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Titanium exposure and gestational diabetes mellitus: associations and potential mediation by perturbation of amino acids in early pregnancy

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Abstract

Background Several recent studies reported the potential adverse effects of titanium exposure on glucose homeostasis among the non-pregnant population, but the association of titanium exposure with gestational diabetes mellitus (GDM) is scarce.

Methods The present study of 1,449 pregnant women was conducted within the Jiangsu Birth Cohort (JBC) study in China. Urine samples were collected in the early pregnancy, and urinary titanium concentration and non-targeted metabolomics were measured. Poisson regression estimated the association of titanium exposure in the early pregnancy with subsequent risk of GDM. Multiple linear regression screened for titanium-related urine metabolites. Mediation analyses assessed the mediating effects of candidate metabolites and pathways.

Results As parameterized in tertiles, titanium showed positive dose–response relationship with GDM risk (*P* for trend = 0.008), with women at the highest tertile of titanium exposure having 30% increased risk of GDM [relative risk (RR) = 1.30 (95% CI: 1.06, 1.61)] when compared to those exposure at the first tertile level. Meanwhile, we identified the titanium-related metabolites involved in four amino acid metabolic pathways. Notably, the perturbation of the aminoacyl-tRNA biosynthesis and alanine, aspartate and glutamate metabolism mediated 27.1% and 31.0%, respectively, of the relative effect of titanium exposure on GDM. Specifically, three titanium-related metabolites, choline, creatine and L-alanine, demonstrated predominant mediation effects on the association between titanium exposure and GDM risk.

Conclusions In this prospective study, we uniquely identified a correlation between early pregnancy titanium exposure and increased GDM risk. We unveiled novel insights into how perturbations in amino acid metabolism may

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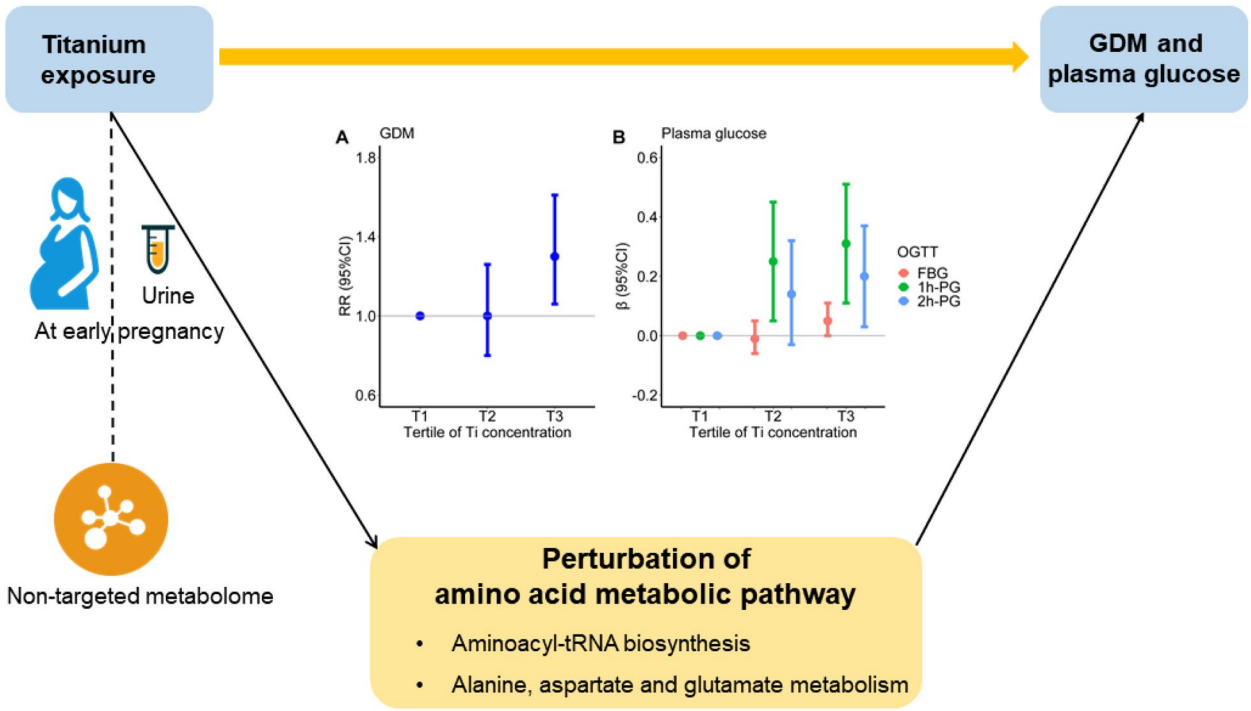
mediate the link between titanium exposure and GDM. Notably, choline, creatine, and L-alanine emerged as key mediators influencing this association. Our findings imply that elevated titanium exposure in early pregnancy can lead to amino acid dysmetabolism, thereby elevating GDM risk.

Keywords Prenatal exposure, Titanium, Gestational diabetes mellitus, Cohort study, Non-targeted metabolomics

Graphical Abstract



Introduction

The global prevalence of Gestational diabetes mellitus (GDM) is increasing at an alarming rate. According to the most recent report from the International Diabetes Federation (IDF), over 16% live births were affected by hyperglycemia in pregnancy, whereby the majority of these pregnancies were GDM [1]. Given the high prevalence and the consequent disease burden for maternal and child health, GDM is emerging as an urgent global public health concern [2]. The increasing prevalence of GDM probably reflects changes in demographic characteristics and lifestyles, as well as alterations in environmental influences. In addition to well-established GDM risk factors, such as advanced maternal age, overweight and obesity, family history of type 2 diabetes mellitus, and poor dietary pattern and lifestyle [2], evidence has been mounting that prenatal exposure to environmental pollutants, including heavy metals and endocrine-disrupting chemicals, also contributes to risk of GDM [3, 4].

Despite the dramatic achievements in the reduction of traditional pollutants over the last decade, a broad and increasing variety of human-made substances have been regularly used but have only lately been recognized as emerging contaminants. Titanium, for instance, is extensively used in food additives, personal care products, plastic production and pigments in paint in the form of microparticle-sized or nanoparticle-sized titanium dioxide (TiO_2) [5]. Recently, concerns have arisen about the potential adverse effects of titanium exposure on glucose homeostasis [6]. A cohort study has established an association between titanium exposure and the onset of type 2 diabetes [7]. Furthermore, an animal experiment has illustrated that prenatal exposure to nanoparticle-sized TiO_2 can lead to elevated fasting blood glucose in

pregnant rats [8]. Nevertheless, population-level investigation of titanium exposure and the incidence of GDM remains scarce. There is only one retrospective cohort study that reported a link between maternal exposure to airborne titanium (constituent of $PM_{2.5}$) during the first trimester and an increased risk of GDM and impaired glucose tolerance [9], while titanium exposure from other sources was not well considered.

Several studies have demonstrated that metabolic disruption caused by exogenous chemical exposure during pregnancy is related to pregnancy outcomes [10, 11]. Chen et al. [12] reported titanium exposure during pregnancy could lead to metabolic perturbations, with amino acid metabolism and carbohydrate metabolism being positively associated with titanium concentration. Though increasing evidence suggested that disturbances in metabolic programming, including amino acid and lipid metabolisms, play key roles in the development of GDM [13–15], whether titanium-related metabolic perturbations are implicated in the development of GDM remains unknown.

In this prospective cohort study, we examined the association of maternal urinary titanium concentration in the early pregnancy with subsequent risk of GDM, and further assessed the mediating effects of titanium-related metabolic alterations. Our findings may help to identify the early metabolic markers involved in biological pathways of GDM induced by titanium exposure.

Methods

Study design and participants

The study was conducted within the Jiangsu Birth Cohort (JBC), a prospective and longitudinal birth cohort in China [16]. The JBC included couples received spontaneous conception or assisted reproductive technology treatment at the Women's Hospital of Nanjing Medical University or Suzhou Affiliated Hospital of Nanjing Medical University. The study design has been described in detail elsewhere [17]. The current study only included pregnant women who conceived spontaneously.

From 2014 to 2017, 1466 pregnant women with a singleton live birth provided urine samples in the first trimester during the pregnancy, and urinary titanium measurements and non-targeted metabolomics testing were performed. Participants with pre-existing diabetes mellitus before pregnancy or diabetes in pregnancy (any fasting blood glucose (FBG) $\geq$ 7.0 mmol/L or 2-h plasma glucose (2-h PG) $\geq$ 11.1 mmol/L in pregnancy) were excluded (N = 17), resulting in a final sample of 1,449 pregnant women with glucose at recruitment. All participants provided written informed consent upon enrollment and the study protocol was approved by the Human Investigation Committees at Nanjing Medical University.

Data collection

At recruitment (gestational weeks [GW] ~ 8–14), face-to-face questionnaires were performed by trained study personnel following standardized protocols to collect baseline characteristics including sociodemographic characteristics (*e.g.*, education, annual household income, date of birth, residence area), lifestyle (*e.g.*, smoking and drinking status), pre-pregnancy weight and height, history of diseases and reproductive history. They were seen at another two antenatal care visits, once during mid-pregnancy (GW ~ 22–26), and again during late-pregnancy (GW ~ 30–34). We collected their lifestyle and health related information during pregnancy by face-to-face questionnaire surveys. Besides, their detailed clinical data including physical examination, clinical test, and pregnancy complications were extracted from electronic health records.

Measurements of urinary titanium

The spot urine samples were drawn at the recruitment in the early pregnancy (GW ~ 8–14) and stored at -20 ℃. After thawing the urine sample at room temperature, 300 μL of urine diluted with 5700 μL of 2% HNO_3 and then nitrated overnight. The concentrations of urinary metals [including titanium, cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), manganese (Mn), nickel (Ni), and antimony (Sb)] were measured by an iCAP Qc inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Fisher Scientific, Germany), which has been described previously [18, 19]. Each 20 samples were interpolated a standard solution to monitor the precision. The limit of detection (LOD), detection rate and quality control (QC) data were present in Table S1. Samples with metal levels below the LOD were substituted with values of LOD/2. The urine specific gravity (SG), was measured by a digital hand-held refractometer (Atago PAL-10S, Co., Ltd., Tokyo, Japan) to adjust for urinary dilution. The formula used for adjustment was $P_c = P\ [(SG_m — 1)/ (SG — 1)]$, where P_c represents the concentration adjusted by SG (μg/L), P represents the measured concentration (μg/L), and SG_m is the median concentration of SG.

Urinary untargeted metabolomic data

Untargeted urinary metabolomics were quantified by the ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF–MS). Details on sample pretreatment and instrumental analysis can be seen in Supplementary methods. Raw data for urine metabolites were converted to the mzXML format using ProteoWizard software. The processed for peak detection, extraction, alignment, and integration were performed based on the XCMS R package. The online database of HMDB (www.hmdb.ca) and

the self-built secondary mass spectrometry database (BiotreeDB, V2.1) were used for substance annotation. The cutoff value for algorithm scoring was set to 0.3. The data QC strategies included: 1) normalized by quality control-based Robust Loess Signal Correction (R-LSC) to correct for potential analytical drift [20]; 2) exclude the metabolic features detected in < 50% of QC samples; 3) exclude metabolic features with relative SD > 30% of QC samples. A total of 683 metabolites (concatenated negative and positive models) were annotated meeting QC criteria. After removing the metabolites whose detection rate < 80%, 640 metabolites were included. The missing value on peak intensities for metabolites were replaced by the half of the minimum peak intensity. Finally, we normalized the data by dividing it by the median value of the peak intensity.

Outcomes

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. In the clinical setting, pregnant women were universally screened for GDM with the 75 g oral glucose tolerance test (OGTT) at GW ~ 24–28. Pregnant women were also tested for fasting glucose during the antenatal care visits. According to the criteria of the World Health Organization (WHO) [21], GDM was ascertained if one or more of the following criteria were met: 1) one or more plasma glucose values in 75 g OGTT meeting or exceeding the thresholds (FBG 5.1–6.9 mmol/L, 1-h plasma glucose (1-h PG) ≥ 10.0 mmol/L, and 2-h PG 8.5–11.0 mmol/L), or 2) FBG meeting 5.1–6.9 mmol/L measured alone or in the 75 g OGTT.

Statistical analysis

Participants were divided into three groups based on the distribution of urinary titanium levels. Tertile 1 includes participants with titanium levels below the 33.3rd percentile, Tertile 2 includes participants with titanium levels between the 33.3rd and 66.7th percentiles, and Tertile 3 includes participants with titanium levels above the 66.7th percentile. Tertile 1, representing the lowest titanium exposure group, was used as the reference group. In the present study, differences in participant characteristics according to the tertiles of titanium concentration were assessed by one-way analysis of variance for continuous variables and χ^2 test for binary/multilevel categorical variables. We utilized natural logarithmic (Ln) transformations to achieve normal distributions of titanium and metabolite concentrations for subsequent analyses. The restricted cubic spline (RCS) was employed to evaluate a potential non-linear association of titanium exposure with risk of GDM and continuous plasma glucose concentrations. Our results indicated a significant non-linear effect of titanium exposure with FBG, while no significant non-linear effects were observed of titanium exposure on GDM risk or 1-h and 2-h PG (Figure S1). We performed robust Poisson regression models [22] to evaluate the associations between urinary SG-corrected titanium concentrations and GDM with titanium concentration as categorical variable (by tertiles and the lowest tertile serving as the reference). Linear regression models were used to evaluate the associations between titanium exposure and plasma glucose (FBG, 1-h PG, and 2-h PG). Relative risks (RRs) were presented. *P* for trend was assessed using the median value of each titanium tertile as a continuous variable.

Potential confounders were identified via direction acyclic graph (Figure S2). The final confounders included maternal age at recruitment and pre-pregnancy BMI (continuous variables), as well as study center (Nanjing or Suzhou), residence area (rural or urban/sub-urban), household income (< 100,000, 100,000–200,000, or ≥ 200,000 CNY), maternal education (< 12 or ≥ 12 years), and parity (0 or ≥ 1). Additionally, we conducted stratification analyses by maternal age and pre-pregnancy BMI to explore whether any factor might modify the effect of titanium on GDM. The tests for statistical interaction were performed with product term of titanium tertiles and the corresponding stratified variable. Two sensitivity analyses were performed to assess the robustness of the results: 1) excluding hypertensive disorders in pregnancy; 2) additionally adjusting for other metals (Cd, Hg, As, Tl, Cr, Mn, Ni, and Sb) that have been reported to be associated with GDM in previous studies [3, 23, 24].

The supervised uniform manifold approximation and projection (UMAP) was used for reducing dimension (neighbors = 50, minimum distance = 0.1) and visualizing the metabolic feature among three titanium tertile groups [25]. The UMAP model was performed by "embed" package in R Version 4.1.3. The permutational multivariate analysis of variance (PERMANOVA) test based on the Euclidean distance was used to assess the metabolite features across three titanium tertile groups [26]. The PERMANOVA test was performed using the "vegan" package in R with 10,000 permutations. In the univariate analysis, we conducted linear regression to investigate the associations of maternal titanium exposure with urinary metabolites after adjusting for aforementioned covariates. The Benjamin-Hochberg false discovery rate (FDR) controlling method was used to adjust for multiple comparisons. To map biochemical clusters and facilitate biologic interpretation, we further conducted KEGG pathway enrichment analysis using MetaboAnalyst 5.0 with metabolites remaining significantly related to titanium concentration after FDR adjustment. Further, we

Table 1 Basic characteristics of study population by tertiles of titanium concentration

Basic characteristics	All participants	Tertiles of SG-corrected titanium (μg/L)			*P*
		T1 (≤ 112.35)	T2 (112.35–184.41)	T3 (≥ 184.41)	
Participants, n	1449	483	483	483	
Study center					
Nanjing	1032 (71.2)	355 (73.5)	338 (70.0)	339 (70.2)	0.399
Suzhou	417 (28.8)	128 (26.5)	145 (30.0)	144 (29.8)	
Maternal age at recruitment, years	29.57 ± 3.74	29.49 ± 3.74	29.62 ± 3.79	29.61 ± 3.68	0.827
Pre-pregnancy BMI, kg/m^2	21.21 (2.87)	21.13 (2.72)	21.45 (3.19)	21.05 (2.65)	0.068
< 18.5	214 (14.8)	71 (14.7)	75 (15.5)	68 (14.1)	0.042
18.5–23.9	1014 (70.0)	353 (73.1)	319 (66.0)	342 (70.8)	
24.0–27.9	176 (12.1)	47 (9.7)	66 (13.7)	63 (13.0)	
≥ 28	44 (3.0)	12 (2.5)	23 (4.8)	9 (1.9)	
Missing	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.2)	
Residence area					
Urban/sub-urban	1087 (75.0)	346 (71.6)	372 (77.0)	369 (76.4)	0.107
Rural	362 (25.0)	137 (28.4)	111 (23.0)	114 (23.6)	
Household income, CNY					
< 100000	416 (28.7)	149 (30.8)	138 (28.6)	129 (26.7)	0.457
100000–200000	679 (46.9)	216 (44.7)	221 (45.8)	242 (50.1)	
> 200000	353 (24.4)	118 (24.4)	123 (25.5)	112 (23.2)	
Missing	1 (0.1)	0 (0.0)	1 (0.2)	0 (0.0)	
Maternal education, years					
< 12	131 (9.0)	45 (9.3)	38 (7.9)	48 (9.9)	0.515
≥ 12	1318 (91.0)	438 (90.7)	445 (92.1)	435 (90.1)	
Parity					
0	1135 (78.3)	383 (79.3)	365 (75.6)	387 (80.1)	0.211
≥ 1	313 (21.6)	100 (20.7)	117 (24.2)	96 (19.9)	
Missing	1 (0.1)	0 (0.0)	1 (0.2)	0 (0.0)	
Smoking during pregnancy	12 (0.8)	5 (1.0)	3 (0.6)	4 (0.8)	0.777
Drinking during pregnancy	26 (1.8)	6 (1.2)	5 (1.0)	15 (3.1)	0.028
Hypertensive disorders in pregnancy[a]	48 (3.3)	22 (4.6)	15 (3.1)	11 (2.3)	0.135
75 g oral glucose tolerance test (n = 1360)					
OGTT screening gestational weeks	25.22 ± 0.95	25.24 ± 0.89	25.24 ± 0.93	25.18 ± 1.02	0.633
FBG, mmol/l	4.40 ± 0.43	4.39 ± 0.41	4.38 ± 0.42	4.44 ± 0.46	0.121
1-h PG, mmol/l	7.59 ± 1.62	7.40 ± 1.57	7.68 ± 1.64	7.71 ± 1.62	0.007
1-h PG, mmol/l	6.62 ± 1.36	6.50 ± 1.34	6.66 ± 1.31	6.70 ± 1.42	0.070

GDM gestational diabetes mellitus, *BMI* body mass index, *SD* standard deviation, *CNY* Chinese Yuan, *OGTT* oral glucose tolerance test, *FBG* fasting blood glucose; 1-h PG, oral glucose tolerance test 1-h plasma glucose; 2-h PG, oral glucose tolerance test 2-h plasma glucose. Data presented as mean ± SD or n (%)

[a] Hypertensive disorders in pregnancy includes chronic, gestational hypertension and pre-eclampsia

explored the associations of titanium-related metabolites with GDM and plasma glucose using the robust Poisson regression and multiple linear regression analysis. We presented adjusted RRs of individual metabolites in relation to GDM using forest plots according to the effect size of RRs and significance level of *P* values.

Additionally, we conducted mediation analyses to explore the potential metabolic mediators (both single and multiple) in the associations between maternal titanium exposure and GDM/plasma glucose. The regression-based approach using R package "CMAverse" was conducted [27] to estimate the total effect, direct effect (DE) and indirect effect. The product term of titanium (categorical variable) was served as the independent variable. Non-parametric bootstrapping (1,000 times) was used to estimate 95% CIs and *P* values. The proportion mediated (%) was estimated as NIE/ [NDE + NIE]).

We conducted all statistical analyses using R Software Version 4.1.3 (The R Foundation). The statistical significance was set at $P < 0.05$ for a two-tailed test.

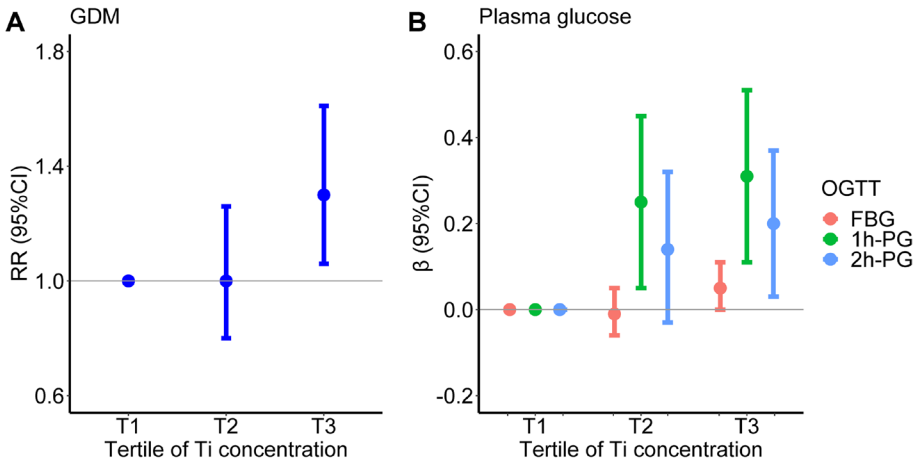


Fig. 1 Associations of titanium tertiles with risk of GDM and plasma glucose concentrations. **A** RR with 95% CI for the risk of GDM; (**B**) β with 95% CI for plasma glucose concentrations in 75 g OGTT. The model was adjusted for study center, maternal age at recruitment, pre-pregnancy BMI, residence area, household income, maternal education, parity. GDM, gestational diabetes mellitus; RR, relative risk; CI, confidential interval. OGTT, oral glucose tolerance test; FPG, fasting blood glucose; 1-h PG, one-hour plasma glucose; 2-h PG, two-hour plasma glucose

Results

Participant characteristics

Baseline characteristics of the study participants by tertiles of titanium were summarized in Table 1. The mean age of the participants at recruitment was approximate 30. The majority of women were nulliparous, lived in urban or sub-urban areas, and received high school education or above.

Among our participants, 25.0% were diagnosed with GDM in their follow-up visits. In the 75 g OGTT, the mean FBG, 1-h PG, and 2-h PG of the participants were 4.40 ± 0.43 mmol/L, 7.59 ± 1.62 mmol/L, and 6.62 ± 1.36 mmol/L, respectively. The median [interquartile range (IQR)] of SG-corrected urinary titanium concentration was 144.70 (93.89, 208.77) μg/L (Table S1). The Spearman correlation coefficient indicated weak correlations between SG-corrected urinary titanium and other metals (Figure S3).

Associations of titanium exposure with risk of GDM and plasma glucose concentrations

As parameterized in tertiles, urinary titanium exposure showed positive dose–response relationship with risk of GDM (*P* for trend across tertiles after the adjustment for conventional covariates = 0.008), with women at the highest tertile of titanium concentration having 30% increased risk of GDM [RR = 1.30 (1.06, 1.61)] when compared to those at the lowest tertile (Fig. 1A, Table S2). Moreover, urinary titanium exposure was significantly associated with post-load glucose levels. Specifically, with the increasing titanium tertile, 1-h PG and 2-h PG displayed an upward trend [1-h PG: β = 0.25 (95% CI: 0.05, 0.45) for the second tertile, β = 0.31 (95% CI: 0.11, 0.51) for the third tertile, *P* for trend = 0.004; 2-h PG: β = 0.14 (95% CI: -0.03, 0.32) for the second tertile; β = 0.20 (95% CI: 0.03, 0.37) for the third tertile, *P* for trend = 0.029]. While the association between titanium and FBG was attenuated, and the dose–response relationship was marginally significant (*P* for trend = 0.042) (Fig. 1B, Table S3).

Stratified analysis demonstrated a significant interaction between maternal pre-pregnancy BMI and titanium exposure on risk of GDM (*P* for interaction = 0.039). Among women who were normal weight or underweight before pregnancy (pre-pregnancy BMI < 24 kg/m^2, N = 1,228), urinary titanium concentration in the highest tertile increased GDM by 35% as compared to the lowest tertile [adjusted RR = 1.35 (95% CI: 1.06, 1.73); *P* for trend = 0.013]. In contrast, null associations were observed among women being overweight/obese before pregnancy (pre-pregnancy BMI ≥ 24 kg/m^2, N = 220) (Table S4). In addition, we did not find maternal age and parity modified the association between urinary titanium concentration and risk of GDM (*P* for interaction = 0.675 and 0.961) (Table S5, Table S6). In the sensitivity analysis, we restricted the analyses in the participants without hypertensive disorders in pregnancy or additional adjusted for metals associated with GDM in previous studies (Cd, Hg, Tl, Cr, Mn, As, Ni and Sb), results remained unchanged (Table S7).

Associations of titanium exposure with individual metabolites

The 640 known metabolites were across 12 classes as follows: 195 organic acids, 153 organoheterocyclics, 78 lipids, 71 organic oxygens, 54 benzenoids, 26 phenylpropanoids, 26 organic nitrogens, 23 nucleosides and

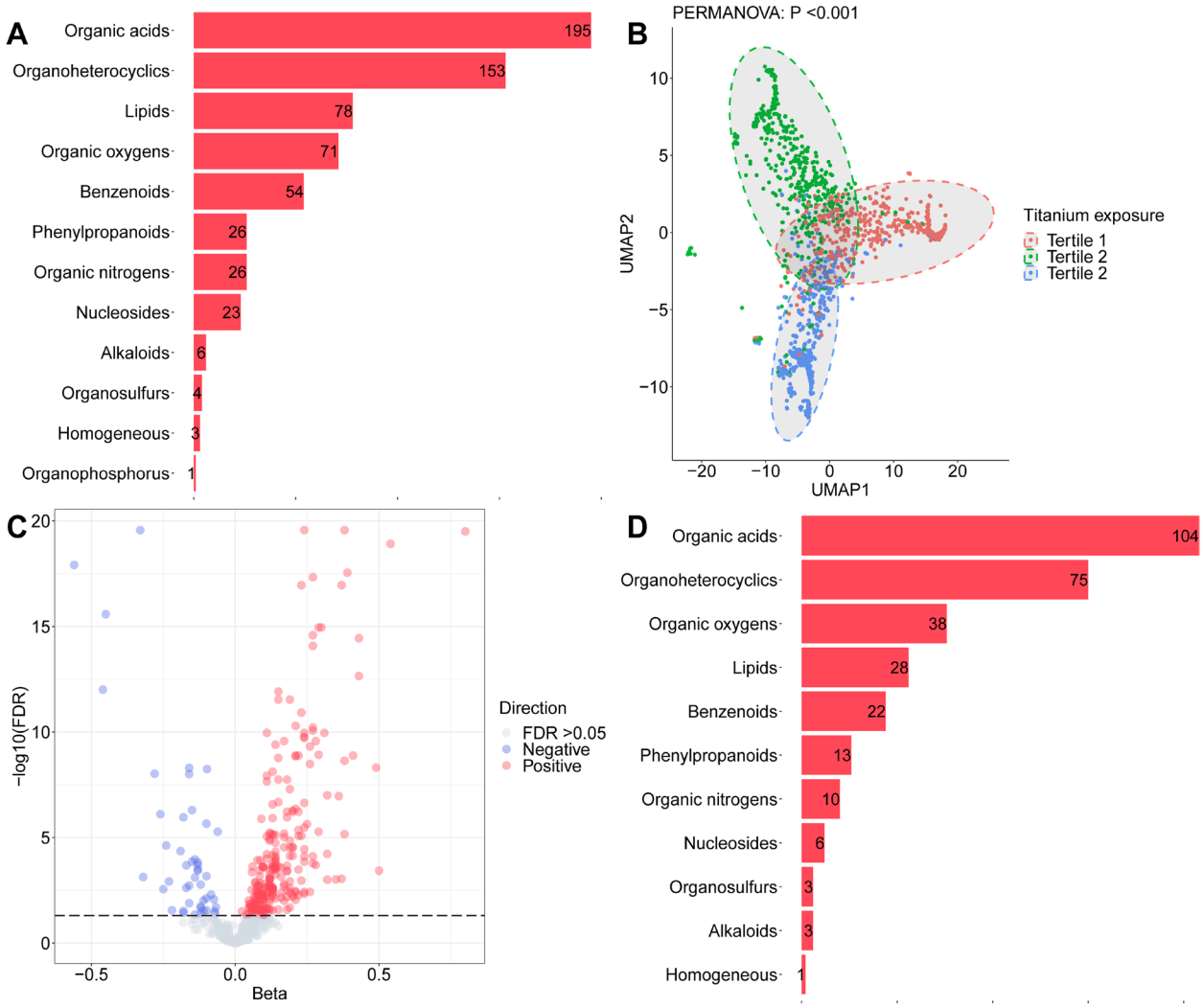


Fig. 2 Summary results for associations of titanium exposure with urinary metabolites. **A** Number of all metabolites per metabolic categories based on annotation in the HMDB database. The x-axis indicates the number of metabolites per category and the y-axis indicates metabolic categories. **B** Supervised Uniform Manifold Approximation and Projection of urinary metabolite profiles across various titanium exposure group. **C** Volcano plot of the associations between maternal urinary SG-corrected titanium concentrations and levels of 640 urinary metabolites. The multivariable linear regression models were adjusted for study center, maternal age at recruitment, pre-pregnancy BMI, residence area, household income, maternal education, parity. The association is considered significant when the FDR-corrected *P* is < 0.05. **D** Number of titanium-related metabolites per metabolic categories based on annotation in the HMDB database. The x-axis indicates the number of metabolites per category and the y-axis indicates metabolic categories

others 4 classes (Fig. 2A). We observed distinct differences in the metabolite profiles across titanium tertiles by PERMANOVA test ($P < 0.001$), suggesting that titanium exposure was in relation to prominent metabolic alterations (Fig. 2B). Univariate associations of titanium exposure with individual metabolites identified a total of 303 significant associations with FDR-corrected *P* for trend < 0.05, among which 256 were up-regulated and 47 down-regulated with increased titanium concentration (Fig. 2C). These 303 titanium-related metabolites spanned 11 classes, including 104 organic acids, 75 organoheterocyclics, 38 organic oxygens, 28 lipids, 22 benzenoids, 13 phenylpropanoids, 10 organic nitrogens, 6 nucleosides, 3 organosulfurs, 3 alkaloids, and 1 homogeneous (Fig. 2D).

Multivariate metabolites enrichment analysis

To facilitate biologic interpretation, we conducted enrichment analysis and identified 4 KEGG pathways predominantly associated with titanium exposure (FDR-corrected $P < 0.05$), including glycine, serine and threonine metabolism ($P = 0.002$), alanine, aspartate and

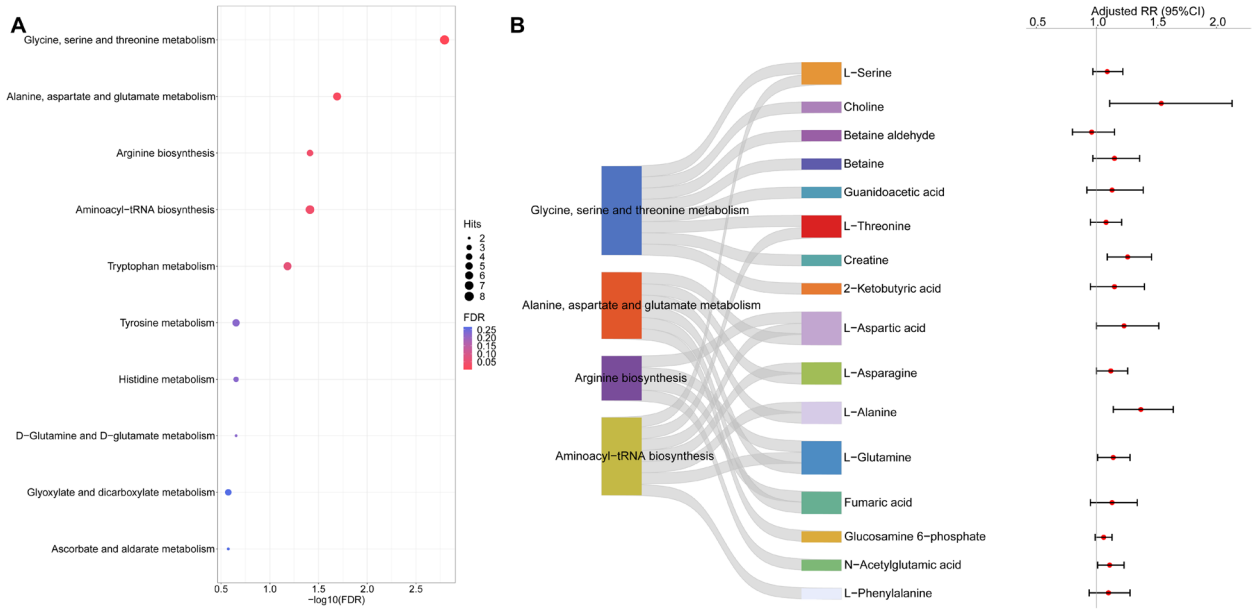


Fig. 3 Pathway enrichment analysis for titanium-related metabolites and the associations of titanium-related metabolites with risk of GDM. **A** Metabolic pathways associated with urinary titanium concentrations. Sizes of circles were mapped to the number of significant metabolites, and color was mapped to the FDR-corrected *P* values. **B** Associations between titanium-related metabolites and risk of GDM. The metabolites were selected from pathway enrichment analysis based on the KEGG database (FDR-corrected $P < 0.05$). The robust Poisson regression models were adjusted for study center, maternal age at recruitment, pre-pregnancy BMI, residence area, household income, maternal education, parity

Table 2 Mediation analyses of titanium-related metabolites on the associations between urinary titanium exposure and risk of GDM

Mediator	Dependent variable	Direct effects		Indirect effects		Proportion mediated	
		RR (95% CI)	*P*	RR (95% CI)	*P*	%	*P*
Choline	GDM	1.23 (1.00, 1.53)	0.050	1.05 (1.01, 1.10)	0.008	21.2%	0.024
Creatine	GDM	1.27 (1.03, 1.57)	0.028	1.03 (1.01, 1.07)	0.002	12.5%	0.016
L-Glutamine	GDM	1.25 (1.01, 1.55)	0.038	1.04 (1.00, 1.11)	0.088	17.5%	0.104
L-Alanine	GDM	1.22 (0.99, 1.52)	0.066	1.06 (1.02, 1.12)	0.006	25.7%	0.022
N-Acetylglutamic acid	GDM	1.29 (1.04, 1.58)	0.020	1.01 (1.00, 1.04)	0.096	5.1%	0.108

GDM gestational diabetes mellitus, *RR* relative risk, *CI* confidential interval. The covariates adjusted for in the mediation analyses included study center, maternal age at recruitment, pre-pregnancy BMI, residence area, household income, maternal education, parity

Table 3 Multiple mediation analyses of titanium-related metabolic pathways on the association between urinary titanium exposure and risk of GDM

Multiple mediator		Direct effects		Indirect effects		Proportion mediated	
Pathway	Hits	RR (95% CI)	*P*	RR (95% CI)	*P*	%	*P*
Glycine, serine and threonine metabolism	8	1.23 (0.99, 1.53)	0.064	1.06 (1.00, 1.14)	0.074	24.9	0.088
Alanine, aspartate and glutamate metabolism	6	1.21 (0.97, 1.50)	0.082	1.08 (1.02, 1.16)	0.010	31.0	0.028
Arginine biosynthesis	4	1.25 (1.01, 1.55)	0.038	1.05 (0.99, 1.12)	0.096	19.6	0.112
Aminoacyl-tRNA biosynthesis	7	1.22 (0.98, 1.51)	0.072	1.07 (1.01, 1.14)	0.014	27.1	0.028

RR relative risk, *CI* confidential interval

The covariates adjusted for in the mediation analyses included study center, maternal age at recruitment, pre-pregnancy BMI, residence area, household income, maternal education, parity

glutamate metabolism ($P=0.020$), Arginine biosynthesis ($P=0.039$), and aminoacyl-tRNA biosynthesis ($P=0.039$) (Fig. 3A and Table S8). The 16 metabolite hits involved in above-mentioned 4 titanium-related pathways were positively associated with titanium concentration (Table S9). Among the 16 metabolites, choline, creatine, L-glutamine, L-alanine, and N-acetylglutamic acid were meanwhile significantly associated with increased risk of GDM with the RRs ranged from 1.11 to 1.54 (Fig. 3B and Table S10). After FDR-adjustment, creatine and L-alanine remained significant associations with increased risk of GDM. Furthermore, urinary creatine was significantly associated with 1-h PG, and L-alanine was associated with 1-h PG and 2-h PG levels after FDR-adjustment (Table S11).

Mediation analyses of titanium-related metabolites in the associations between titanium exposure and risk of GDM

The mediation analyses revealed that choline, creatine, and L-alanine displayed mediating effects on the association between urinary titanium concentrations and GDM risk. The proportions of mediation were recorded as 21.2%, 12.5%, and 25.7% for choline, creatine, and L-alanine, respectively (Table 2). In addition, the mediating effects of creatine and L-alanine on the relationship between titanium exposure and 1-h PG was 8.1% and 16.2%, respectively, and the mediating effect of L-alanine on the relationship between titanium exposure and 2-h PG was 20.3%, respectively (Table S12). The direct and indirect effects, as well as the mediated proportion of the metabolites involved in 4 titanium-related pathways were presented in Table 3. The joint effects of metabolites involved in aminoacyl-tRNA biosynthesis and alanine, aspartate and glutamate metabolism mediated 27.1% and 31.0% of the total effect of titanium exposure on GDM risk, respectively.

Discussion

In this well-characterized prospective study, we provide, to our knowledge, the first population-based epidemiological evidence to date of the associations between titanium exposure in early pregnancy and subsequent risk of GDM. Further, we reported novel findings that perturbation of amino acid metabolism, particularly alanine, aspartate and glutamate metabolism, as well as aminoacyl-tRNA biosynthesis, might underlie the association between titanium exposure and development of GDM. Specifically, three metabolites, choline, creatine and L-alanine, demonstrated predominant mediation effects on the association between titanium exposure and GDM risk.

Humans have been extensively exposed to titanium with the application of the microparticle-sized or nanoparticle-sized titanium dioxide [5]. Recently, the European food safety authority (EFSA) has concluded that titanium dioxide can no longer be considered as safe when used as food additive [28, 29]. Since then, concerns regarding the safety of titanium dioxide consumption have been growing. Previous evidence has suggested that titanium exposure disturbed the glucose homeostasis and increased risk of diabetes [6]. Our findings illustrated that higher exposure to titanium in early pregnancy was related to subsequent increased risk of GDM and elevated post-load blood glucose in mid-pregnancy. Consistently, animal experiments have shown that prenatal exposure to nanoparticle-sized TiO_2 increased fasting blood glucose in pregnant rats [8]. Overall, our findings extended the literature with epidemiological evidence on the association of titanium exposure with GDM. Additionally, we observed that the relationship between titanium and GDM risk was modified by pre-pregnancy BMI with titanium robustly associated with GDM among women being normal weight/underweight before pregnancy. Our findings were in line with some previous data which revealed that the correlation between metals and risk of GDM only existed in pregnant women of pre-pregnancy BMI below 24 kg/m^2 [3, 30]. A probable interpretation is that overweight/obesity before pregnancy has more prominent contribution to the development of GDM, which may mask the effect of titanium exposure [31].

Furthermore, our study illustrated that titanium exposure was related to significant alterations of four amino acid metabolism and signaling pathways (glycine, serine and threonine metabolism; alanine, aspartate and glutamate metabolism; arginine biosynthesis; and aminoacyl-tRNA biosynthesis). Data in animal models has demonstrated that oral intake of nanoparticle-sized TiO_2 impaired amino acid metabolism [32]. Chen et al. [33] has also demonstrated that rats exposed to nanoparticle-sized TiO_2 led to significant changes in gut aminoacyl-tRNA biosynthesis metabolic pathway, which involved the metabolism of many amino acids. Our findings were consistent with a prior cohort study that reported urinary alanine, aspartate and glutamate metabolism, arginine metabolism, as well as glycine and serine metabolism in late pregnancy were significantly and positively associated with titanium exposure in early pregnancy [12].

Amino acid metabolism has been implicated in GDM development starting in early pregnancy in several studies [13, 34, 35]. In the present study, out of the 20 metabolite hits in the titanium-related amino acid metabolic pathways, five (choline, creatine, L-glutamine, L-alanine, and N-acetylglutamic acid) showed positive associations

with GDM risk. Our findings were consistent with a previous prospective test and validation study in the PETALS cohort that higher alanine and glutamine levels in early to mid-pregnancy were associated with increased risk of GDM, and alanine levels in early pregnancy might serve as the predictive metabolic marker for GDM risk [36]. Additionally, in a metabolomics study involving 51 pregnant women, creatine and choline were recognized as potential indicators of GDM development at GW 14–25 [37].

Further, we demonstrated that choline, creatine and L-alanine displayed considerable mediation effects in the association between titanium exposure and GDM risk. Multiple mediation analyses further indicated that the joint mediation effect of metabolites involved in aminoacyl-tRNA biosynthesis as well as alanine, aspartate and glutamate metabolism could explain almost one third of the association. To our knowledge, the mechanisms underlying titanium exposure and metabolic disorders remain unclear. Previous evidence has demonstrated that titanium exposure may induce alterations in gut microbiota [6], which further impact choline and trimethylamine N-oxide (a choline-derived gut-microbiota-dependent metabolite) and finally lead to insulin resistance and disturbance in glucose homeostasis [38]. In addition, circulating amino acids play important roles in glucose homeostasis, insulin activity, inflammation, and oxidative stress, which are pivotal hypotheses regarding the etiology of GDM [39]. For L-alanine, as one of glucogenic amino acids, it regulates gluconeogenesis and glycolysis and plays a critical role in maintaining blood glucose. Our findings suggested the link between titanium exposure, perturbation of amino acid metabolism, and the development of GDM. Nonetheless, additional independent epidemiological validation and mechanistic studies via animal models are warranted.

The primary strength of our research includes its methodologic rigor. The prospective cohort design ensures the accuracy of data collection, including exposures, multiple confounders, outcomes, and the biological processes prior to the onset of the disease, therefore might be able to infer the potential causality of titanium exposure and GDM. Additionally, in the hospital-based cohort, all participants received universal GDM screening and were diagnosed based on the WHO criteria, which largely minimized case–control misclassification and clinical heterogeneity and improved generalizability of our findings. Furthermore, we profiled untargeted metabolomics in early pregnancy, ensuring the metabolomic profiling prior to GDM diagnosis in mid-pregnancy. Our findings introduced novel insights into the metabolic mechanisms linking titanium exposure to the development of GDM. However, potential limitations should be recognized. First, the use of spot urine measurement for titanium may lead to exposure misclassification during early pregnancy. However, given the long biological half-life (320 days) of titanium [40] and the stable lifestyle most pregnant women remained, the urinary titanium concentrations might be able to represent long-term exposures. Second, we lack information regarding the source or particle size distribution of titanium exposure, which might exert distinct health effects. Third, the urinary titanium concentration and metabolic profiles were both measured in early pregnancy. Therefore, we cannot determine the causal effect of titanium exposure on metabolism dysregulation. Functional studies focusing on targeted pathways and metabolites are warranted to shed further light on the mechanisms underlying titanium exposure and the development of GDM. Lastly, the participants of our study were predominantly residents of Jiangsu, China, hence confirmation of our findings in other pregnant populations is warranted.

Conclusions

In summary, our research yields epidemiological evidence that impaired amino acid metabolism induced by higher titanium exposure in early pregnancy are associated with the subsequent development of GDM. Our findings highlight that environmental-level exposure to titanium during pregnancy might have profound effects on maternal health, and suggest potential metabolic markers and pathways involved in the onset of GDM triggered by environmental pollutants. Additional studies to investigate whether these metabolites may serve as early preventive or intervention targets are warranted.

Supplementary Information

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Supplementary Material 1

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Authors' contributions

Y.L., J.D., H.Z. and Y.J. conceptualized the study design. Y.J., T.S. and Y.J. drafted the manuscript. T.S. and J.Y. conducted statistical analysis and designed the figures. Y.D., X.X., C.L., Q.X., X.W., S.X., B.X. and X.H. performed data quality control. Z.H., J.D., H.M. and H.L. supervised data collection. Y.L. revised the manuscript. Z.H., and Y.J. obtained fundings. All authors approved the final version of the manuscript.

Data Availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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