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Original article

Complex patterns of circulating fatty acid levels in gestational diabetes mellitus subclasses across pregnancy

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SUMMARY

Background & aims: To investigate the relationship between maternal serum fatty acid levels and gestational diabetes mellitus (GDM) subtypes across pregnancy.

Methods: A total of 680 singleton mothers enrolled in the Complex Lipids in Mothers and Babies (CLIMB) study in Chongqing, China were included. Clinical information and serum samples were collected at gestational weeks (GWs) 11–14, 22–28, and 32–34. 75 g Oral Glucose Tolerance Test (OGTT) was conducted at GW 24–28 and GDM subtypes divided into three groups using International Association of Diabetes and Pregnancy Study Group (IADPSG) guidelines criteria: elevated fasting plasma glucose (FPG group; n = 59); 1-h and/or 2-h post-load glucose (1h/2h-PG group; n = 94); combined group (FPG&1h/2h-PG group; n = 42). Non-GDM pregnancies were included (n = 485) as controls. Twenty fatty acids were quantified in serum using gas chromatography-mass spectrometry (GC–MS) analysis.

Results: Overall, most serum fatty acid concentrations increased rapidly from the first to second trimester, followed by a plateauing or reduction in the third trimester ($p < 0.001$). In cross sectional analysis, fatty acid concentrations were significantly higher in the FPG group at GW 11–14 and decreased in the 1h/2h-PG group at GW 32–34, relative to controls. Moreover, higher α-linolenic acid (ALA; the second tertile: adjusted odds ratio [aOR] = 2.53, 95% CI: 1.17 to 5.47; the third tertile: aOR = 2.60, 95% CI: 1.20 to 5.65) and docosahexaenoic acid (DHA; the second tertile: aOR = 2.34, 95% CI: 1.10 to 4.97; the third tertile: aOR = 2.16, 95% CI: 1.00 to 4.63) were significantly associated with a higher risk of GDM in women with elevated fasting plasma glucose at GW 11–14 (first tertile as reference).

Conclusions: Our findings highlight the importance of considering GDM subtypes for the individualised management of GDM in pregnancy. ALA and DHA in early pregnancy are associated with a higher risk of FPG-GDM subtype. This has widespread implications when recommending n-3 PUFAs supplementation for women with GDM.

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1. Introduction

In accordance with traditional Chinese culture, women are encouraged to consume animal products more frequently during pregnancy because these foods are a good source of high-quality protein, fat, and micronutrients [1]. In combination with increasing economic development and urbanisation, this has seen a shift in pregnancy dietary patterns, with greater consumption of animal derived products in recent decades [2,3]. Physiologic adaptations seen in pregnancy include a degree of insulin resistance and a modest increase in blood lipid concentrations (physiologic hyperlipidemia). These metabolic alterations are required to fulfil higher energy demand in order to supply adequate nutrient for intrauterine growth and development [4,5]. Indeed, n-3 polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA), play an essential role in the support the development of the brain and central nervous system of a fetus [6,7].

Gestational diabetes mellitus (GDM), characterised by any poor glucose regulation during pregnancy [8], affects between 12.8% and 16.7% of pregnant women in China [9]. GDM is associated with increased risks of short- and long-term complications for both the mother and the offspring, such as type 2 diabetes mellitus (T2DM) and obesity later in life [10]. Indeed, women with a history of GDM appear to have a nearly 10-fold higher risk of developing T2DM than those with a normoglycemic pregnancy [11]. Fluctuating fatty acid (FA) levels have been linked to insulin resistance and β-cell dysfunction in type 2 diabetes mellitus (T2DM) [12], and have been widely investigated in women with GDM [13–16]. A systematic review and meta-analysis of twelve studies assessed a total of 2426 individuals (507 women with GDM and 1919 controls) found elevated FA levels in women with GDM [17]. However, previous studies have considered GDM status as a dichotomous outcome, despite evidence suggesting that GDM may present as distinct clinical subtypes. For example, different subtypes have been associated with different risks for a range of pregnancy complications and adverse outcomes, including needing insulin therapy, hypertension and pre-eclampsia, preterm birth, caesarean delivery, neonatal hypoglycemia and hyperbilirubinemia, and being born large for gestational age (LGA) and macrosomia [18–22]. Furthermore, GDM defined by elevated fasting plasma glucose (FPG) appears to be a stronger predictor of LGA infants than elevated post-load glucose (PG), while elevated PG in GDM pregnancies is more likely to result in preterm birth [18,20,21]. As for prediabetes and T2DM, numerous studies have shown fasting and post-load glycaemia manifesting as different metabolic and pathophysiological mechanisms that differ in insulin target tissues (such as liver, adipose tissue, and muscle) and β-cell responses [23,24]. Although previous studies have indicated different pathophysiologies for individual oral glucose tolerance test (OGTT) time points, no specific study has examined the potential link between fatty acid levels and specific subtypes of GDM, classified according to elevated glucose levels at baseline (fasting), 1 h and/or 2 h post OGTT (e.g. FPG, PG, and combined).

To address this, we prospectively 1) characterised the longitudinal profiles of serum fatty acids across trimesters among GDM subtypes and 2) examined the associations of individual serum fatty acids in early to mid-pregnancy with subsequent risk of different GDM subtypes.

2. Methods

2.1. Study participants

This study uses data from the Complex Lipids in Mothers and Babies (CLIMB) study, conducted at the First Affiliated Hospital of Chongqing Medical University and Chongqing Health Centre for Women and Children in China from September 2015 to June 2017. The details of the CLIMB study have been published previously [25]. All procedures performed in this study were in accordance with the principles in the Declaration of Helsinki 1964 and the International Conference on Harmonisation Good Clinical Practice E6 (ICH-GCP). The study was approved by the Ethics Committee of Chongqing Medical University (2014034). Written informed consent was obtained from all participants included in the study at enrolment.

Of the 1500 women recruited into the CLIMB study, 750 were randomly selected for fatty acids analysis at three different timepoint (the 11–14th, 22–28th, and 32–34th weeks of gestation). We excluded women lost to follow up (n = 24), those who had pre-existing diabetes mellitus (n = 1), those who had no OGTT data (n = 19) and those who had pregnancy-induced hypertension, preeclampsia, or preterm birth (n = 26). This resulted in 680 women who were eligible for inclusion in the study.

2.2. Categorisation of four GDM groups based on OGTT glucose data

All participants underwent a 75-g 2-h OGTT between 24 and 28 weeks following at least 8 h of fasting. The diagnosis of GDM was based on the criteria recommended by the International Association of Diabetes and Pregnancy Study Group (IADPSG) of one or more abnormal glucose levels (i.e., fasting, 1 h, or 2 h plasma glucose concentrations $\geq$ 5.1, 10.0, or 8.5 mmol/L, respectively) [26]. Subgroups were generated for subsequent analyses in light of previous studies indicating that different abnormal glucose levels at different OGTT time points have disparate effects on pregnancy outcomes [18–22]. Four mutually exclusive categories, were defined: 1) Non-GDM group (no elevated glucose levels, n = 485); 2) FPG group (isolated elevated glucose level at fasting with normal glucose levels at 1 h and 2 h, n = 59); 3) 1h/2h-PG group (elevated glucose levels at either 1 h and/or 2 h with normal fasting glucose, n = 94); and 4) FPG&1h/2h-PG group (elevated glucose levels at fasting and either 1 h or 2 h, n = 42). It is important to note that the determination of GDM subgroup type was based on OGGT measurements in the second trimester and these groups were used to study the longitudinal profiles of fatty acids throughout pregnancy. The detailed definition for each group is described in Table 1 and a flowchart of the study participants is shown in Fig. 1.

2.3. Clinical information and sample collection

The clinical information and sample collection have been fully described previously [27]. Briefly, clinical information such as demographic factors (maternal age, education, last menstrual period (LMP), gravidity) were collected at enrolment. Maternal anthropometry (body mass index (BMI) and blood pressure (BP)) and maternal blood samples were collected at three visits during pregnancy (11–14, 22–28, and 32–34 gestational weeks) by trained nurses. The prepared serum samples were aliquoted and stored at −80 °C until assayed.

2.4. Fatty acids analysis and quantification

We measured the serum concentration of 20 fatty acids by gas chromatography-mass spectrometry (GC–MS) (Agilent 5977A mass spectrometer/7890B gas chromatography, Agilent Technologies, USA) analysis described previously [27]. Briefly, an internal standard, heptadecanoic acid (C17:0), was added to each sample before the extraction of fatty acids. The fatty acid separation was performed on a DB-23 capillary column (20 m × 0.18 mm × 0.20 μm, Agilent Technologies, USA). The chromatographic peak height of each of the fatty acids was extracted using Agilent ChemStation software

Table 1
The detailed definition for each participant group.

Group	Definition
Non-GDM	Normal FPG and normal 1 h and 2 h glucose
FPG	FPG levels ≥5.1 mmol/L and normal 1 h and 2 h glucose
1h/2h-PG	1-h glucose ≥10.0 mmol/L and/or 2 h glucose ≥8.5 mmol/L and normal FPG
FPG&1h/2h-PG	FPG levels ≥5.1 mmol/L and 1 h glucose ≥10.0 mmol/L and/or 2 h glucose ≥8.5 mmol/L

Abbreviation: GDM: gestational diabetes mellitus, FPG: fasting plasma glucose, PG: post-load glucose.

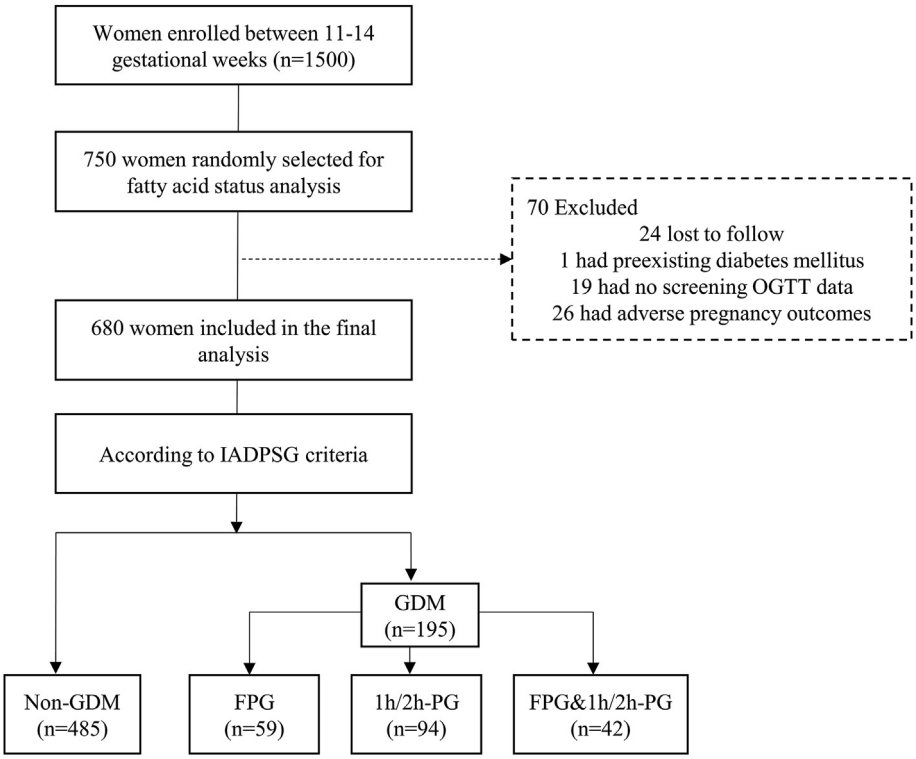

Fig. 1. Flowchart of study participants. OGTT: oral glucose tolerance test; IADPSG: International Association of Diabetic Pregnancy Study Group; GDM: gestational diabetes mellitus; FPG: fasting plasma glucose; 1h/2h-PG: 1 h and/or 2 h post-load plasma glucose.

(version 2.6). Levels of individual serum fatty acids were first normalised using the internal standard and then quantified to absolute concentrations using calibration curves derived from the corresponding chemical standard.

2.5. Statistical analysis

For maternal clinical characteristics, non-parametric Kruskal–Wallis with Bonferroni test for post-hoc analysis was used for comparisons of continuous variables between the four groups. Chi-square or Fisher's exact test was used for pairwise comparisons of categorical variables, as appropriate. Repeated measures ANOVA was applied to assess the longitudinal trends of serum fatty acid concentrations from the first trimester to the third trimester across GDM subtypes and the non-GDM group. Post hoc multiple-comparison adjustment for p-value (q-value) was performed using the Benjamini-Hochberg false-discovery rate (FDR)-controlling method [28]. For associations between fatty acids and GDM subtypes, multivariable logistic regression models were conducted to estimate odds ratios (OR) and their 95% confidence intervals (CI) across the four groups. The levels of fatty acids were analysed as continuous variables and categorical variables based on the tertile distributions, and the first tertile was used as the reference group. Potential confounding variables including maternal age, primiparity, maternal educational level, diastolic BP (dBP) (only adjusted at the second trimester), and BMI at each trimester were adjusted in multivariable logistic regression models. The area under the receiver operating characteristic (ROC) curve was analysed using the pROC R-package [29]. A p-value <0.05 or adjusted p-value (q-value) < 0.05 were considered statistically significant. Data were described as mean ± SD or median with interquartile range (IQR) for continuous variables, or as proportions for categorical variables. Heatmaps were illustrated using ggplot2 R-based packages [30], and forest plots were illustrated using GraphPad prism 8.0 software (GraphPad Co. Ltd., USA).

3. Results

3.1. Clinical characteristics

Clinical characteristics for the study population are shown in Table 2. The fasting, 1 h, and 2 h plasma glucose levels following an OGTT were significantly different between non-GDM and three GDM subtypes ($p < 0.001$). For other demographic factors including maternal age ($p < 0.001$), maternal educational level ($p = 0.005$), primiparity ($p = 0.019$), BMI at each trimester ($p < 0.001$), and dBP

Table 2
Clinical characteristics of the study participants (n = 680).

	Non-GDM	GDM subtypes according to IADPSG thresholds			*P* Value
		FPG	1h/2h-PG	FPG&1h/2h-PG	
All (n = 680)	485	59	94	42	
Age, years	28 (26, 30)[c]	28 (26, 31)	30 (28, 32)[a]	29 (26.3, 33)	**<0.001***
Education, years	16 (15, 16)	16 (15, 16)	16 (15, 16)[d]	15 (15, 16)[c]	**0.005***
Nationality (% Han)					0.454
Yes	472 (97.32)	59 (100.00)	92 (97.87)	40 (95.24)	
No	13 (2.68)	0 (0.00)	2 (2.13)	2 (4.76)	
Marital status (% married)					1.000
Yes	477 (98.35)	58 (98.31)	93 (98.94)	42 (100.00)	
No	8 (1.65)	1 (1.69)	1 (1.06)	0 (0.00)	
Primiparity (%)					**0.019***
Yes	388 (80.00)	41 (69.49)	64 (68.09)	29 (69.05)	
No	97 (20.00)	18 (30.51)	30 (31.91)	13 (30.95)	
Smoking or drinking during pregnancy (%)					1.000
Yes	481 (99.18)	59 (100.00)	94 (100.00)	42 (100.00)	
No	4 (0.82)	0 (0.00)	0 (0.00)	0 (0.00)	
BMI (kg/m^2)					
1st trimester	20.8 (19.3, 22.7)[d]	21.8 (19.8, 24.0)[d]	21.5 (19.8, 23.0)[d]	23.4 (22.4, 25.6)[a,b,c]	**<0.001***
2nd trimester	22.9 (21.3, 25.1)[d]	23.3 (21.8, 25.5)[d]	23.5 (21.9, 25.9)[d]	26.0 (24.1, 28.0)[a,b,c]	**<0.001***
3rd trimester	24.6 (22.6, 26.9)[d]	24.5 (23.3, 26.8)[d]	25.6 (23.4, 27.8)[d]	27.2 (26.0, 29.6)[a,b,c]	**<0.001***
sBP (mmHg)					
1st trimester	111 (106, 120)	113 (108 120)	112 (106, 119)	115 (109, 121)	0.120
2nd trimester	115 (108, 121)	115 (110, 122)	113 (109, 119)	118 (112, 126)	0.280
3rd trimester	115 (108, 120)	118 (111, 121)	117 (110, 121)	120 (111, 128)	0.113
dBP (mmHg)					
1st trimester	70 (65, 76)	71 (66, 78)	70 (66, 76)	73 (65, 80)	0.579
2nd trimester	70 (68, 74)[d]	70 (68, 74)	69 (67, 75)	70 (68, 78)[a]	**0.032***
3rd trimester	69 (67, 75)	71 (69, 77)	71 (68, 76)	72 (68, 76)	0.289
GA at sampling (weeks)					
1st trimester	12.7 (12.1, 13.3)	12.7 (12.3, 13.3)	12.7 (12.1, 13.7)	12.7 (12.3, 13.4)	0.766
2nd trimester	24.1 (23.7, 24.7)	24.3 (23.9, 24.7)	24.1 (23.6, 24.7)	24.1 (23.6, 24.5)	0.474
3rd trimester	32.3 (31.9, 32.7)	32.1 (31.5, 32.6)	32.3 (31.9, 32.9)	32.6 (32.0, 33.1)	0.121
OGTT values (mmol/L)					
fasting	4.6 (4.4, 4.8)[b,c,d]	5.2 (5.1, 5.3)[a,c]	4.7 (4.5, 4.9)[a,b,d]	5.3 (5.1, 5.5)[a,c]	**<0.001***
1 h	7.3 (6.4, 8.2)[b,c,d]	8.0 (7.1, 9.0)[a,c,d]	10.1 (9.4, 10.8)[a,b]	10.4 (9.8, 11.7)[a,b]	**<0.001***
2 h	6.7 (5.9, 7.3)[b,c,d]	7.3 (6.6, 7.7)[a,c,d]	8.8 (8.4, 9.4)[a,b]	9.0 (8.6, 9.5)[a,b]	**<0.001***

GDM: gestational diabetes mellitus, IADPSG: International Association of Diabetes and Pregnancy Study Groups, FPG: fasting plasma glucose, PG: post-load glucose. BMI: body mass index, BP: blood pressure. Data are median (25th percentile, 75th percentile) or n (%), $^*p < 0.05$. *P* values based on $\chi 2$ or Fisher's exact test were used for pairwise comparisons of proportions, Kruskal–Wallis with Bonferroni test for post-hoc analysis was used for comparisons of continuous variables between the four groups.

[a] Significantly different from the Non-GDM.
[b] Significantly different from the FPG.
[c] Significantly different from the 1h/2h-PG.
[d] Significantly different from the FPG&1h/2h-PG.

at second trimester ($p = 0.032$) at least one of the four groups was significantly different. Women in the 1h/2 h-PG group had significantly higher maternal age than those who were non-GDM; maternal educational level was significantly lower in the FPG&1h/2h-PG group than in the 1h/2h-PG group; BMI at each trimester was significantly higher in the FPG&1h/2h-PG group compared to those with non-GDM, FPG or 1h/2h-PG group; women in the FPG&1h/2h-PG group had a significantly higher dBP at second trimester than those in the non-GDM group. There were no significant differences among these four groups with respect to nationality, marital status, history of abortion, smoking or alcohol consumption during pregnancy, systolic blood pressure, or gestational age at sampling. Similarly, little evidence for differences in adverse pregnancy outcomes were observed among different GDM groups except for weak evidence of reduced low birth weight in GDM groups relative to controls, as shown in Supplementary Table S1.

3.2. Longitudinal serum fatty acids profile across trimesters

A total of 20 fatty acids were quantified in first, second, and third trimester serum samples, as shown in Fig. 2. Overall, the longitudinal profile of all fatty acids was complex, (Supplementary Excel Sheet S1, $p < 0.001$), with several distinct variation in FA levels both within the non-GDM group and between GDM subgroups over time. Group 1 (Fig. 2A) showed a consistent rise across pregnancy from first to third trimester, irrespective of GDM status. This included hexadecanoic acid, octadecenoic acid, octadecanoic acid, eicosenoic acid, and tetracosenoic acid. Group 2 (Fig. 2B) also showed increasing levels across pregnancy in non-GDM pregnancies, with some evidence of variation in association with GDM subtype. For example, third trimester ALA and tetradecanoic acid were higher specifically in the FPG group relative to non-GDM pregnancies, whereas third trimester eicosadienoic acid was lower in FPG and 1h/2h-PG groups relative to non-GDM or FPG groups. Hexadecenoic and arachidonic acids were higher in FPG&1h/2h-PG pregnancies specifically in the second trimester.

Fluctuations in fatty acid concentrations were also observed from the second to third trimester in association with GDM status: γ-linolenic acid and eicosatrienoic acid reduced in three GDM subtype groups from second to third trimester, but not in the non-GDM group; ALA, arachidonic acid, and DHA were reduced only in FPG&1h/2h-PG group, but increased in the other three groups; Lastly, eicosapentaenoic acid (EPA) were reduced in both FPG and FPG&1h/2h-PG groups but elevated in 1h/2h-PG group from the second to third trimester. The most striking differences between groups were seen in FPG&1h/2h-

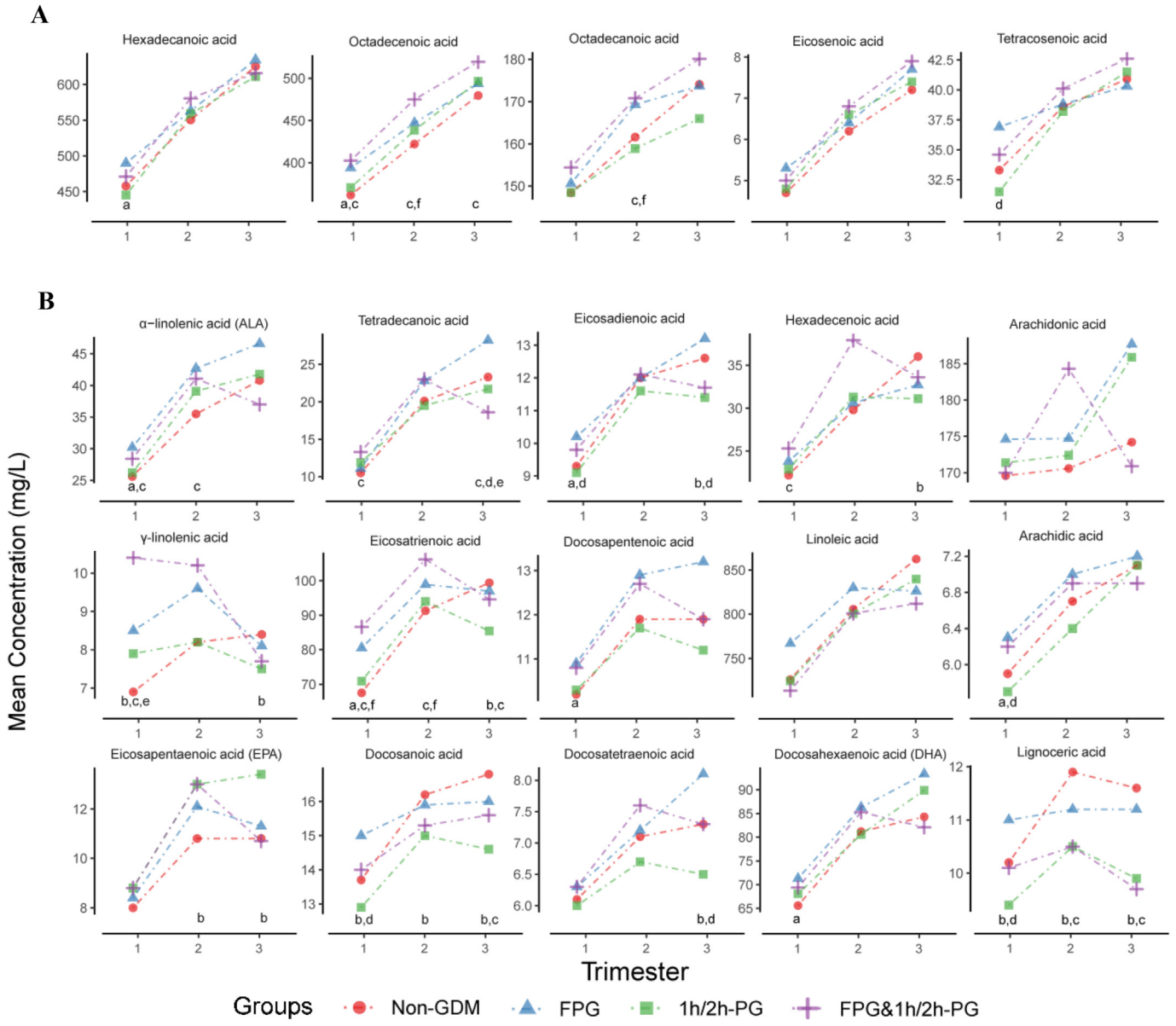

Fig. 2. Longitudinal mean concentrations of serum fatty acids in the first, second, and third trimesters collected from non-GDM pregnancies and pregnancies with GDM subtype groups (FPG, 1h/2h-PG and FPG&1h/2h-PG). Red colours indicate non-GDM, blue colours indicate the FPG group, green colours indicate the 1h/2h-PG group, and purple colours indicate the FPG&1h/2h-PG group. GDM: gestational diabetes mellitus, FPG: fasting plasma glucose, PG: post-load glucose. Significant differences for fatty acids between each group (p-values < 0.05): a: significantly different between Non-GDM vs. FPG; b: significantly different between Non-GDM vs. 1h/2h-PG; c: significantly different between Non-GDM vs. FPG&1h/2h-PG; d: significantly different between FPG vs. 1h/2h-PG; e: significantly different between FPG vs. FPG&1h/2h-PG; f: significantly different between 1h/2h-PG vs. FPG&1h/2h-PG. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PG pregnancies which showed decreasing ALA, tetradecanoic, eicosadienoic, eicoatrienoic, hexadecenoic and arachidonic acid levels between the second and third trimesters.

3.3. Pairwise comparisons of serum fatty acid concentrations between non-GDM and GDM subtypes

Differences in fatty acids detected among the four GDM groups at each trimester are shown in Fig. 3, and the results of the six-pairwise comparisons are presented in Supplementary Table S2. Concentrations of a total of 14, 7, and 10 fatty acids were found to be significantly altered between groups in the first, second, and third trimesters, respectively (Supplementary Table S2). Furthermore, after post hoc FDR correction (q < 0.05): the majority of significantly elevated fatty acids (eicosatrienoic acid, γ-linolenic acid, ALA, and hexadecenoic acid) were detected in the GDM subtypes especially in the FPG or FPG&1h/2h-PG group in the first trimester, while all the fatty acids showing significantly decreased concentrations (γ-linolenic acid, hexadecenoic acid, eicosadienoic acid, docosatetraenoic acid, docosanoic acid, and lignoceric acid) were only found in the 1 h/2 h-PG group in the third trimester (Fig. 3). Specifically, compared to non-GDM controls, women in the FPG group had significantly higher levels of the n-3 PUFAs (ALA and DHA) in the first trimester although the significance of DHA did not persist after post hoc FDR correction. On the other hand, women in the 1h/2h-PG group had significantly higher levels of EPA in the third trimester compared to non-GDM.

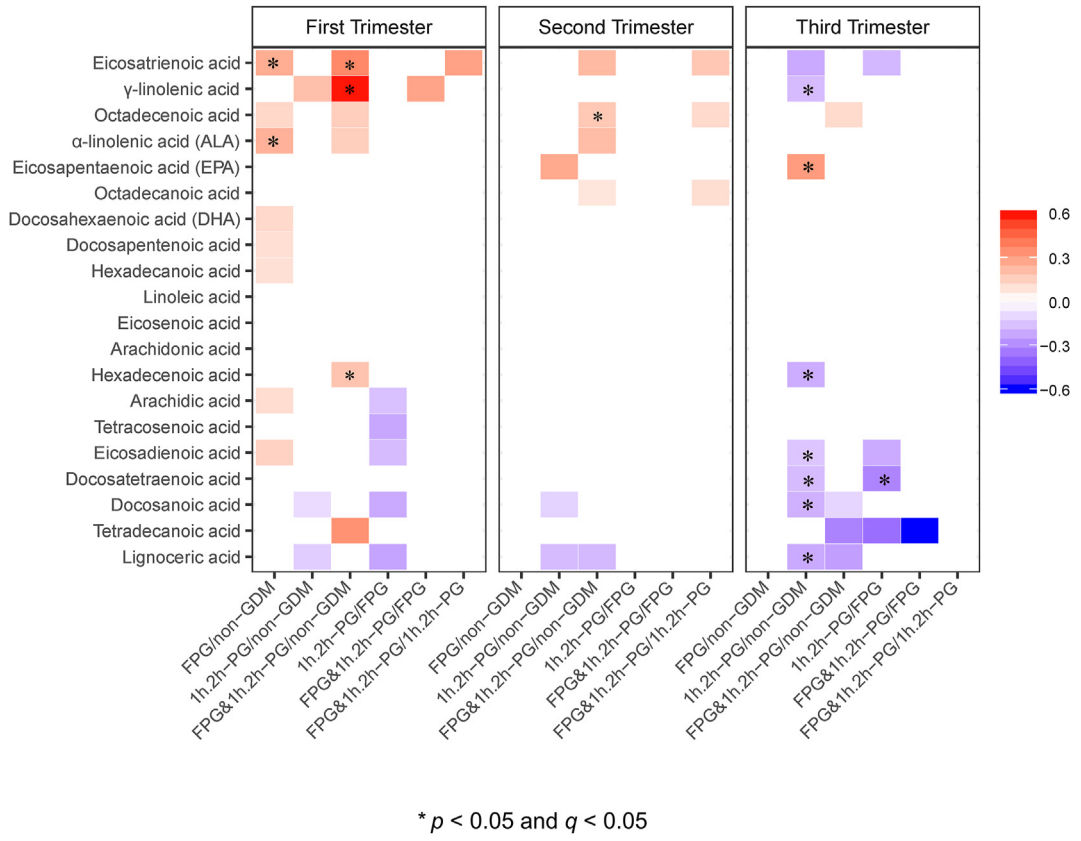

Fig. 3. Heat map of the fatty acids detected in each participant group showing the ratio of fatty acid levels among the four groups. Red colours represent higher fatty acid concentrations in dividend groups than the divisor groups, while blue colours indicate lower fatty acid levels in dividend groups than the divisor groups. The relative concentration of fatty acids was plotted using a log2 scale, only the significant fatty acids with *p*-values less than 0.05 are shown, and *q*-values less than 0.05 are labelled with *. GDM: gestational diabetes mellitus, FPG: fasting plasma glucose, PG: post-load glucose. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4. The adjusted odds ratios (OR) between each fatty acid concentration and risks of GDM subtypes in the first and second trimester

We examined associations between maternal serum fatty acids and risks of GDM subtypes as continuous variables and they are presented in Supplementary Table S3. After adjusting for potential confounding factors, the significance ($p < 0.05$ and $q < 0.05$) of aOR only observed in the first trimester (Fig. 4A). Comparing to non-GDM women, higher concentrations of the four fatty acids (ALA: aOR = 1.03, 95% CI: 1.01 to 1.04; eicosenoic acid: aOR = 1.11, 95% CI: 1.02 to 1.21; arachidic acid: aOR = 1.22, 95% CI: 1.06 to 1.40; and DHA: aOR = 1.01, 95% CI: 1.00 to 1.02) were associated with higher risk of GDM in the FPG group (Fig. 4A and Supplementary Table S3). We further analysed these fatty acid levels as categorical variables and found that ALA and DHA displayed promising ORs ($p < 0.05$): the adjusted ORs (95% CIs) of FPG-GDM subtype risk for the second and third tertiles for ALA were 2.53 (95% CI: 1.17 to 5.47) and 2.60 (95% CI: 1.20 to 5.65) respectively; the adjusted ORs (95% CIs) of FPG-GDM subtype risk for the second and third tertiles for DHA were 2.34 (95% CI: 1.10 to 4.97) and 2.16 (95% CI: 1.00 to 4.63) compared to the first tertile as reference (Fig. 4B and Supplementary Excel Sheet S2).

4. Discussion

To our knowledge, this is the first longitudinal study to assess the profiles of serum fatty acids between GDM subtypes and non-GDM women, in addition to testing their associations of their levels early in pregnancy with subsequent risks of different GDM subtypes. All fatty acids rapidly increase as the pregnancy progresses from first to the second trimester, followed by a wide variation in patterns observed in the third trimester. Of particular interest, higher levels of two diet-derived n-3 PUFAs fatty acids (ALA and DHA) were associated with a higher risk of GDM subtype in early pregnancy, which may be potentially modifiable risk factors for GDM in women with elevated fasting plasma glucose.

4.1. Changes in fatty acid profile throughout gestation

The initial elevation of fatty acid levels could be explained by physiologic adaptations of hyperlipidemia and insulin resistance in early pregnancy. During this time, serum levels of lipids such as triglycerides and fatty acids are induced by progesterone, estrogen, and lactogen, which in turn act as precursors and energy supply for fetal growth [4,5]. This pattern of insulin resistance reaches its maximum in the second trimester via a placental hormonal mechanism such as elevated placental lactogen, resulting in the uptake of free fatty acids by insulin target organs being reduced [17]. In the later period of pregnancy, the reduced circulation of fatty acids can be accounted for hypervolemia (dilution of circulating fatty acids in healthy pregnancy) in the third trimester [31]. Further reductions in fatty acid concentrations in GDM subtypes might occur due to the dysregulation of lipid metabolism influenced by GDM, despite conditions of satisfactory glycaemic control [32]. Additionally, a 40% decrease in insulin sensitivity was reported in women with GDM in comparison with a pregnant control group

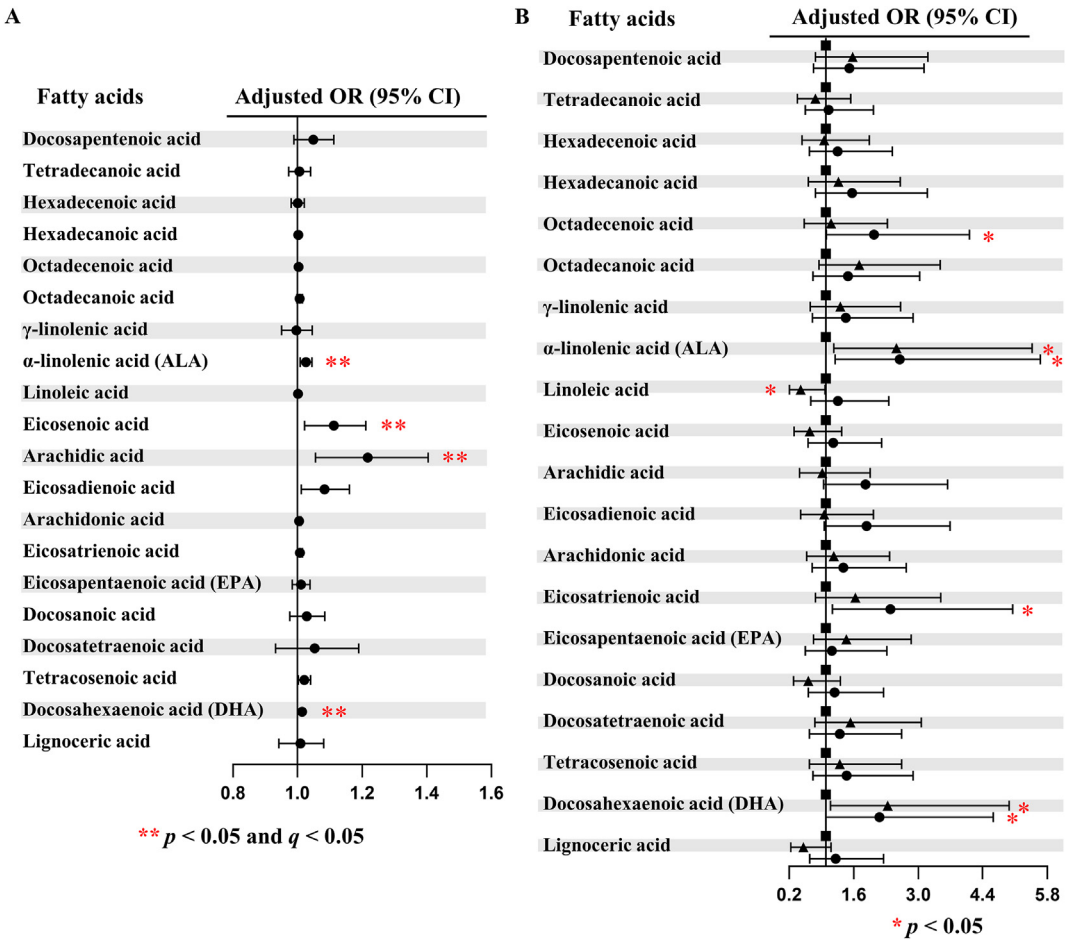

Fig. 4. The significance of associations between fatty acid concentrations and the risk of the FPG-GDM subtype in the first trimesters. (A) Adjusted ORs (95% CIs) for the risk of the FPG-GDM subtype using fatty acids variations as continuous variables. Circles indicate adjusted ORs which were estimated for the non-GDM vs. the FPG group. Error bars indicate 95% confidence intervals (CI). Red asterisks (**) indicate both *p*- and *q*-values less than 0.05. (B) Adjusted ORs (95% CIs) for the risk of the FPG-GDM subtype using fatty acids variations as categorical variables. Squares represent the first tertile of fatty acids (as the reference). Triangles and circles represent the estimated ORs of the second vs. first tertile and the third vs. first tertile, respectively. Error bars indicate 95% confidence intervals (CI). Red asterisks (*) indicate *p*-values less than 0.05. Models were adjusted for maternal age, primiparity, maternal educational level, and BMI at each trimester. GDM: gestational diabetes mellitus, FPG: fasting plasma glucose, PG: post-load glucose. Numerical estimations are presented in Supplementary Table S3 and Supplementary Excel Sheet S2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in late pregnancy [33], and the decreased insulin sensitivity was associated with reduced concentrations of PUFAs [34]. Therefore, GDM subtypes could potentially reduce specific fatty acid concentrations in the third trimester.

4.2. Fatty acids and the risk of different GDM subtypes

Among the GDM subtypes, the highest risk being in the of FPG group was associated with elevated concentrations of ALA and DHA in the first trimester. Despite the fact that no previous studies have explored the association between fatty acids and different GDM subtypes, our findings are biologically plausible. Chen *et al.* [35] investigated the relationship of eleven individual maternal FFA with insulin resistance and insulin secretion at 15.8 weeks of gestation in 1368 pregnant women (81 women with GDM and 1287 controls). They found that DHA was positively associated with HOMA-IR and C-peptide (indicated insulin secretion) those were positively associated with a two-to four-fold increased risk for developing GDM. Previous studies also showed that elevated FFA levels may cause peripheral (muscle) and hepatic insulin resistance [36,37]. Furthermore, a recent nested case–control study conducted in China at gestation week of 13.2 in 610 pregnant women (including 305 GDM cases and 305 controls) reported somewhat similar result in that total n-3 PUFAs showed significant elevation of OR in GDM women [13]. However, contradictory results for the role of individual fatty acids in GDM risk have been reported in other studies [13–16]. For instance, Zhu *et al.* [15,16] assessed individual plasma fatty acids in early to mid-pregnancy (gestational weeks of 10–14 and 15–26) in 321 pregnant women (107 GDM cases and 214 non-GDM controls) demonstrated that γ-linolenic acid and eicosatrienoic acid (dihomo-γ-linolenic acid) concentrations were positively correlated with GDM, while hexadecanoic acid (palmitic acid) were associated with reduced risks of GDM. Li *et al.* and Huang *et al.* [13,14] reported that γ-linolenic acid and EPA levels resulted in significant elevation of ORs in the GDM group, whereas linolenic acid, arachidic acid, docosanoic acid (behenic acid), and lignoceric acid were associated with attenuated ORs in the GDM group. The inconsistency between our findings and previous studies might be explained by the insufficient consideration of the different physiologies of GDM subtypes in previous findings. Future investigations into the role of individual fatty acids in GDM subtype risk are warranted.

4.3. Warrant for the usage of n–3 PUFAs in GDM

In the present study, we found that two n-3 PUFAs (ALA and DHA) were associated with an increased risk of GDM subtype with

elevated FPG. The World Health Organization recommends an intake of 300 mg of n-3 PUFAs per day in pregnant women [38], and in China, the 2016 Dietary Guidelines for Women and Children recommends that pregnant women should consume deep-sea fish, which contain a high level of n-3 PUFAs, about 2–3 times per week [39]. While the importance of an appropriate intake of n-3 PUFAs for fetal neurodevelopment is well-established, there is evidence for impaired placental uptake of maternal fatty acids in GDM pregnancies [40,41]. Mechanisms involved in the dysregulation of omega-3 levels and altered placental transfer in the risk of GDM are not completely known. Previous randomised controlled trials have also failed to demonstrate any benefit of consuming n-3 PUFAs on the incidence of GDM [42,43]. Moreover, a meta-analysis including 2064 who consumed fish oil and 2053 women who did not, showed that fish oil supplementation was not related to a reduced risk of GDM [44]. In addition, a systematic review and meta-analysis of randomised controlled trials assessed effects of n-3 PUFA supplementation in individuals with T2DM, including trials randomising 121,070 participants in 83 trials, also concluding that n-3 PUFAs seem to have little improvement or no effect on T2DM symptoms [45]. Thus, the evidence from human intervention trials does not support the role of n-3 PUFAs in preventing diabetes or improving insulin sensitivity. In combination with our data, these outcomes suggest caution may be warranted in monitoring GDM risk following ALA or DHA supplementation during pregnancy, especially in diabetic and obese women prone to a higher risk of GDM.

4.4. Strengths and limitations

The present study has several strengths. It is the first prospective cohort study to investigate the serum fatty acid profiles across three GDM subtypes and non-GDM women. Longitudinal data were collected throughout pregnancy that allowed temporal examination of fatty acid variations. Further, the levels of individual fatty acids were measured and expressed as absolute concentrations, not semi-quantifications. However, several limitations also merit discussion. Firstly, maternal dietary intake data was not included to evaluate how maternal diets influence serum fatty acid levels. Secondly, all our subjects diagnosed with GDM were managed with dietary counselling and/or treated with insulin, which may interfere with the serum fatty acid outcomes in the third trimester. However, this would not interfere with the associations between fatty acids and risks of GDM subtypes in the first and second trimester. Thirdly, our study did not account the effect of total triglyceride (TG) levels on serum fatty acid concentrations by adjusting for TG levels in each analysis or by reporting fatty acids as a percent of total. Lastly, although we carefully adjusted for several potential confounders, we cannot fully rule out the possibility of residual confounding by other unmeasured factors. Further investigation is warranted into whether relative fatty acid composition, in addition to the absolute concentrations investigated in this study, is important in the risk and/or pathophysiology of GDM subtypes, as well as follow up studies on different postpartum outcomes in these subgroups and examining the effect of maternal FAs on the childrens' neurodevelopment.

5. Conclusions

In conclusion, various characteristics of free fatty acids are associated with fasting, post-load, and combined plasma glucose levels. A careful reconsideration of GDM with individualised management according to three GDM subtypes is warranted. ALA and DHA seem to be associated with a higher risk of developing the FPG-GDM subtype in later pregnancy, and it would be worthwhile emphasising caution for n-3 PUFA supplementation during pregnancy for GDM women with GDM.

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Author contributions

T.Z. contributed to data collection, interpreted the results, and wrote the manuscript. W.R.J. contributed to interpreted the results and wrote the manuscript. Y.Y.X. contributed to data collection and interpreted the results. T.Z. and T.L.H. performed the statistical analysis. R.S, R.D.C, J.D.S, Z.Z., G.X. and P.B. revised the manuscript, commented on the design, and interpreted the results. T. M. revised the manuscript. T.L.H. and H.Z. devised the original clinical study, interpreted the results, supported the writing of the manuscript, and directed the project. All authors read, commented, and approved the final version of the manuscript. T.L.H. and H.Z. are the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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Appendix A. Supplementary data

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