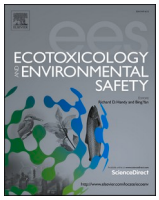
Ecotoxicology and Environmental Safety 290 (2025) 117596

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Maternal neonicotinoid pesticide exposure impairs glucose metabolism by deteriorating brown fat thermogenesis

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ARTICLE INFO

Keywords:
Neonicotinoids
Gestational diabetes mellitus
Glucose metabolism
Brown adipose tissue
Thermogenesis

ABSTRACT

Background: Neonicotinoids (NEOs) are well-designed highly selective pesticides that target nicotinic acetylcholine receptors. However, their extensive use, accumulation, and biomagnification pose significant risks to humans. Increasing evidence has suggested that NEOs may affect glucose homeostasis, but little research has linked NEOs exposure to gestational diabetes mellitus (GDM), which is the most common disease in pregnancy. We here aimed to investigate the association between NEOs exposure and GDM occurrence.

Methods: 100 pregnant women who completed a 75 g oral glucose tolerance test (OGTT) at 24–28 weeks of gestation were enrolled. Urinary concentrations of seven widely used NEOs were quantified using ultra-high performance liquid chromatography multiple reaction monitoring mass spectrometry (UHPLC-MRM-MS/MS). Correlation analysis revealed the associations between NEOs concentrations and glucose homeostasis parameters. The toxic effects of thiamethoxam (TMX) and clothianidin (CLO) were assessed using pregnant mice, and the potential mechanism in impairing glucose disposition regarding brown adipose tissue (BAT) thermogenesis has been elucidated.

Results: Among the 100 urine samples, 88 % were contaminated by NEOs with concentrations ranging from 2.50 to 491.34 nmol/L. TMX and CLO were the most frequently detected NEOs, highly detected in women with GDM. Moreover, we found statistically significant associations between TMX concentrations and 1hBG, and 2hBG. Exposure to mixed NEOs during gestation resulted in elevated glucose levels and impaired insulin sensitivity in normal pregnant and GDM mice models. In addition, we found the metabolic disorders induced by NEOs were linked to the deterioration of BAT thermogenesis *in vivo*.

Conclusion: In general, we demonstrated that prenatal exposures to NEOs were associated with an increased risk of GDM by deteriorating the thermogenic capacity of BAT

1. Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy, which begets serious adverse impacts for current and future generations, such as macrosomia, birth complications, and type 2 diabetes mellitus (Johns et al., 2018). According to the International Diabetes Federation, GDM affects 16.7 % of births worldwide and over 21 million infants annually (Magliano et al., 2021).

Intriguingly, male fetus exhibits a strong association with elevated risk of GDM potentially by influencing maternal glucose metabolism (Retnakaran et al., 2015). Traditionally, the etiology of GDM mainly focuses on advanced maternal age, excessive gestational weight gain, prior obesity and inactivity (Juan and Yang, 2020). Recently, environmental exposure has emerged as a potentially important risk factor. For example, numerous studies have reported that GDM risk increases with exposure to air pollutants (e.g., PM2.5, PM10, SO_2 and NO_2) (Niu et al., 2023). A prospective cohort study demonstrated that PFOS (Perflurooctane sulfonic acid) and PFHpA (Perfluoroheptanoic acid), two environmental perfluoroalkyl substances, significantly disrupt glucose homeostasis during pregnancy (Yu et al., 2021). In addition, endocrine-disrupting chemicals such as 2-tert-octylphenol (2-t-OP) were

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https://doi.org/10.1016/j.ecoenv.2024.117596
Received 2 September 2024; Received in revised form 6 December 2024; Accepted 19 December 2024
Available online 21 December 2024
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significantly associated with a higher risk of GDM (Hou et al., 2021). Thus, a better understanding of how these environmental exposures influence human health will provide novel avenues for GDM prevention.

Neonicotinoid insecticides (NEOs) are the most widely used pesticides in the world representing 25 % of the global insecticides market, (Bass et al., 2015) used in more than 120 countries and with a global market value of ~$2.6 billion (Fouad and Abdel-Raheem, 2024). They are soluble in water and are readily absorbed by plants through their roots or leaves. They are then transported throughout the tissues of the plant (Bass et al., 2015). The widespread use of NEOs in the air, water, soil, and agricultural resources environment leads to increasing bioaccumulation due to consuming contaminated food (Bonmatin et al., 2021; Cui et al., 2021). Notably, an estimated 2.3 billion individuals may exceed acceptable pesticide intake levels, with 1.1 billion potentially surpassing these levels by tenfold (Maggi et al., 2021). A nationwide survey of NEOs residues in 13 daily-consumed foods and found that 62.21 % foodstuffs contaminated by 7 bioactive NEOs (acetamiprid (ACE), clothianidin (CLO), dinotefuran (DIN), imidacloprid (IMI), nitenpyram (NIT), thiacloprid (THI), and thiamethoxam (TMX)) at concentrations ranging from 0.1 to 1471.43 μg/kg (Cui et al., 2021). Several studies have reported the emergence of NEOs in human urine from many countries (Zhang et al., 2018; Zhang and Lu, 2022). NEOs target the nicotinic acetylcholine receptor (nAChR) in the central nervous system, exhibiting high selective toxicity to insects (Bass et al., 2015). Moreover, *in vitro* and *in vivo* studies suggest that the accumulation of NEOs has adverse effects on human health, e.g., neurotoxicity, hepatotoxicity, embryotoxicity, and genotoxicity in mammals (Benchikh et al., 2024). However, very little research has been done on gestational exposure to NEOs and maternal metabolism and biochemical status. Therefore, more research is necessary to evaluate the harm that NEOs exposure causes to humans.

Rising evidence has suggested NEOs could pose risks to mammals and humans. A recent study documented that daily exposure to NEOs causes oxidative stress and lipid accumulation, resulting in amino acid metabolism disorders (Yan et al., 2020). Human epidemiological studies have shown that NEOs are associated with insulin and glucose homeostasis in *US* adults (Vuong et al., 2022). The toxic effects of NEOs during pregnancy have received enormous interest as fetuses and pregnant women are sensitive to environmental chemical exposures. A nested case-control study reported that DIN and ACE exposure significantly increased the risk of fetal growth restriction (Pan et al., 2023). Animal studies show that gestational exposure to IMI induces long-lasting changes in behavior and brain function in the offspring (Burke et al., 2018). Although NEOs have been theoretically linked to glucose metabolism, very few studies reported the association between serum NEO levels and the pathogenicity of GDM.

To fill the abovementioned gap, we conducted a series of studies: 1) Characterize the NEO exposures in urine from women with or without GDM and determine the relationship between differences in NEOs and GDM onset; 2) Evaluate the effects of specific NEOs exposure in impairing glucose disposition using mice model of GDM; 3) Explore potential toxic mechanisms of NEOs in the view of brown adipose tissue (BAT) thermogenesis after gestational exposure. Our findings would help better understand the impacts of NEOs on the health of pregnant women.

2. 2. Materials and methods

2.1. Study population

From July to September of 2022, we collected urine samples at 24–28 weeks of gestation from those having a GDM screening at Nanjing Women and Children's Healthcare Hospital (Nanjing, China). 100 pregnant women were enrolled in our study, including 50 GDM women and 50 normal controls (NC). The diagnosis of GDM is based on the blood glucose threshold of the 75 g oral glucose tolerance test (OGTT): fasting blood glucose (FBG) $\geq$ 5.1 mmol/L, 1 h blood glucose (1hBG) $\geq$ 10.0 mmol/L, or 2 h blood glucose (2hBG) $\geq$ 8.5 mmol/L. All participants had a single pregnancy and excluded a history of type 1 diabetes, type 2 diabetes, hypertension, eclampsia, preeclampsia, polycystic ovary syndrome or cholestasis syndrome before pregnancy. The urine samples were aliquoted into centrifuge tubes, 1 mL per tube, and were transferred to a −80℃ refrigerator for storage until further use. This project was approved by the Ethics Committee of Nanjing Women and Children's Healthcare Hospital (Permit number: 2020KY075). All provided signed informed consent before participating in the study.

2.2. Chemicals and reagents

The seven most widely used NEOs in the Chinese market were selected as representatives. The sources of standard reagents were illustrated as follows: IMI (CAS: 138261–41–3, 99.5 % purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). ACE (CAS: 160430–64–8, 97.8 % purity), TMX (CAS: 153719–23–4, 99.5 % purity), and DIN (CAS: 165252–70–0, 98.5 % purity) were obtained from Macklin (Shanghai, China). NIT (CAS: 150824–47–8, 99.0 % purity), THI (CAS: 111988–49–9, 99.5 % purity), and CLO (CAS: 210880–92–5, 99.5 % purity) were commercially available from Aladdin Ltd (Shanghai, China). The HPLC grade of ethyl acetate, acetonitrile, and formic acid was purchased from CNW Technologies (Duesseldorf, Germany). Experimental water was prepared using a Milli-Q water purification system (Millipore, USA).

2.3. Assessment of urine NEOs

200 μL urine samples were taken for NEOs quantification by the internal standard curve method using ultra-high performance liquid chromatography multiple reaction monitoring mass spectrometry (UHPLC-MRM-MS/MS) (Biotree, Shanghai). As described before, details regarding the analytical method include target analytes extraction, liquid chromatography separation, tandem mass spectrometry analysis, calibration standards, and quality control, (Cui et al., 2021). Briefly, NEOs in urine were detected using a liquid-liquid extraction method, followed by instrumental analysis utilizing an Agilent 1290 Infinity II series UHPLC System (Agilent Technologies, USA) equipped with a Waters ACQUITY UPLC CSH C18 (150 × 2.1 mm, 1.7 μm, Waters, USA). The limit of detection (LOD) was determined by the ratio of the peak signal intensity (S) to the noise intensity (N), S/N = 3. The LOD was 9.77 nmol/L for IMI, 1.22 nmol/L for ACE, 0.61 nmol/L for NIT, 0.31 nmol/L for THI, 4.88 nmol/L for TMX, 9.77 nmol/L for CLO and 2.44 nmol/L for DIN, respectively. The absolute concentrations of detected NEOs considering dilution ratio were provided in Supplemental Table 1. "ND" means the compound could not be detected in this sample.

2.4. Animal experiments

C57BL/6 J mice (7 weeks old) were purchased from the Animal Center of Nanjing Medical University and housed in a standard temperature/ humidity-controlled environment (24 ± 2 ℃/ 50 ± 10 %) with free access to food and water. Male mice weighted 18–20 g, and female mice weighted 16–18 g, respectively. All mice were adapted for one week before initiating any experimental procedures. The animal studies conformed to guidelines issued by the Institutional Animal Ethics Committee and approved by the Institute of Animal Care and Use Committees at the Nanjing Medical University (IACUC-1907011).

For normal pregnancy (NP), female mice were mated with male (2:1) fed on a chow diet. For GDM pregnancy, mice were fed a high-fat diet (HFD, 60 % kcal) for 4 weeks before mating and continued with HFD throughout the gestation period. Mating was determined by the presence of a vaginal plug and designated as gestation day 0. Streptozotocin (STZ, 30 mg/kg) was injected intraperitoneal (i.p.) to induce maternal

hyperglycemia as previously described (Yin et al., 2022). The GDM mice were qualified as random blood glucose ≥ 11.1 mmol/L. Total 20 pregnant mice (5 mice per group) were gavaged daily with NEOs (an equal mass mixture of TMX and CLO, 20 mg/kg) or vehicle 2 weeks before conception and throughout the 3 weeks of gestation. The dose referred to similar study shown to induce biochemical alterations without causing behavioral signs of toxicity or mortality (Ndonwi et al., 2020). Mice were euthanized by CO_2 gas at the 18th day of pregnancy and BAT was collected.

2.5. Glucose tolerance and insulin tolerance tests

For the glucose tolerance test (GTT), pregnant mice fasted for 16 h were injected i.p. with glucose at 2 g/kg or 1.5 g/kg for NP or GDM mice, respectively. For the insulin tolerance test (ITT), mice were injected i.p. with insulin after 6 h of fasting. The dose of insulin was 0.5 U/kg in the NP group and 0.75 U/kg in the GDM group. Tail sampling was performed at 0, 15, 30, 60, 90, and 120 min after administrating glucose detection using a glucose meter (Contour Blood Glucose Meter, Bayer, Mishawaka, IN).

2.6. Infrared thermal imaging

Surface temperatures were measured with an infrared camera (Thermo GEAR G120/G100, NEC Avio Infrared Technologies Co., Ltd., Tokyo, Japan) and analyzed with FLIR Tools Software (FLIR System).

2.7. Western blot analysis

The protein of BAT was extracted using RIPA buffer (Beyotime Biotechnology, Beijing, China) and quantified by BCA Protein Assay Kit (Thermo Fisher Scientific, USA). For Western blot analysis, forty nanograms of protein were loaded and separated by SDS-PAGE gel and then transferred onto a PVDF membrane (Millipore, USA). The membrane was probed overnight with an antibody against uncoupling protein-1 (UCP1) (23763–1-AP, Proteintech) and then visualized by horseradish peroxidase (HRP) -conjugated secondary antibodies. Heat shock protein-90 (HSP90, Santa Cruz) was a loading control. Images were captured by the ChemiDoc MP imaging system (Bio-rad, USA). The quantitative analysis was performed using the NIH Image J software (version: 1.52i).

2.8. Histology and immunohistochemistry

Fresh BAT was fixed in 4 % paraformaldehyde at room temperature for 24 h, followed by embedded in paraffin. For immunochemical staining, the 5 μm thick tissue sections were deparaffinized, hydrated, and processed for antigen retrieval using 1 × Antigen Retrieval Buffer (Abcam, USA). Slides were blocked in 5 % donkey serum in 1 × PBS for 1 h at room temperature and then probed with primary antibody against UCP1 (23763–1-AP, Proteintech, 1:300) in 0.1 % Triton X-100/ 1 % BSA (Sigma-Aldrich) overnight at 4 ℃. The HRP-tagged secondary antibody was used to amplify signals of bonded antibody in combination with a commercially available DAB kit (Vector Laboratories). Then, the slides were counterstained with hematoxylin. All pictures were acquired with Pannoramic 250 FLASH from 3DHISTECH Ltd.

2.9. Statistical analysis

Data were visualized and analyzed by using GraphPad Prism 8 software. Results were expressed as mean ± SD. A two-tailed unpaired Student's *t*-test was applied for comparison when appropriate. $P < 0.05$ was considered significant in all tests. We used Spearman rank correlation analysis to assess the relationships among GDM-related NEOs, glycated hemoglobin (HbA1c), and 75 g-OGTT values. The logistic regression model was employed to construct a diagnostic model through receiver operating characteristic (ROC) curves for NEO exposure on GDM prevalence.

3. Results

3.1. General information of participants

Of the 100 pregnant women, 50 were diagnosed with GDM and 50 were recruited as NC. The demographic characteristics of participants in the current analysis were summarized in Table 1. The GDM and NC groups were similar in age, gestational days, gravidity and parity, while the body mass index (BMI) was moderately higher in the GDM group. In contrast, diabetic state indicators that included FBG, 1hBG, 2hBG and HbA1c were significantly higher in the GDM group compared with the NC group. Additionally, we observed no other difference among the biochemical indices such as TAG (triglyceride), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) between the two groups.

3.2. NEOs concentrations in urine from women with or without GDM

The NEO concentrations in urine were detected by UHPLC-MRM-MS/MS, and detailed data were provided in Supplemental Table 1. Only TMX, CLO, and DIN can be detected in the NC and GDM groups. At the same time, IMI, NIT, ACE or THI could not be examined, suggesting the limit species of NEOs in urine (Fig. 1A). All the data were valid as the value was more prominent than the LOD of each analyte (ranged from 0.31 to 9.77 nmol/L). Interestingly, the concentrations of TMX and CLO were significantly higher in the GDM group than in the NC group (Fig. 1B), which may be related to the pathology of GDM.

3.3. NEOs were positively related to elevated glucose level

Correlation analysis was performed to find the inner association between NEOs and the four clinical measurements that were often employed for diagnosing GDM (HbA1c, FBG, 1hBG, and 2hBG). The concentration of TMX was shown to be positively correlated with 1hBG and 2hBG, while CLO and DIN were irrelevant with aberrant glucose levels (Fig. 2A). To ascertain whether these lipids could recognize GDM, the ROC curve was calculated. The NEOs (TMX, CLO, and DIN) both had a larger median area under the ROC curve (AUCs) than 0.5, indicating that they would be more useful in the diagnosis of GDM (Fig. 2B). All of the data above pointed to a potential role for NEOs in the etiology of GDM and shown that the concentration of NEOs in urine can distinguish GDM patients with greater accuracy.

Table 1
Maternal clinical information of the participants.

Characteristics	GDM (n = 50)	NC (n = 50)	P value
Age, years	30.61 ± 3.04	29.93 ± 2.52	0.250
BMI, kg m^{-2}	25.39 ± 3.17*	23.94 ± 3.03	0.022
Gestational age, days	179.68 ± 8.64	178.16 ± 9.23	0.502
Gravidity, n	1.68 ± 0.96	1.54 ± 0.79	0.426
Parity, n	0.24 ± 0.43	0.26 ± 0.49	0.828
FBG, mmol L^{-1}	4.74 ± 0.56**	4.29 ± 0.27	< 0.01
1hBG, mmol L^{-1}	9.83 ± 1.21**	7.24 ± 1.12	< 0.01
2hBG, mmol L^{-1}	7.90 ± 1.89**	6.06 ± 1.06	< 0.01
HbA1c, %	5.19 ± 0.29**	4.88 ± 0.20	< 0.01
TAG, mmol L^{-1}	2.54 ± 0.73	2.31 ± 1.00	0.198
HDL, mmol L^{-1}	2.08 ± 0.44	2.19 ± 0.36	0.205
LDL, mmol L^{-1}	3.21 ± 0.74	3.25 ± 0.76	0.811

Values were presented as mean ± SD. The unpaired student's *t*-test was used for continuous variables. $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs* NC group.
Abbreviations: GDM, gestational diabetes mellitus; NC, normal control; BMI, body mass index; FBG, fasting blood glucose; 1hBG, 1 h blood glucose of OGTT; 2hBG, 2 h blood glucose of OGTT; HbA1c, glycosylated hemoglobin; TAG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein.

A

		IMI	NIT	ACE	THI	TMX	CLO	DIN
Detection rate (%)	NC	0	0	0	2	50	82	22
	GDM	0	0	0	0	78	94	14
LOD (nmol/L)	/	9.77	1.22	0.61	0.31	4.88	9.77	2.44
Concentration range (nmol/L)	NC	/	/	/	0.47	2.55~14.47	32.78~306.30	2.50~16.79
	GDM	/	/	/	/	5.18~64.75	44.11~491.34	2.86~6.16
Mean ± SD	NC	/	/	/	/	7.43±3.09	148.28±73.08	6.05±4.93
	GDM	/	/	/	/	13.76±11.21	192.89±110.58	4.46±1.00
P value	/	/	/	/	/	0.008	0.031	0.410

B

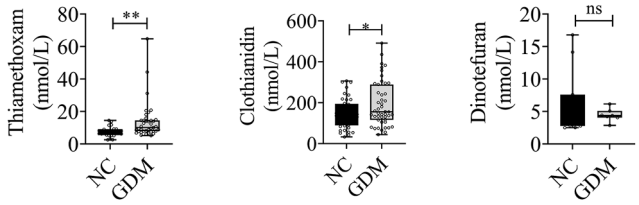
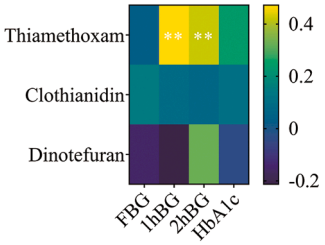


Fig. 1. NEOs concentrations were detected in urine from women with or without GDM. (A) The detailed information of 7 NEOs in urine was examined using UHPLC-MRM-MS/MS. NC, normal control; GDM, gestational diabetes mellitus; LOD, limit of detection; IMI, imidacloprid; NIT, nitenpyram; ACE, acetamiprid; THI, thiacloprid; TMX, thiamethoxam; CLO, clothianidin; DIN, dinotefuran. (B) Box-plot of TMX, CLO, and DIN in NC and GDM group. Data were shown as mean ± SD and analyzed by two-tailed unpaired student's *t*-test. n = 50 per group, $^{*}p < 0.05$, $^{**}p < 0.01$, and "ns" means no significant difference *vs* NC group.

A

	FBG		1hBG		2hBG		HbA1c	
	r	*p*	r	*p*	r	*p*	r	*p*
Thiamethoxam	0.031	0.809	0.468**	0.001	0.422**	0.001	0.235	0.062
Clothianidin	0.143	0.186	0.095	0.443	0.075	0.490	0.109	0.312
Dinotefuran	-0.157	0.534	-0.213	0.444	-0.323	0.191	-0.037	0.885



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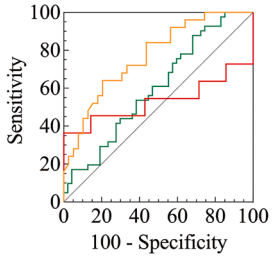

Color	Insecticides	AUC	95%CI
	Thiamethoxam	0.769	0.654 to 0.885
	Clothianidin	0.602	0.484 to 0.720
	Dinotefuran	0.533	0.260 to 0.805

Fig. 2. NEOs were associated with GDM occurrence in pregnant women. (A) Correlation analysis of NEOs with blood glucose level using Spearman's method. The yellow color indicates a positive correlation coefficient (r), whereas the black purple denotes a negative correlation in the heatmap. $^{**}p < 0.01$ *vs* NC group. (B) ROC curve showing the performance of the logistic ridge regression model of NEOs. The AUC and 95 % CI of each model were depicted.

3.4. NEOs impaired insulin sensitivity in pregnant mice

To explore whether NEOs could affect the occurrence of GDM, mice were gavaged daily with NEOs or vehicles 2 weeks before mating and 3 weeks throughout the gestation. As shown in Fig. 3A-C, fasting blood glucose levels were continuously higher after being treated with NEOs for 1 week, 2 weeks and at pregnancy 14d, indicating that NEOs may rapidly impair glucose metabolism. The glucose levels were precisely regulated by insulin, GTT and ITT were used to evaluate insulin sensitivity. GTT analysis showed lower glucose tolerance in the NP-NEOs group compared with the NP-Vehicle group (Fig. 3D). Meanwhile, mice treated with NEOs had impaired insulin sensitivity examined by ITT (Fig. 3E). In short, NEOs could impair insulin sensitivity, leading to the occurrence of GDM.

3.5. NEOs worsen glucose metabolism in GDM mice model

To reveal the effect of NEOs on glucose metabolism in GDM mice, we established a GDM mice model using HFD combined with repeated STZ injections. After gavage with NEOs for two weeks, the fasting blood glucose levels were significantly increased in the GDM-NEOs group compared to the GDM-vehicle group (Fig. 4A). This is consistent with Fig. 3B, illustrating that NEOs impaired glucose levels both in NP and GDM model. Subsequently, STZ injection successfully established the GDM mice model as the random blood glucose was > 11.1 mmol/L (Fig. 4B). The impaired glucose metabolism continued to pregnancy 14d (Fig. 4C), and GTT and ITT analysis showed lower glucose tolerance and insulin sensitivity in the NEOs treated group (Fig. 4D-E). These results suggested that NEOs may worsen hyperglycemia and insulin resistance in the GDM mice model.

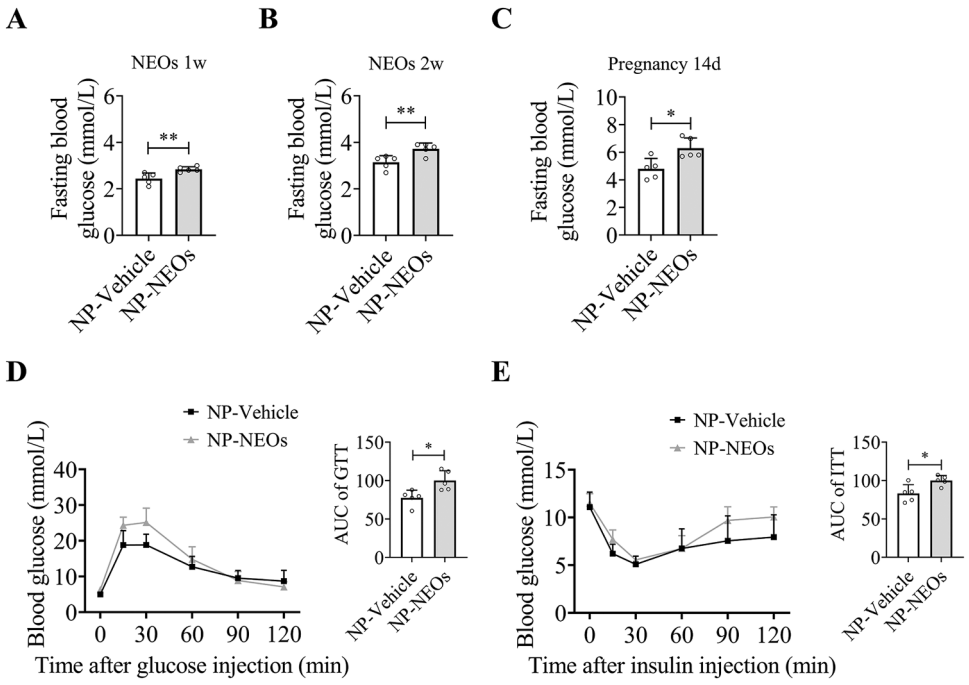

Fig. 3. NEOs impaired insulin sensitivity in pregnant mice. Fasting blood glucose level of mice treated with NEOs for 1 week (A), 2 weeks (B), or continued to day 14 of gestation (C). (D-E) GTT and ITT assays of mice treated with NEOs or vehicle to day 16–18 of gestation. Data were shown as mean ± SD. n = 5 mice per group, $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs* NP-vehicle group.

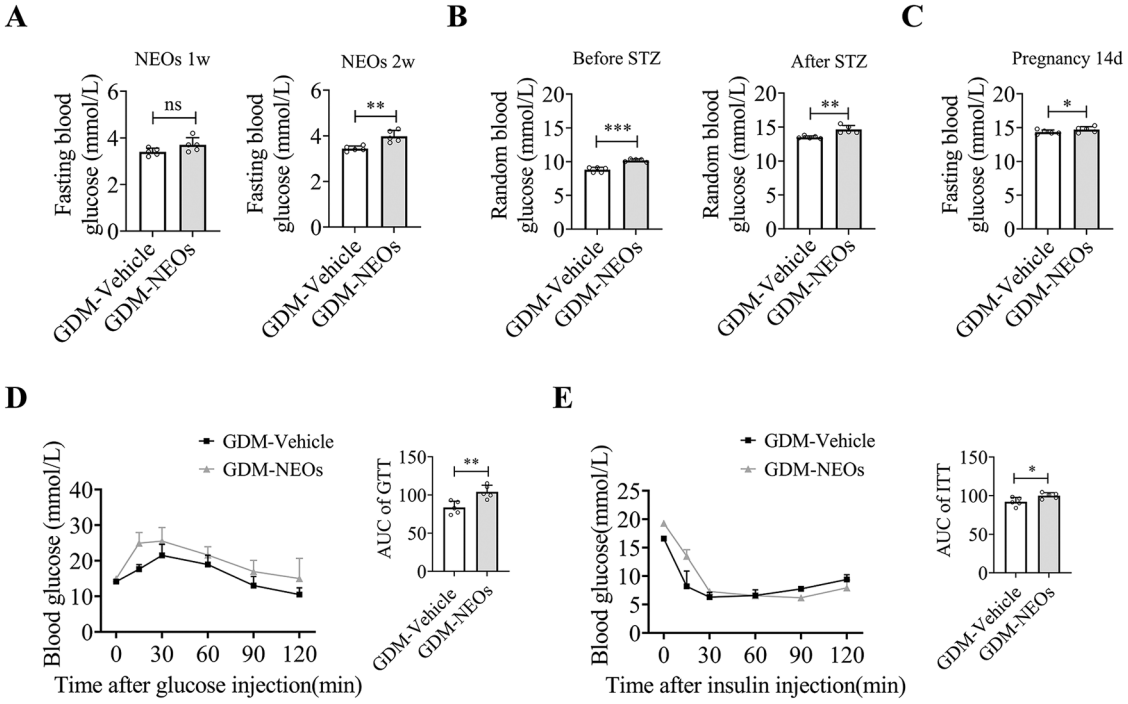

Fig. 4. NEOs worsen symptoms in the GDM mice model. (A) Fasting blood glucose level of HFD-mice treated with NEOs for 1 week or 2 weeks. (B) Random blood glucose levels of HFD-mice before and after STZ injection. (C) The fasting blood glucose level of GDM mice treated with NEOs continued to day 14 of gestation. (D-E) GTT and ITT assays of GDM mice treated with NEOs or vehicle to day 16–18 of gestation. Data were shown as mean ± SD. n = 5 mice per group, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ *vs* GDM-vehicle group. "ns" means no significant difference between groups.

3.6. NEOs deteriorated the thermogenic capacity of BAT

The development of GDM might involve the function of BAT, which can improve glucose homeostasis, mitigate insulin resistance, and protect against obesity (Cohen and Kajimura, 2021; Maliszewska and Kretowski, 2021). Thus, it is vital to assess the function of BAT in NEOs treated mice. Using infrared thermal imaging, we found that NEOs reduced body surface temperature both in NP and GDM groups, especially at the shoulder of the BAT accumulation area (Fig. 5A-B), suggesting that NEOs may destroy the thermogenic activity in BAT. Indeed,

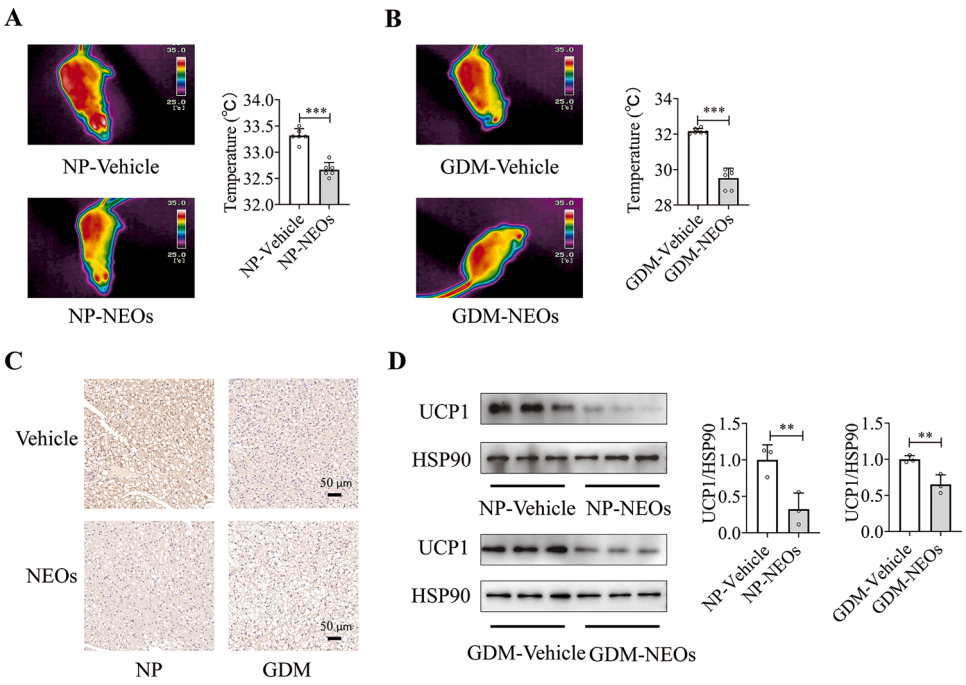

Fig. 5. NEOs deteriorated maternal thermogenic capacity of BAT. Infrared thermal images at day 18 of gestation for normal mice (A) or GDM mice (B) treated with NEOs or vehicle. (B) Infrared thermal images of GDM mice treated with NEOs or vehicle. (C) Immunohistochemical staining of UCP1 level in BAT. (D) Western blot analysis to determine UCP1 expression in BAT. The data were shown as mean ± SD. n = 5 mice per group, $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs* NP-vehicle or GDM-vehicle group.

the UCP1 levels (marker gene of thermogenic function) in BAT, examined by immunohistochemistry staining (Fig. 5C) or western blot analysis (Fig. 5D), were considerably decreased in the NP-NEOs and GDM-NEOs group, indicating the whitening of BAT. In a word, NEOs played an essential role in the pathology of GDM by deteriorating the thermogenic capacity of BAT.

4. Discussion

GDM is a widespread disease that seriously threatens people's health, and maternal NEOs exposure status has emerged as a potentially important risk factor. In this study, we detected contaminated NEOs in urine from pregnant women and found higher levels of TMX and CLO in GDM. The concentration of TMX was significantly corrected with 1hBG and 2hBG, and both TMX and CLO may help diagnose and predict GDM. In the pregnant mice, exposure to mixed NEOs resulted in elevated glucose levels and impaired insulin sensitivity. Besides, NEOs worsen glucose metabolism in the GDM mice model. Furthermore, maternal metabolic disorder induced by NEOs was linked to the deterioration of thermogenic capacity in BAT.

With the fast development of modern industrial and agricultural sectors, many chemical pollutants have been released into the environment (Hladik et al., 2018). When NEOs are applied to crops, only a small portion (on average 5 %) being absorbed and ~95 % of the active ingredient available in the soil and soil water or lost as dust during planting (Tapparo et al., 2012). NEOs accumulated in the soil of fields planted with treated seeds, and can still be detected several years later after the cessation of use (Hladik et al., 2017). It can affect earthworms and micro-organisms in the soil environment, and is further facilitated by leaching and run-off, leading to water contamination and subsequent adverse effects on aquatic ecosystems (Zhang et al., 2024). NEO-contaminated food consumption is considered to be the main route of human exposure to NEOs. Ingested through contaminated plants or water, destroys human health, including neurological, endocrine, immunological, and reproductive systems (Benchikh et al., 2024; Hoy et al., 2024). Recently, researchers have determined the potential harmful of organic pollutants on hormone homeostasis, causing dysglycemia, inflammation, oxidative stress, β-cell dysfunction, insulin resistance, and neurohormonal dysfunction (Vuong et al., 2022; Martelli et al., 2020) and resulting in obesity, diabetes, and other health issues (Ndonwi et al., 2020; Miranda et al., 2023). Given the similarities in the pathophysiology of GDM and diabetes, (Lowe, 2023) environmental contaminants may also be significant in the onset and course of GDM. Pregnant women may be particularly vulnerable to alterations due to the influence of pregnancy hormones, (Gingrich et al., 2020) which may encourage us to focus on the association between NEOs and the etiology of GDM. Most research looked at the relationship between a single chemical and GDM; however, examining certain chemicals fails to capture the complexity of natural exposure. Various environmental pollutants that result in qualitative synergistic interactions and quantitative accumulation expose pregnant women. As a result, we first detected maternal exposure of seven worldwide popular NEOs in women with or without GDM.

Throughout the history of NEOs development, IMI was once the most popular product on the market for many years (Tapparo et al., 2012; Van Dijk et al., 2013). TMX progressively occupies the lead position in the market of insecticides, and the amount of TMX residue in food, water, and human tissue has grown (Maienfisch et al., 2001). CLO, the primary metabolite of TMX in soil, insects, and plants, is also registered as an insecticide worldwide (Fan and Shi, 2017). Various studies have detected NEOs by UHPLC-MRM-MS/MS and found that urinary levels in China range from 0.38 to 5.6 ng/mL (Zhang and Lu, 2022). Consistent with these findings, we detected TMX (range 2.55–64.75 nmol/L), CLO (range 32.78–491.34 nmol/L), and DIN (range 2.50–16.79 nmol/L), respectively. However, we did not measure the effective residue of IMI, NIT and ACE in maternal metabolites; this might be due to the area where the urine samples were taken or the few use of these NEOs in China. Moreover, our results revealed that TMX and CLO were significantly higher in GDM women, and TMX concentration was closely positively corrected with 1hBG and 2hBG. Consistently, Veedu SK et al. found that in a freshwater fish Catla catla, exposure to TMX showed significant increased blood glucose (Veedu et al., 2022). In the mice

model, we proved that the mixed NEOs may affect glucose metabolism in normal and GDM pregnancy.

Although many factors are involved in controlling maternal metabolic adaptation during pregnancy, energy intake and expenditure status play an essential role. Prior research has demonstrated that pregnancy alone can lower a mouse's basal body temperature, energy expenditure, and UCP1 expression in BAT (Qiao et al., 2018). BAT is specialized to consume the body's excess energy through UCP1, released in the form of heat, and contributes to weight loss and glucose homeostasis (Cohen and Kajimura, 2021; Maliszewska and Kretowski, 2021). Improved glucose tolerance and insulin sensitivity were not linked to weight reduction in SM/J mice, (Carson et al., 2020) while more mice can successfully recover from type 1 diabetes by BAT transplanting, (Gunawardana and Piston, 2012) indicating the critical role of BAT in maintaining maternal energy homeostasis. Emerging evidence suggests that environmental pollutants influence energetic homoeostasis and metabolic diseases development by altering BAT thermogenic activity, such as dichlorodiphenyltrichoroethane (DDT), perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) (Di Gregorio et al., 2018). Noteworthy, pesticide chlorpyrifos impairs BAT thermogenesis and contribute to increasing susceptibility to metabolic syndrome (Wang and Steinberg, 2022). However, minimal information is available regarding the role of BAT function in NEOs affecting glucose metabolism. Our results revealed that the UCP1-mediated thermogenic capacity of BAT was whitened by NEOs in the pregnanct mice, leading to decreased heat release and further resulting in increased blood glucose level, impaired insulin sensitivity, and worsened symptoms of GDM.

Our study does, however, have certain shortcomings. First of all, although our sample size was more robust in representing the contamination of NEOs in pregnant women in Nanjing, a larger sample size with an expanded area would probably enable the detection of more subtle concentrations of NEOs in future research. Second, the research subjects were limited to the top seven NEOs most indicative of the local market. A more thorough assessment of all NEOs metabolites would better reflect the exposure level in the pregnant. Third, we neglected the data of accumulation and persistence or removal of NEOs from mice after gavage.

5. Conclusion

In summary, we found that TMX and CLO were significantly higher in GDM women, and TMX was closely corrected with the levels of 1hBG and 2hBG. NEOs exposure impaired insulin sensitivity and worsened glucose metabolism in the pregnant mice. UCP1-mediated BAT thermogenesis might play a significant role in illuminating the pathophysiology of GDM. Consequently, it is the first time to reveal the effects of NEOs on glucose metabolism by deteriorating the thermogenic capacity of BAT. Further research on exposure to NEOs should be paid to the transmission pathways of NEOs in pregnant women to minimize the risks on human health.

CRediT authorship contribution statement

Chenbo Ji: Validation, Project administration. **Hong Zhong:** Writing – review & editing, Investigation. **Wenwen Zhu:** Writing – original draft, Investigation, Formal analysis. **Jiali Fang:** Visualization, Methodology, Investigation, Data curation. **Tianying Zhong:** Supervision, Funding acquisition, Conceptualization. **Xianwei Cui:** Writing – review & editing, Funding acquisition, Data curation.

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.

Acknowledgments

This work was financially supported by Natural Science Foundation of Jiangsu Province (BK20241734), Medical Research Project of Jiangsu Province Health Commission in 2019 (K2019014) and Nanjing Medical Science and Technique Development Foundation (ZKX23041).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117596.

Data Availability

Data will be made available on request.

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