expression levels were detected by western blot analysis as previously described (Fan et al., 2022). Briefly, extracts from cultured cells were resolved by SDS-PAGE, transferred to PVDF membrane (Roche, BW, Germany), and then incubated with specific primary antibodies including rabbit anti-PEPCK (dilution 1:1000; CST, MA, USA), GSK3β (dilution 1:1000; CST, MA, USA), p-GSK3β (Ser 9) (dilution 1:1000; CST, MA, USA), nuclear factor erythroid 2-related factor 2 (Nrf2; dilution 1:250; CST, MA, USA), and β-actin (dilution 1:5000; Proteintech, Hubei, China) followed by HRP-conjugated anti-rabbit IgG antibody (dilution 1:4000; Proteintech, Hubei, China). Protein bands were visualized with ECL (Thermo Fisher Scientific, MA, USA) and quantified using Quantity-One software (Bio-Rad, CA, USA). Relative protein expressions were normalized to β-actin.

2.10. Dual-luciferase reporter assay

Wild and mutant type of 3′-UTR of Nrf2 were cloned into the GP-miRGLO vector (Promega, WI, USA) to construct reporter system of GP-miRGLO-Nrf2-WT and GP-miRGLO-Nrf2-MUT, respectively. Cells of 293T and HepG2 were co-transfected with miR-144-3p mimic or negative control and reporter vector of GP-miRGLO-Nrf2-WT for 6 h, and then incubated in DMEM for 18 h. Simultaneously, groups of co-transfections with mimic or negative control and reporter vector of GP-miRGLO-Nrf2-MUT were set. Dual Luciferase Reporter Gene Assay Kit (GenePharma, Shang, China) were used to detect Firefly and Renilla luciferase activity in accordance with the manufacture's instruction.

2.11. Statistical analysis

Kolmogorov-Smirnov test was used to exam the distribution of numeric variables. To improve the normal distribution, concentrations of urinary zinc and plasma miRNA measured by qRT-PCR in population-based study were normalized by natural logarithm (ln) transformation and $\log_2$ transformation, respectively.

In the discovery stage, paired Student's *t*-test or Wilcoxon signed-rank test for continuous variables and Chi-square test for categorical variables were used to compare the differences of basic characteristics between T2DM patients with matched controls. For the sequencing data, we conducted read counts processing with Cutadapt, quality assessment with FastQC, and sequences alignment in miRBase V21 and Genome Reference Consortium Human Build 38 (GRCh38). The treated data was further filtrated by excluding miRNAs without detection in over 40% samples and normalized via R package "edgeR" (Robinson et al., 2010). Log_2 transformed counts per million (logCPM) was used in generalized linear models to identify urinary zinc level-related miRNAs at P value $<$ 0.05 (Krauskopf et al., 2019). Models were adjusted for age, sex, BMI, education degree, smoking status, drinking status, physical activity, city, and urinary creatinine. Differential expression miRNAs identification between T2DM and control groups were performed via exact tests with the criteria of P value $<$ 0.05 and fold change (FC, up- or down-regulated) $\geq$ 1.5 (Hu et al., 2022; Jardim et al., 2009).

In the validating stage, continuous and categorical analyses (first quantile as the reference) were performed using generalized linear models for continuous outcomes and logistic regression model for dichotomous outcome to quantify the associations among urinary zinc, candidate miRNAs, and FPG or risk of T2DM. All models were adjusted for age, sex, BMI, education degree, smoking status, drinking status, physical activity, family history of diabetes, city, and urinary creatinine (only for urinary zinc). Potential confounders were selected based on a priori knowledge about the risk factors for zinc exposure or T2DM, as well as previous studies investigating miRNAs expression levels

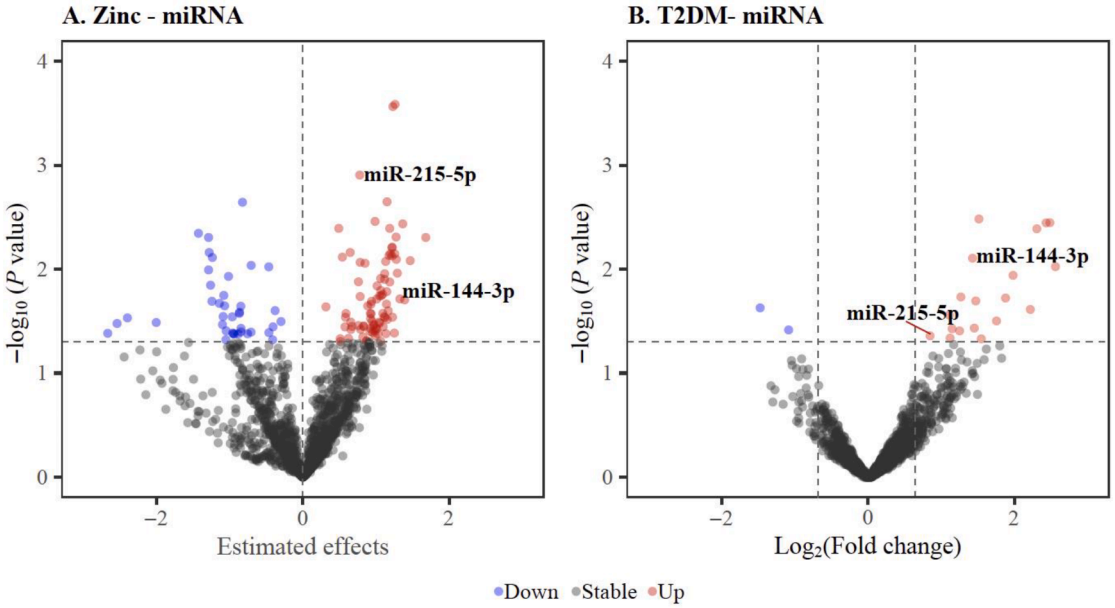

Fig. 1. miRNA expression profiles for zinc exposure and T2DM in the discovery stage. Volcano plots showing the differential miRNA expression associated with zinc exposure (A) and T2DM (B). The horizontal and vertical lines indicate 0.05 significance level and estimated effects = 0 (A) or fold change ≥ 1.5 (B), respectively; the red and blue dots indicate significantly up-regulated and down-regulated miRNAs, respectively, with increasing levels of zinc exposure (A) or among patients with T2DM (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

associated with pollutants exposure or T2DM (Barr et al., 2005; Bollati et al., 2010; Ma et al., 2020; Ortega et al., 2014; Ye et al., 2022; Zheng et al., 2018). Restricted cubic spline (RCS) regression model was conducted with R package "rms" to graphically visualize the relationships of candidate miRNAs with urinary zinc, FPG, and T2DM risk and assess the robustness of findings from continuous and categorical analyses. Knots were set at the 10th, 50th, and 90th percentiles in RCS regression models with the reference value of 50th percentile. The interaction effects between urinary zinc and plasma miR-144-3p on FPG and risk of T2DM were tested by modeling an interaction term in generalized linear models. We further performed mediation analyses with R package "mediation" (Tingley et al., 2014) to assess the mediating role of miR-144-3p in the relationships of zinc with FPG and T2DM risk. Based on the exposure-mediator model and mediator-outcome model, the proportion mediated and corresponding 95% confidence intervals (CIs) were calculated by 1000 times quasi-Bayesian Monte Carlo simulations.

In the cellular experiments, the data are presented as the mean ± SEM. Statistical significance of differences for more than two groups was assessed using one-way ANOVA, further analyzed by Tukey's post-hoc test. And unpaired Student's *t*-test was used for two groups.

All analyses were performed with SAS 9.4 (SAS Institute, NC, USA), R software 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria), and GraphPad Prism 8 (GraphPad Software, CA, USA). Two-tailed $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Subject characteristics

General characteristics of participants included in the discovery and validating stages are summarized in Table 1. Urinary zinc concentration [median (IQR)] of zinc-miRNAs discovery set was 391.03 (276.49, 599.75) μg/L. The mean (SD) value of FPG in patients with T2DM [7.98 (0.86) mmol/L] was significantly higher than that among healthy controls [5.08 (0.37) mmol/L] ($P < 0.001$). No statistical difference in basic characteristics including age, sex, BMI, smoking status, drinking status, and physical activity between patients with T2DM and healthy controls was observed.

Table 2
Associations of urinary zinc with candidate miRNAs, FPG, and T2DM in the validating stage (N = 161)*.

Outcomes	Estimated effects by continuous urinary zinc	Estimated effects by quartiles of urinary zinc				
		Q1	Q2	Q3	Q4	*P* trend†
miR-144-3p	1.23 (0.55, 1.91)	0 (ref.)	0.09 (-0.91, 1.09)	1.13 (0.13, 2.12)	2.08 (0.85, 3.32)	<0.001
miR-215-5p	0.40 (-0.41, 1.21)	0 (ref.)	0.53 (-0.66, 1.72)	0.10 (-1.09, 1.29)	0.63 (-0.85, 2.10)	0.555
FPG	0.56 (0.18, 0.93)	0 (ref.)	0.44 (-0.12, 1.00)	0.59 (0.04, 1.15)	0.61 (-0.08, 1.30)	0.043
T2DM	6.56 (1.65, 26.15)	1 (ref.)	4.41 (0.39, 49.93)	10.69 (1.02, 111.56)	6.29 (0.38, 104.51)	0.101

Abbreviations: FPG, fasting plasma glucose; T2DM, type 2 diabetes mellitus.
* Models were adjusted for age, sex, BMI, education degree, smoking status, drinking status, physical activity, family history of diabetes, city, and urinary creatinine.
† *P* trend values across quartiles of zinc were estimated by including urinary zinc category as a continuous variable.

The mean age of 161 subjects (44 males, 27.33%) included in the validating stage was 56.35 years (Table 1). The concentration of urinary zinc among validating population was 350.45 (225.26, 537.50) μg/L, with the FPG level of 5.49 (1.27) mmol/L.

3.2. Plasma miRNA expression profiles in the discovery stage

A total of 1457 miRNAs were detected for participants in zinc-miRNAs discovery set, as well as 1385 miRNAs in both of T2DM and healthy control groups. In the stage of screening zinc exposure-related miRNAs, we observed that 121 miRNAs were significantly associated with urinary zinc levels. Among them, 82 miRNAs significantly increased and 39 miRNAs decreased with increasing urinary zinc (Fig. 1

Table 3
Associations of candidate miRNAs with FPG and T2DM in the validating stage (N = 161)*.

miRNAs	Outcomes	Estimated effects by continuous miRNAs	Estimated effects by quartiles of miRNAs				
			Q1	Q2	Q3	Q4	*P* trend†
miR-144-3p	FPG	0.19 (0.11, 0.27)	0 (ref.)	0.40 (-0.11, 0.92)	0.52 (0.01, 1.03)	1.22 (0.70, 1.74)	<0.001
	T2DM	1.68 (1.25, 2.26)	1 (ref.)	5.05 (0.46, 56.03)	7.44 (0.71, 78.33)	26.89 (2.63, 274.62)	0.002
miR-215-5p	FPG	0.10 (0.04, 0.17)	0 (ref.)	0.34 (-0.15, 0.83)	0.33 (-0.18, 0.83)	0.65 (0.15, 1.14)	0.018
	T2DM	1.92 (1.37, 2.70)	NA‡	NA‡	NA‡	NA‡	NA‡

Abbreviations: FPG, fasting plasma glucose; T2DM, type 2 diabetes mellitus; NA, not applicable.
* Models were adjusted for age, sex, BMI, education degree, smoking status, drinking status, physical activity, family history of diabetes, and city.
† *P* trend values across quartiles of plasma miRNA were estimated by including miRNA category as a continuous variable.
‡ Not applicable due to too large linear estimated effects.

Table 4
Interaction effects of urinary zinc with miR-144-3p on FPG and T2DM and mediation effects by miR-144-3p in the validating stage (N = 161)*.

Outcomes	Interaction effects	Mediation effects			
		Direct effects	Mediated effects	Total effects	Proportion mediated
FPG	0.11 (-0.02, 0.25)	0.36 (-0.03, 0.71)	0.21 (0.07, 0.38)	0.56 (0.20, 0.94)	36.21%
T2DM	0.68 (0.35, 1.32)	3.87 (1.42, 10.52)	1.57 (1.06, 2.32)	6.07 (2.03, 18.11)	43.46%

Abbreviations: FPG, fasting plasma glucose; T2DM, type 2 diabetes mellitus.
* Models were adjusted for age, sex, BMI, education degree, smoking status, drinking status, physical activity, family history of diabetes, city, and urinary creatinine.

and Supplementary Table S1). Likewise, 22 miRNAs significantly altered in patients with T2DM when compared with healthy controls (Fig. 1 and Supplementary Table S2). Among above differentially altered miRNAs, miR-144-3p and miR-215-5p showed significantly increased expression level in both zinc exposure elevation and T2DM (Fig. 1).

3.3. Associations among zinc, candidate miRNAs, FPG and T2DM risk and role of miR-144-3p in the validating stage

The associations among urinary zinc, candidate miRNAs, FPG, and risk of T2DM were further analyzed in 161 participants and the results are presented in Table 2 and Table 3. After adjustment for potential confounders, significantly positive association between urinary zinc and plasma miR-144-3p was observed and the category analysis indicated a monotonic increment in plasma miR-144-3p across quartiles of urinary zinc (*P* and *P* trend < 0.05; Table 2). Each 1-unit increase in log-transformed concentration of miR-144-3p was related to increased FPG with β (95% CI) of 0.19 (0.11, 0.27) and elevated T2DM risk with OR (95% CI) of 1.68 (1.25, 2.26) (all *P* < 0.05; Table 3). In the category analysis, we found significantly monotonic FPG and T2DM risk elevations for plasma miR-144-3p increment (Table 3). Each 1-unit increase in log-transformed concentration of miR-215-5p was related to increased FPG with β (95% CI) of 0.10 (0.04, 0.17) (*P* and *P* trend < 0.05; Table 3). The results of sensitivity analyses by RCS models were consistent with the primary models (Supplementary Fig. S1 and S2).

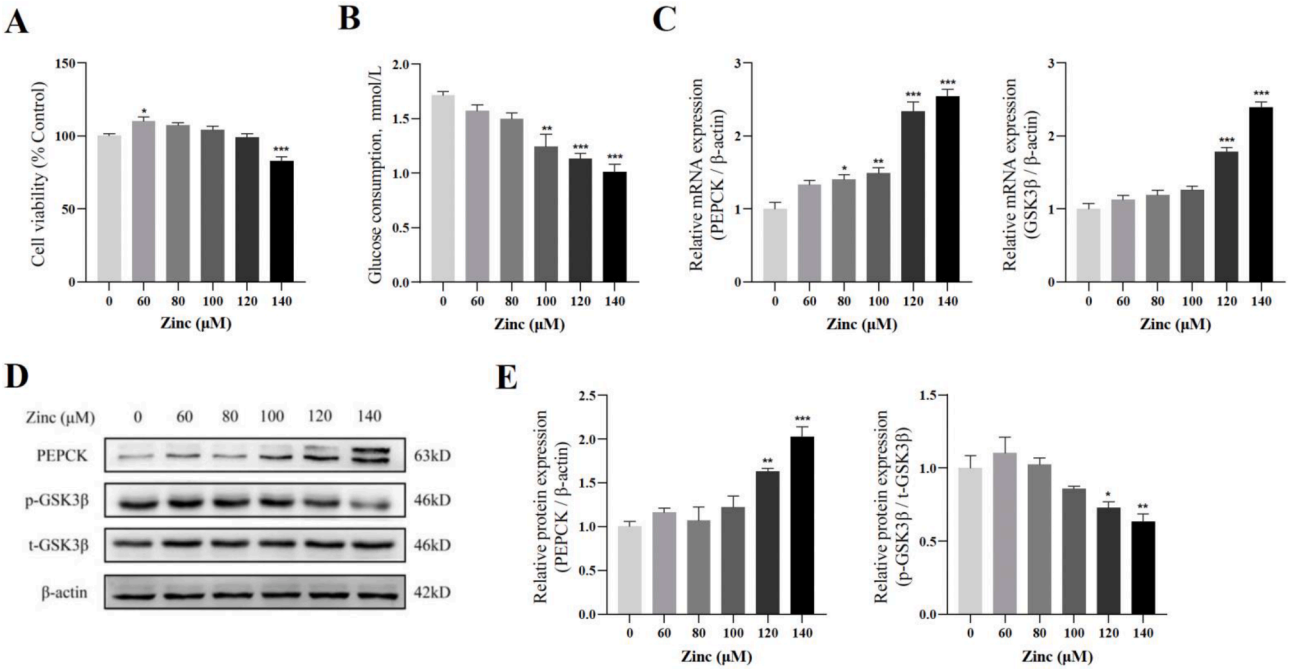

Fig. 2. Effect of zinc on insulin resistance in HepG2 cells. HepG2 cells were incubated with different concentrations of zinc sulfate from 0 to 140 μM for 24 h. For measurements of insulin resistance indicators, cells were further incubated with 100 nM insulin for 30 min. (A) Cell viability of HepG2 cells (n = 6). (B) Glucose consumption in HepG2 cells (n = 3). (C) Relative mRNA levels of PEPCK and GSK3β (n = 4). (D and E) Representative immunoblots and relative protein levels of PEPCK and p-GSK3β (Ser 9) (n = 4). Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control group. PEPCK, phosphoenolpyruvate carboxy kinase; GSK3β, glycogen synthase kinase 3 beta.

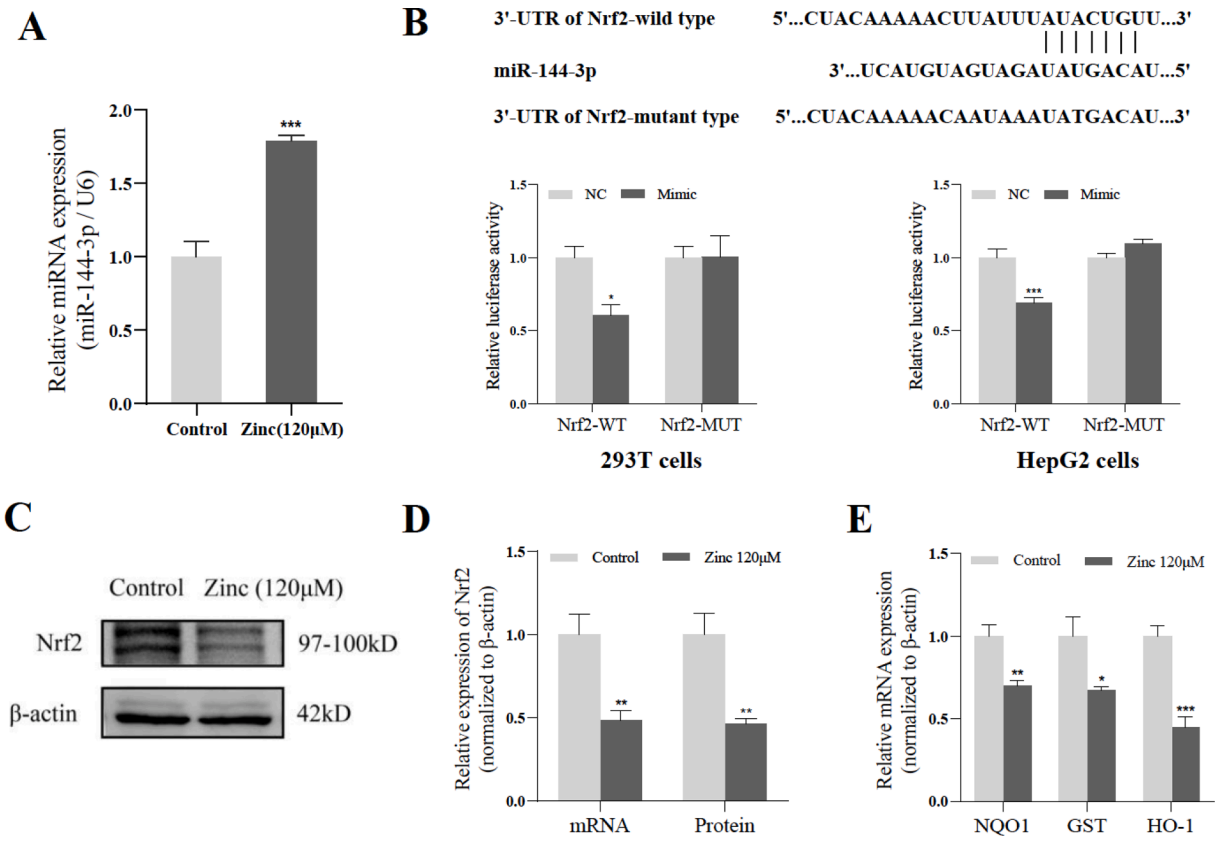

Fig. 3. Zinc enhances miR-144-3p expression targeting Nrf2 in HepG2 cells. HepG2 cells were incubated with 0 or 120 μM of zinc sulfate for 24 h. (A) Relative expression levels of miR-144-3p (n = 4). (B) MiR-144-3p Putative binding sites and corresponding mutant sites of Nrf2. Dual-luciferase reporter assay of miR-144-3p with 3-′UTR vectors (wild type or mutant) of Nrf2 in 293T and HepG2 cells (n = 6). (C and D) Representative immunoblots and relative expression levels of Nrf2 (n = 4). (E) Relative mRNA levels of NQO1, GST, and HO-1 (n = 4). Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control or NC group. Nrf2, nuclear factor erythroid 2-related factor 2; NQO1, quinone oxidoreductase 1; GST glutathione S-transferase; HO-1, hemeoxygenase-1.

Significantly positive relationships of urinary zinc with FPG and T2DM risk were observed in continuous and/or category analyses (Table 2).

Due to insignificant association between urinary zinc and plasma miR-215-5p, we only included miR-144-3p in the interaction effect estimation and mediation analysis which presented in Table 4. No statistically significant interaction effect was observed between urinary zinc and plasma miR-144-3p on FPG or risk of T2DM. In addition, plasma miR-144-3p mediated 36.21% and 43.46% of the associations of urinary zinc with FPG and T2DM risk, respectively.

3.4. GO and KEGG enrichment analyses

A total of 1587 target genes were predicted for miR-144-3p and the results of bioinformatic analyses are presented in Supplementary Fig. S3 and S4. For miR-144-3p, its target genes were mainly involved in DNA-binding transcription activator activity in molecular function, neuronal cell body in cellular component, and regulation of neuron projection development in biological process. In addition, the KEGG pathway enrichment analysis indicated that the T2DM and insulin resistance signaling pathways were related to target genes of miR-144-3p (Supplementary Fig. S4).

3.5. Zinc induced insulin resistance in HepG2 cells

As shown in Fig. 2A, the cell viability of HepG2 cells slightly increased when zinc concentration was 60 μM, while significantly decreased in zinc of 140 μM group, indicating mild cytotoxicity of zinc on HepG2 cells. As presented in Fig. 2B, significant decline in glucose consumption were observed in 100, 120 and 140 μM zinc treatment groups. Compared with blank group, significant increases in mRNA and protein levels of PEPCK, significantly increased GSK3β mRNA expression, and reduced GSK3β phosphorylation at Ser 9 were observed in HepG2 cells with 120 or 140 μM zinc treatment (Fig. 2C-E).

These results indicated that zinc exposure could induce hepatic insulin resistance with altered glucose metabolism. Combining cell viability and the changes of glycometabolic homeostasis, we selected 120 μM zinc to treat HepG2 cells for 24 h in the following miR-144-3p-related experiments.

3.6. Zinc enhanced miR-144-3p expression targeting Nrf2 to inhibit antioxidant response elements and induce insulin resistance in HepG2 cells

Compared with the control group, miR-144-3p expression level significantly increased with zinc exposure (Fig. 3A). Fig. 3B confirmed the binding relationship between miR-144-3p and 3′-UTR of Nrf2 mRNA. In 293T and HepG2 cells transfected with Nrf2-wild type, the luciferase activity was significantly decreased by miR-144-3p mimic. However, no significant alteration was observed when miR-144-3p mimic was co-transfected with Nrf2-mutant type. Moreover, both of mRNA and protein levels of Nrf2 were markedly decreased after zinc exposure (Fig. 3C and D). Given the function of Nrf2 as a transcription factor, the mRNA levels of its major downstream antioxidant enzyme genes including quinone oxidoreductase 1 (NQO1), glutathione S-transferase (GST), and hemeoxygenase-1 (HO-1) were also detected. In zinc-induced HepG2 cells, remarkably declined expressions of these genes were observed (Fig. 3E).

Figs. 4 and 5 shows the regulating role of miR-144-3p in zinc-induced insulin resistance in HepG2 cells. Transfection efficiency of miR-144-3p

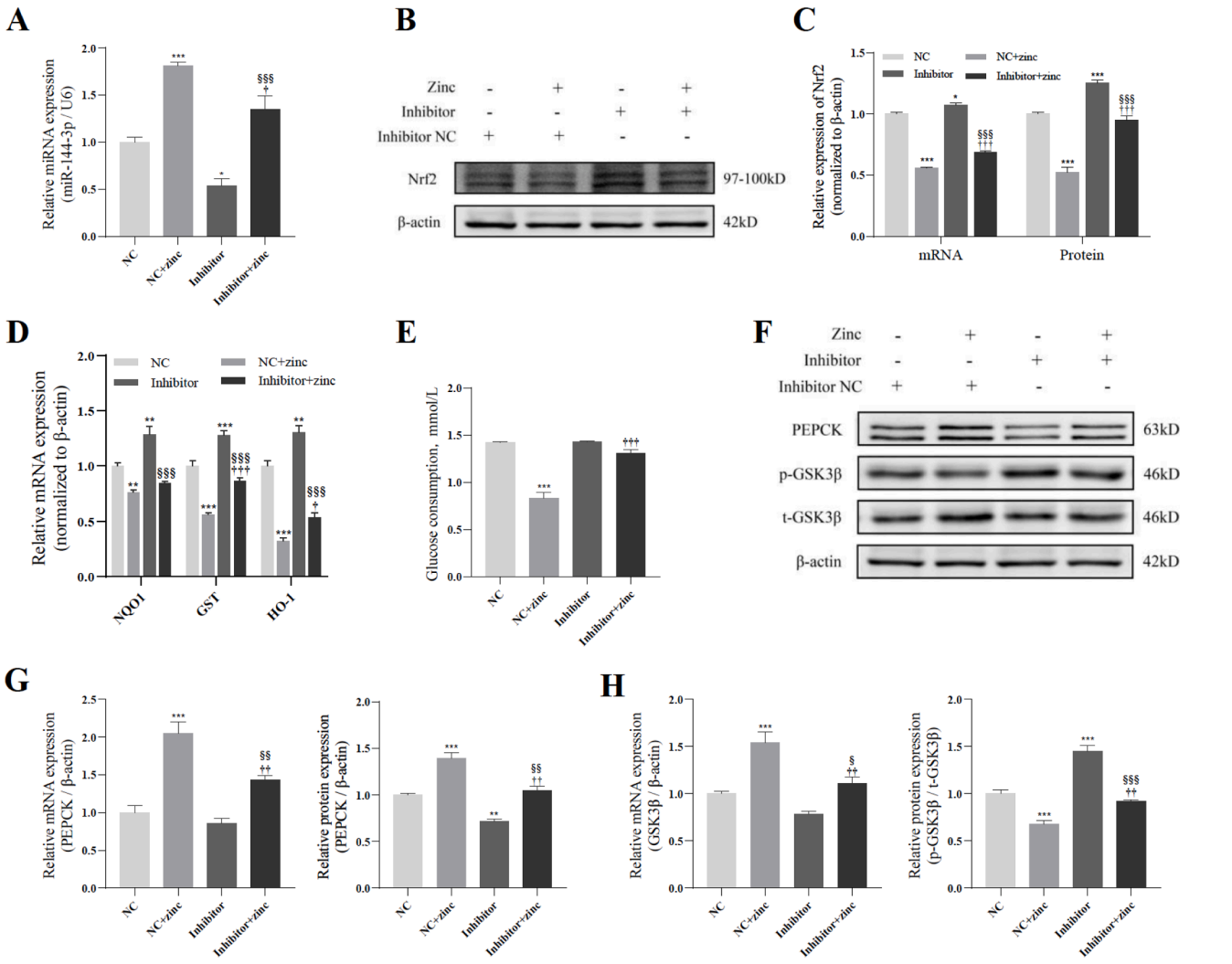

Fig. 4. Inhibition of miR-144-3p enhances Nrf2 pathway and attenuates insulin resistance in zinc sulfate-induced HepG2 cells. HepG2 cells were transfected with 200 nM miR-144-3p inhibitor or negative control followed with 0 or 120 μM zinc sulfate treatment for 24 h. For measurements of insulin resistance indicators, cells were further incubated with 100 nM insulin for 30 min. (A) Relative expression levels of miR-144-3p (n = 4). (B and C) Representative immunoblots and relative expression levels of Nrf2 (n = 4). (D) Relative mRNA levels of NQO1, GST, and HO-1 (n = 4). (E) Glucose consumption in HepG2 cells (n = 3). (G) Relative mRNA levels of PEPCK and GSK3β (n = 4). (F and H) Representative immunoblots and relative expression levels of PEPCK and p-GSK3β (Ser 9) (n = 4). Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus NC group. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ versus NC + zinc group. $^{\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{\S\S\S}P < 0.001$ versus inhibitor group. Nrf2, nuclear factor erythroid 2-related factor 2; NQO1, quinone oxidoreductase 1; GST glutathione S-transferase; HO-1, hemeoxygenase-1; PEPCK, phosphoenolpyruvate carboxy kinase; GSK3β, glycogen synthase kinase 3 beta.

inhibitor and mimic in HepG2 cells was measured by qRT-PCR and presented in Fig. 4A and Fig. 5A. In zinc-induced HepG2 cells transfected with miR-144-3p inhibitor, the mRNA and protein levels of Nrf2 and the mRNA of GST and HO-1 significantly increased (Fig. 4B-D). Furthermore, miR-144-3p inhibitor transfection elevated glucose consumption and protein level of GSK3β phosphorylation in zinc-treated HepG2 cells, accompanied with declined levels of PEPCK mRNA and protein and GSK3β mRNA (Fig. 4E-H). Above results indicated that miR-144-3p inhibition could ameliorate zinc-induced insulin resistance. On contrary, miR-144-3p mimic transfection remarkably inhibited Nrf2 expression as well as downstream antioxidant genes and aggravated zinc exposure-resulted insulin resistance (Fig. 5B-H).

These results demonstrated that zinc upregulated miR-144-3p expression to suppress Nrf2 antioxidant pathway, which resulted in the occurrence of hepatic insulin resistance.

4. Discussion

Zinc has been linked with glucose metabolic disorder and the development of T2DM in epidemiological studies including our previous research (Qu et al., 2020; Yary et al., 2016; Ye et al., 2022). In the present study, we identified plasma miRNA expression profiles for zinc exposure and T2DM and found that plasma miR-144-3p was significantly associated with elevated levels of zinc exposure and risk of T2DM. Furthermore, miR-144-3p partially mediated the positive associations of zinc with FPG and T2DM risk in 161 participants randomly extracted from the Wuhan-Zhuhai cohort. We further confirmed that zinc exposure caused abnormal up-regulation of miR-144-3p in HepG2 cell model. Additionally, with miR-144-3p expression interference, we observed that zinc may induce insulin resistance by enhancing miR-144-3p expression targeting Nrf2 and inhibiting downstream antioxidant genes. These results provide new clues for pathophysiological process of zinc-induced glucose metabolic disorder and T2DM.

Circulating miRNAs stemmed from various tissues were expected to be potential biomarkers for physiological states alteration in view of the stability and specific to tissue and disease states (Chen et al., 2008; Laterza et al., 2009). Recently, a series of researches have indicated that environmental chemicals may cause adverse health effects by changing

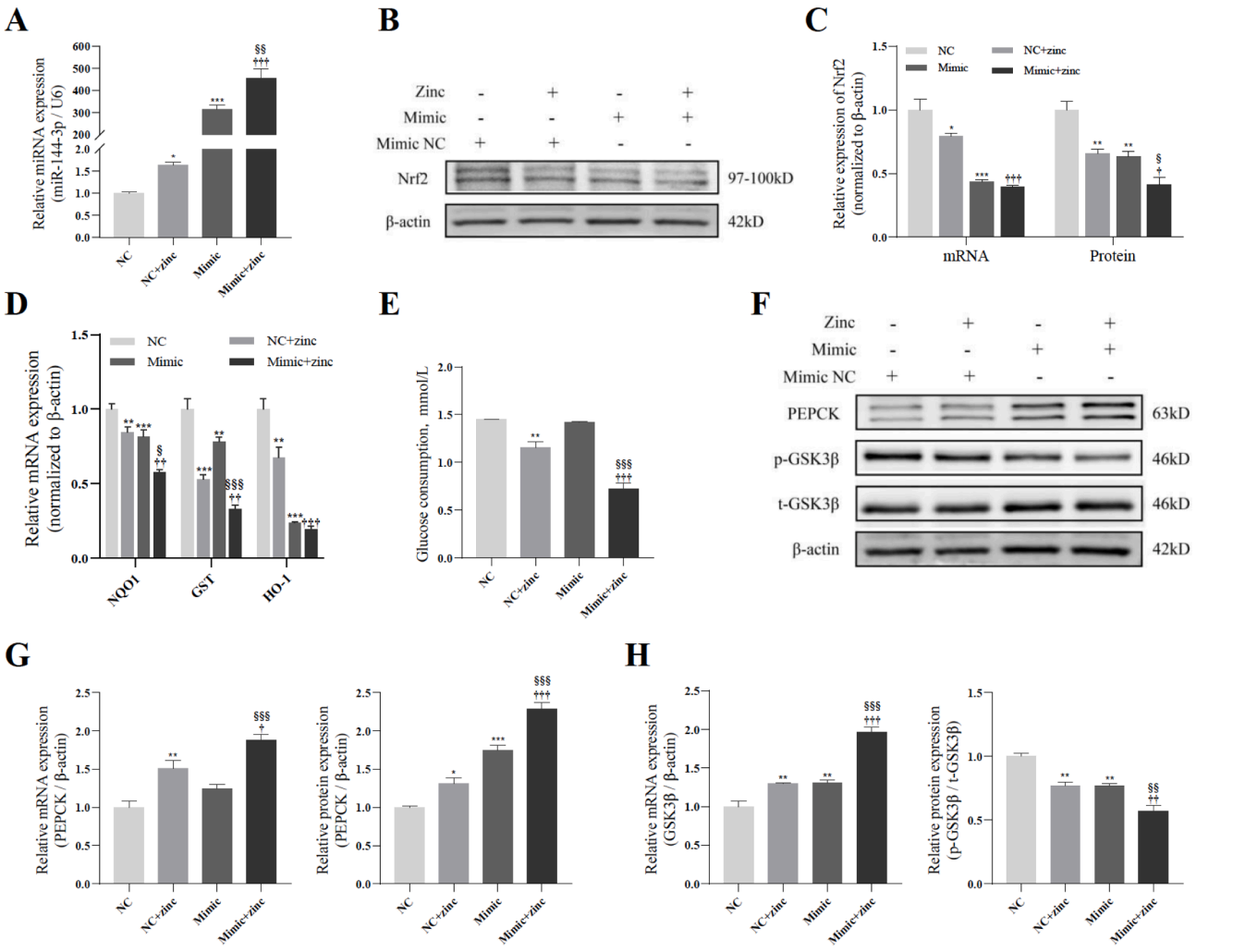

Fig. 5. Overexpression of miR-144-3p suppresses Nrf2 pathway and promotes insulin resistance in zinc sulfate-induced HepG2 cells. HepG2 cells were transfected with 25 nM miR-144-3p mimic or negative control followed with 0 or 120 μM zinc sulfate treatment for 24 h. For measurements of insulin resistance indicators, cells were further incubated with 100 nM insulin for 30 min. (A) Relative expression levels of miR-144-3p (n = 4). (B and C) Representative immunoblots and relative expression levels of Nrf2 (n = 4). (D) Relative mRNA levels of NQO1, GST, and HO-1 (n = 4). (E) Glucose consumption in HepG2 cells (n = 3). (F) Relative mRNA levels of PEPCK and GSK3β (n = 4). (G and H) Representative immunoblots and relative expression levels of PEPCK and p-GSK3β (Ser 9) (n = 4). Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus NC group. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ versus NC + zinc group. $^{\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{\S\S\S}P < 0.001$ versus mimic group. Nrf2, nuclear factor erythroid 2-related factor 2; NQO1, quinone oxidoreductase 1; GST glutathione S-transferase; HO-1, hemeoxygenase-1; PEPCK, phosphoenolpyruvate carboxy kinase; GSK3β, glycogen synthase kinase 3 beta.

the level of miRNA and activating related pathways (Krauskopf et al., 2019; Mancini et al., 2020; Nie et al., 2022; Yamamoto et al., 2013). However, there is a lack of systematic studies on zinc-related miRNA. Only a double-blind randomized crossover study observed upregulated miR-144 after acute exposure to diesel exhaust (Yamamoto et al., 2013), which was an important source of environmental zinc (Hou et al., 2019). In the present study, we screened 121 miRNAs associated with increasing zinc exposure and validated the relationship between internal zinc level and plasma miR-144-3p in a community population. To our knowledge, this is the first report of zinc-related miRNA in a general population in China.

Published literatures have also identified circulating miRNAs as sensitive indicators for T2DM and vital regulators for signaling pathway involved in glycometabolism and insulin resistance in T2DM (Agbu and Carthew, 2021). Zhu et al. performed a *meta*-analysis of T2DM-related miRNAs expression profile studies and indicated that upregulated miR-144 was a blood biomarker for T2DM (Zhu and Leung, 2015). Another study based on T2DM patients and in vitro experiments confirmed that miR-144 downregulated insulin receptor substrate 1 (IRS1) resulting in impaired insulin signaling (Karolina et al., 2011). Consistently, we found that the level of plasma miR-144-3p was positively associated with FPG and T2DM risk. Moreover, the bioinformatic analysis for predicted target genes of miR-144-3p supporting the regulating role of miR-144-3p in insulin resistance pathway and T2DM development.

To better understand the role of miR-144-3p in zinc exposure and T2DM, we performed interaction effects assessment and mediation analyses in 161 participants. Our results indicated that miR-144-3p did not modify the relationship of zinc exposure with increased FPG and T2DM risk but might be one of the intermediate pathways underlying disordered glycometabolism and elevated risk of T2DM in relation to zinc exposure. Few study has explored potential mechanism of zinc exposure affecting glucose metabolism and T2DM development. In our previous study, systemic inflammation showed mediating role in the positive associations of zinc exposure with FPG (9.09%) and insulin resistance index (17.67%) (Ye et al., 2022). Consistently, the bioinformatic analysis in this study revealed that predicted target genes of miR-144-3p might be involved in TGF-beta signaling pathway which was demonstrated to activate inflammatory cascades and promote glucose intolerance (Chen et al., 2019; Yadav et al., 2011). In addition, a study among

184 non-diabetic participants found positive association between TGF-beta1 levels and insulin resistance index (Yadav et al., 2011). Above evidence suggested that the role of miR-144-3p in relationships of zinc with hyperglycemia and T2DM deserved high attention.

MiR-144-3p, a redox sensitive miRNA, has been demonstrated to play a vital role in redox balance (Jadeja et al., 2020), while dysregulated antioxidant system and consequent oxidative stress are implicated in the pathogenesis of insulin resistance and T2DM. Jadeja et al. confirmed that miR-144-3p regulated antioxidant signaling through regulating Nrf2 and downstream antioxidant target genes (Jadeja et al., 2020). *TargetScan* also predicts the binding sites for miR-144-3p in 3′-UTR of Nrf2 mRNA, which was confirmed in this study with dual-luciferase reporter assay. Nrf2 is recognized as a key redox regulator, acting as a transcription factor to activate the expression of various antioxidant enzymes such as NQO1, GST, and HO-1. Under non-stressed condition, Nrf2 tightly binds to kelch-like ECH-associated protein 1 (keap1), degrades continuously, and maintains low abundance. Under the circumstance of oxidative stress, keap1 is oxidized and covalently modified, resulting in nucleus translocation of Nrf2 and activation of antioxidant enzymes transcription. Increased antioxidant enzymes enhance free radical scavenging activity and protect cells from oxidative damage. Thus, dysregulated Nrf2 and downstream antioxidant response elements are one of the main pathological processes of oxidative stress-mediated diseases including insulin resistance in T2DM (Matzinger et al., 2018). In the present study, we observed that miR-144-3p overexpression downregulated Nrf2 mRNA and protein levels and further suppressed transcription of downstream antioxidative enzymes NQO1, GST, and HO-1, while miR-144-3p inhibitor ameliorated these abnormalities in zinc-induced HepG2 cells. Our results suggested that elevated miR-144-3p expression might decrease Nrf2 expression to break redox balance and aggravate insulin resistance induced by zinc exposure.

To our knowledge, this is the first time to explore circulating miRNAs expression profile for zinc exposure and to evaluate potential role of candidate miRNAs in zinc-related hyperglycemia and T2DM. Moreover, next-generation high-throughput sequencing technology was adopted in this study to extensively screen plasma miRNAs in association with zinc exposure and T2DM development, which was further validated among extended population and in vitro experiments, making our findings more convincing. There are still limitations should be noted. First, previous study has suggested the network of multiple miRNAs in regulation of pathophysiological processes while we only focused on miR-144-3p, ignoring possible interaction of other miRNAs in zinc-related glucose metabolic disorder. Second, only HepG2 cells were used in the in vitro experiments lacking evidence from another type of cell. However, our study design combined epidemiological and experimental methods to provide more reliable evidence for our hypothesis. In addition, the sample size is relatively small and further researches with large-scale population are needed to confirm our results.

5. Conclusions

In conclusion, this study indicated that miR-144-3p elevation play a vital role in zinc exposure-related hyperglycemia and T2DM. Zinc upregulated miR-144-3p expression, thereby suppressed the target gene Nrf2 and downstream antioxidant enzymes levels, and contributed to glucose metabolic disorder and insulin resistance in T2DM. Our results provided new insight into T2DM pathogenesis caused by zinc exposure.

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CRediT authorship contribution statement

Zi Ye: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Man Cheng:** Investigation, Data curation, Validation. **Lieyang Fan:** Investigation, Methodology, Resources. **Jixuan Ma:** Formal analysis, Methodology, Writing – review & editing. **Yingdie Zhang:** Investigation. **Pei Gu:** Investigation. **Yujia Xie:** Investigation. **Xiaojie You:** Investigation. **Min Zhou:** Investigation, Data curation. **Bin Wang:** Supervision, Funding acquisition. **Weihong Chen:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107807.

References

Agbu, P., Carthew, R.W., 2021. MicroRNA-mediated regulation of glucose and lipid metabolism. Nat. Rev. Mol. Cell Biol. 22 (6), 425–438. https://doi.org/10.1038/s41580-021-00354-w.

American Diabetes Association, 2021. 2. Classification and Diagnosis of Diabetes. Diabetes Care 44(Suppl 1), S15-S33. doi: 10.2337/dc21-S002.

Barr, D.B., Wilder, L.C., Caudill, S.P., et al., 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 113 (2), 192–200. https://doi.org/10.1289/ehp.7337.

Belgardt, B.-F., Ahmed, K., Spranger, M., et al., 2015. The microRNA-200 family regulates pancreatic beta cell survival in type 2 diabetes. Nat. Med. 21 (6), 619–627. https://doi.org/10.1038/nm.3862.

Bollati, V., Marinelli, B., Apostoli, P., et al., 2010. Exposure to metal-rich particulate matter modifies the expression of candidate microRNAs in peripheral blood leukocytes. Environ Health Perspect 118 (6), 763–768. https://doi.org/10.1289/ehp.0901300.

Chen, X., Ba, Y., Ma, L., et al., 2008. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 18 (10) https://doi.org/10.1038/cr.2008.282.

Chen, P.-Y., Qin, L., Li, G., et al., 2019. Endothelial TGF-β signalling drives vascular inflammation and atherosclerosis. Nat Metab 1 (9), 912–926. https://doi.org/10.1038/s42255-019-0102-3.

Eze, I.C., Hemkens, L.G., Bucher, H.C., et al., 2015. Association between ambient air pollution and diabetes mellitus in Europe and North America: systematic review and meta-analysis. Environ Health Perspect 123 (5), 381–389. https://doi.org/10.1289/ehp.1307823.

Fan, L., Li, W., Ma, J., et al., 2022. Benzo(a)pyrene induces airway epithelial injury through Wnt5a-mediated non-canonical Wnt-YAP/TAZ signaling. Sci. Total Environ. 815, 151965 https://doi.org/10.1016/j.scitotenv.2021.151965.

Fernández-Cao, J.C., Warthon-Medina, M., Moran, H., V.,, et al., 2019. Zinc Intake and Status and Risk of Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. Nutrients 11 (5). https://doi.org/10.3390/nu11051027.

Hou, S., Zheng, N., Tang, L., et al., 2019. Pollution characteristics, sources, and health risk assessment of human exposure to Cu, Zn, Cd and Pb pollution in urban street dust across China between 2009 and 2018. Environ. Int. 128, 430–437. https://doi.org/10.1016/j.envint.2019.04.046.

Hu, H., Zhao, M., Li, Z., et al., 2022. Plasma miR-193b-3p Is Elevated in Type 2 Diabetes and Could Impair Glucose Metabolism. Front. Endocrinol. 13, 814347 https://doi.org/10.3389/fendo.2022.814347.

Jadeja, R.N., Jones, M.A., Abdelrahman, A.A., et al., 2020. Inhibiting microRNA-144 potentiates Nrf2-dependent antioxidant signaling in RPE and protects against oxidative stress-induced outer retinal degeneration. Redox Biol. 28, 101336 https://doi.org/10.1016/j.redox.2019.101336.

Jardim, M.J., Fry, R.C., Jaspers, I., Dailey, L., Diaz-Sanchez, D., 2009. Disruption of microRNA expression in human airway cells by diesel exhaust particles is linked to tumorigenesis-associated pathways. Environ Health Perspect 117 (11), 1745–1751. https://doi.org/10.1289/ehp.0900756.

Karolina, D.S., Armugam, A., Tavintharan, S., et al., 2011. MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2

diabetes mellitus. PLoS One 6 (8), e22839. https://doi.org/10.1371/journal.pone.0022839.
Krauskopf, J., van Veldhoven, K., Chadeau-Hyam, M., et al., 2019. Short-term exposure to traffic-related air pollution reveals a compound-specific circulating miRNA profile indicating multiple disease risks. Environ. Int. 128, 193–200. https://doi.org/10.1016/j.envint.2019.04.063.
Laterza, O.F., Lim, L., Garrett-Engele, P.W., et al., 2009. Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. Clin. Chem. 55 (11), 1977–1983. https://doi.org/10.1373/clinchem.2009.131797.
Li, X., Wang, M., Song, Y., et al., 2021. Obesity and the relation between joint exposure to ambient air pollutants and incident type 2 diabetes: A cohort study in UK Biobank. PLoS Med 18 (8), e1003767. https://doi.org/10.1371/journal.pmed.1003767.
Ma, J., Zhou, Y., Wang, D., et al., 2020. Associations between essential metals exposure and metabolic syndrome (MetS): Exploring the mediating role of systemic inflammation in a general Chinese population. Environ. Int. 140, 105802 https://doi.org/10.1016/j.envint.2020.105802.
Mancini, F.R., Laine, J.E., Tarallo, S., et al., 2020. microRNA expression profiles and personal monitoring of exposure to particulate matter. Environmental Pollution (Barking, Essex : 1987) 263(Pt B), 114392. doi: 10.1016/j.envpol.2020.114392.
Matzinger, M., Fischhuber, K., Heiss, E.H., 2018. Activation of Nrf2 signaling by natural products-can it alleviate diabetes? Biotechnol. Adv. 36 (6), 1738–1767. https://doi.org/10.1016/j.biotechadv.2017.12.015.
Mayer-Davis, E.J., Lawrence, J.M., Dabelea, D., et al., 2017. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002–2012. N. Engl. J. Med. 376 (15), 1419–1429. https://doi.org/10.1056/NEJMoa1610187.
Nie, H., Hu, H., Li, Z., et al., 2022. Associations of plasma metal levels with type 2 diabetes and the mediating effects of microRNAs. Environmental Pollution (Barking, Essex : 1987) 292(Pt B), 118452. doi: 10.1016/j.envpol.2021.118452.
Ortega, F.J., Mercader, J.M., Moreno-Navarrete, J.M., et al., 2014. Profiling of circulating microRNAs reveals common microRNAs linked to type 2 diabetes that change with insulin sensitization. Diabetes Care 37 (5), 1375–1383. https://doi.org/10.2337/dc13-1847.
Plum, L.M., Rink, L., Haase, H., 2010. The essential toxin: impact of zinc on human health. Int. J. Environ. Res. Public Health 7 (4), 1342–1365. https://doi.org/10.3390/ijerph7041342.
Qu, X., Yang, H., Yu, Z., et al., 2020. Serum zinc levels and multiple health outcomes: Implications for zinc-based biomaterials. Bioact. Mater. 5 (2), 410–422. https://doi.org/10.1016/j.bioactmat.2020.03.006.
Renzi, M., Cerza, F., Gariazzo, C., et al., 2018. Air pollution and occurrence of type 2 diabetes in a large cohort study. Environ. Int. 112, 68–76. https://doi.org/10.1016/j.envint.2017.12.007.
Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26 (1), 139–140. https://doi.org/10.1093/bioinformatics/btp616.
Safiri, S., Karamzad, N., Kaufman, J.S., et al., 2022. Prevalence, Deaths and Disability-Adjusted-Life-Years (DALYs) Due to Type 2 Diabetes and Its Attributable Risk Factors in 204 Countries and Territories, 1990–2019: Results From the Global Burden of Disease Study 2019. Front. Endocrinol. 13, 838027 https://doi.org/10.3389/fendo.2022.838027.
Skalny, A.V., Aschner, M., Tinkov, A.A., 2021. Zinc. Advances in food and nutrition research 96, 251–310. https://doi.org/10.1016/bs.afnr.2021.01.003.
Song, Y., Hou, J., Huang, X., et al., 2014. The Wuhan-Zhuhai (WHZH) cohort study of environmental air particulate matter and the pathogenesis of cardiopulmonary diseases: study design, methods and baseline characteristics of the cohort. BMC Public Health 14, 994. https://doi.org/10.1186/1471-2458-14-994.
Sun, H., Saeedi, P., Karuranga, S., et al., 2022. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res. Clin. Pract. 183, 109119 https://doi.org/10.1016/j.diabres.2021.109119.
Tattikota, S.G., Rathjen, T., McAnulty, S.J., et al., 2014. Argonaute2 mediates compensatory expansion of the pancreatic β cell. Cell Metab 19 (1), 122–134. https://doi.org/10.1016/j.cmet.2013.11.015.
Tingley, D., Yamamoto, T., Hirose, K., Keele, L., Imai, K., 2014. Mediation: R package for causal mediation analysis.
Tsou, T.-C., Chao, H.-R., Yeh, S.-C., Tsai, F.-Y., Lin, H.-J., 2011. Zinc induces chemokine and inflammatory cytokine release from human promonocytes. J. Hazard. Mater. 196, 335–341. https://doi.org/10.1016/j.jhazmat.2011.09.035.
Wang, Y.-H., Li, K.-J., Mao, L., et al., 2013. Effects of exogenous zinc on cell cycle, apoptosis and viability of MDAMB231, HepG2 and 293 T cells. Biol Trace Elem Res 154 (3), 418–426. https://doi.org/10.1007/s12011-013-9737-1.
Willeit, P., Skroblin, P., Moschen, A.R., et al., 2017. Circulating MicroRNA-122 Is Associated With the Risk of New-Onset Metabolic Syndrome and Type 2 Diabetes. Diabetes 66 (2), 347–357. https://doi.org/10.2337/db16-0731.
Xiao, L., Zhou, Y., Ma, J., et al., 2018. Oxidative DNA damage mediates the association between urinary metals and prevalence of type 2 diabetes mellitus in Chinese adults. Sci Total Environ 627, 1327–1333. https://doi.org/10.1016/j.scitotenv.2018.01.317.
Xu, L., Li, Y., Yin, L., et al., 2018. miR-125a-5p ameliorates hepatic glycolipid metabolism disorder in type 2 diabetes mellitus through targeting of STAT3. Theranostics 8 (20), 5593–5609. https://doi.org/10.7150/thno.27425.
Yadav, H., Quijano, C., Kamaraju, A.K., et al., 2011. Protection from obesity and diabetes by blockade of TGF-β/Smad3 signaling. Cell Metab 14 (1), 67–79. https://doi.org/10.1016/j.cmet.2011.04.013.
Yamamoto, M., Singh, A., Sava, F., et al., 2013. MicroRNA expression in response to controlled exposure to diesel exhaust: attenuation by the antioxidant N-acetylcysteine in a randomized crossover study. Environ Health Perspect 121 (6), 670–675. https://doi.org/10.1289/ehp.1205963.
Yan, J., Wang, C., Jin, Y., et al., 2018. Catalpol ameliorates hepatic insulin resistance in type 2 diabetes through acting on AMPK/NOX4/PI3K/AKT pathway. Pharmacol. Res. 130, 466–480. https://doi.org/10.1016/j.phrs.2017.12.026.
Yary, T., Virtanen, J.K., Ruusunen, A., Tuomainen, T.-P., Voutilainen, S., 2016. Serum zinc and risk of type 2 diabetes incidence in men: The Kuopio Ischaemic Heart Disease Risk Factor Study. J. Trace Elements Med. Biol. : Org. Soc. Min. Trace Elements (GMS) 33, 120–124. https://doi.org/10.1016/j.jtemb.2015.11.001.
Ye, Z., Liang, R., Wang, B., et al., 2022. Cross-sectional and longitudinal associations of urinary zinc with glucose-insulin homeostasis traits and type 2 diabetes: Exploring the potential roles of systemic inflammation and oxidative damage in Chinese urban adults. Environ. Pollut. (Barking, Essex : 1987) 314, 120331. doi: 10.1016/j.envpol.2022.120331.
Zampetaki, A., Kiechl, S., Drozdov, I., et al., 2010. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ. Res. 107 (6), 810–817. https://doi.org/10.1161/CIRCRESAHA.110.226357.
Zheng, Y., Ley, S.H., Hu, F.B., 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat. Rev. Endocrinol. 14 (2), 88–98. https://doi.org/10.1038/nrendo.2017.151.
Zhu, H., Leung, S.W., 2015. Identification of microRNA biomarkers in type 2 diabetes: a meta-analysis of controlled profiling studies. Diabetologia 58 (5), 900–911. https://doi.org/10.1007/s00125-015-3510-2.