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Association between maternal metabolic profiles in pregnancy, dietary patterns during lactation and breast milk leptin: a retrospective cohort study

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Abstract

Breast milk leptin plays a potential role in preventing childhood obesity. However, the associations of breast milk leptin with maternal metabolism in pregnancy and dietary patterns during lactation are still unclear. We aimed to explore associations of breast milk leptin with maternal metabolic profiles in pregnancy and dietary patterns during lactation. A total of 332 participants were recruited for this retrospective cohort study. Breast milk samples were collected at approximately 6 weeks postpartum. Breast milk leptin and twenty-three metabolic profiles in pregnancy were measured in this study. A semi-quantitative FFQ was used to gather dietary information during lactation. Both principal component analysis and the diet balance index were used to derive dietary patterns. Among twenty-three maternal metabolic profiles, maternal serum glucose ($\beta = 1{\cdot}61$, $P = 0{\cdot}009$), γ-glutamyl transferase ($\beta = 0{\cdot}32$, $P = 0{\cdot}047$) and albumin ($\beta = -2{\cdot}96$, $P = 0{\cdot}044$) in pregnancy were correlated with breast milk leptin. All dietary patterns were associated with breast milk leptin. Given the joint effects of maternal metabolism in pregnancy and dietary patterns during lactation, only diet quality distance was significantly associated with leptin concentrations in breast milk (low level *v.* almost no diet problem: $\beta = -0{\cdot}46$, $P = 0{\cdot}011$; moderate/high level *v.* almost no diet problem: $\beta = -0{\cdot}43$, $P = 0{\cdot}035$). In conclusion, both maternal metabolism in pregnancy and dietary patterns during lactation were associated with breast milk leptin. Maternal diet balance during lactation was helpful to improve breast milk leptin concentration.

Key words: **Metabolism: Pregnancy: Dietary patterns: Lactation: Human milk: Breast-feeding: Leptin**

Breast milk is widely considered as the optimal food for infants in early life. Exclusive breast-feeding can meet all the nutritional needs of infancy in the first 6 months. Continued breast-feeding and complementary foods are recommended until 2 years of age or beyond[1]. Breast milk contains various nutrients and bioactive components, such as leptin. Emerging research suggests that breast milk leptin plays a vital role in preventing excessive weight gain during infancy[2–4]. Given that obesity has become a global public health issue associated with increased risks of adverse health outcomes, it is crucial to investigate what can affect leptin concentrations in human milk. However, only a few observational studies have explored the influencing factors of breast milk leptin. Most of them focused on maternal demographic characteristics, such as maternal age, parity and BMI[3,5–7]. In addition, few studies have found that breast milk leptin might be associated with specific food or nutrient intakes during lactation[8–10]. Overall, there is still a lack of research on the influencing factors of breast milk leptin.

During pregnancy, maternal metabolism changes to maintain fetus nutrition and prepare for lactation. The emerging studies showed that human milk compositions might associate with maternal metabolic status in pregnancy[11–13]. Nevertheless, the relationship between maternal metabolism in pregnancy and breast milk leptin is still limited. Maternal metabolism in pregnancy was usually considered to have differences between obese and normal weight women[14]. Several studies have reported a correlation between maternal pre-pregnancy obesity and breast milk leptin[5,6,15]. It indicated that metabolic change in

Abbreviations: ALB, albumin; DBI, diet balance index; DQD, diet quality distance; GGT, γ-glutamyl transferase; GLU, glucose; HBS, high bound score; LASSO, least absolute shrinkage and selection operator; LBS, low bound score; PCA, principal component analysis.

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pregnancy might affect leptin concentration in breast milk. Only a few studies investigated whether mothers' metabolic diseases during pregnancy altered leptin concentrations in human milk with small sample sizes[16,17]. However, the current research did not find that gestational diabetes or gestational hypertension might change breast milk leptin[16,17]. Given that metabolism is a complex process with numerous biochemical reactions. There remains a need for using multiple biomarkers to comprehensively assess the correlation between maternal metabolism in pregnancy and breast milk leptin.

Maternal diet during lactation is a modifiable protective factor for maternal and infant health. Despite the limited amount of available information, some studies have observed the association between maternal nutrition and human milk compositions[18–20]. Furthermore, maternal dietary restrictions can lead to the depletion of maternal nutritional reserves, further changing the volume and composition of breast milk[21]. Several cross-sectional or dietary intervention studies showed that human milk leptin differed from maternal dietary intakes during lactation[8–10]. However, current study of the association between lactating mothers' diet and breast milk leptin usually focused on specific food and nutrient intakes employing a single food or nutrient approach[8]. Given individuals consume meals including combinations of various foods and nutrients, traditional analysis of a single food or nutrient neglects the complex interactions among the entire diet[22]. Dietary pattern analysis is an alternative and complementary approach to explaining the relationship between authentic dietary intake and health outcomes. Dietary patterns consider the complexity of different foods or nutrients and are more appropriate for investigating their relation to health outcomes and providing evidence for disease prevention and nutritional recommendations[23–25].

However, there is a lack of research on the association between dietary patterns during lactation and breast milk components. There are two approaches commonly used to derive dietary patterns. One method is to employ dimensionality reduction techniques to derive patterns from dietary intake data[23]. Among various data reduction method, principal component analysis (PCA) has been widely used to identify dietary patterns[24,26–29]. Another method is to calculate indices relying on the prior information of healthy diet characteristics[30]. Due to the differences in dietary habits among countries, dietary habits of the population should be considered when selecting dietary indices.

Therefore, we aimed to explore the association of breast milk leptin with maternal metabolic profiles in pregnancy and dietary patterns during lactation using a retrospective cohort study. In addition, we investigated the joint effect of maternal metabolism in pregnancy and dietary patterns during lactation on human milk leptin to better understand the influencing factors of breast milk leptin.

Methods

Study population

This retrospective cohort study was conducted at Peking University People's Hospital. Postpartum women were included in this study if they received regular prenatal examinations and intended to breast-feed. We excluded participants who had severe liver or kidney disease, mental disorders and infectious diseases during pregnancy or lactation. Maternal medical records were used to obtain demographic characteristics and laboratory metabolic data in pregnancy. Participants were recommended to visit the hospital and collect milk samples at approximately 6 weeks postpartum. Diet and physical activity information during lactation were collected by face-to-face interviews at the same time.

From 2018 to 2021, we recruited 332 women who met the inclusion and exclusion criteria. A total of 296 women provided breast milk samples to detect leptin concentrations. Among 296 participants, only one woman had missing laboratory metabolic data during pregnancy, and twenty-six participants did not complete the food survey. Figure 1 shows the flow chart of this study.

This study was conducted according to the principles of Declaration of Helsinki. All procedures involving participants were approved by the ethics committee of the Peking University People's Hospital (ethics number 2020PHB113-01). Written informed consent was obtained from all participants.

Sample size calculation

R-square for multiple linear regression was used to estimate the sample size. Previous investigations did not provide an R-square between human milk leptin and its potential influencing factors. We hypothesised independent variables (metabolic profiles in pregnancy, dietary patterns in lactation) and covariates could explain 10 % of the variance for breast milk leptin, the power was selected as 0·8, the significance level was set as 0·05 and the covariate number was fixed as 13, which was the maximum confounding variable of this study. The required sample size was 173 on the above condition. Sample size calculation was performed by StataMP (version 16.0; Stata Corp.).

Biochemical analysis of metabolic profiles during pregnancy

Blood samples were collected after overnight fasting at around 24 weeks gestation by professional nurses. Serum samples were collected for subsequent detection by centrifugation. We used twenty-three biochemical biomarkers to represent participants' metabolism in pregnancy from the following aspects: (1) protein metabolism; (2) glucolipid metabolism; (3) calcium and phosphorous metabolism; (4) bilirubin metabolism; (5) enzymatic activity and (6) renal function. Specifically, the metabolic profiles included total protein, albumin (ALB), globulin, glucose (GLU), TAG, total cholesterol, HDL-cholesterol, LDL-cholesterol, Ca, inorganic phosphorus, total bilirubin, direct bilirubin, indirect bilirubin, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase (GGT), alkaline phosphatase, lactate dehydrogenase, α-hydroxybutyrate dehydrogenase, creatine kinase, urea, creatinine and uric acid. All twenty-three biomarkers were analysed with the use of an automatic biochemical analyser (Beckman AU5832).

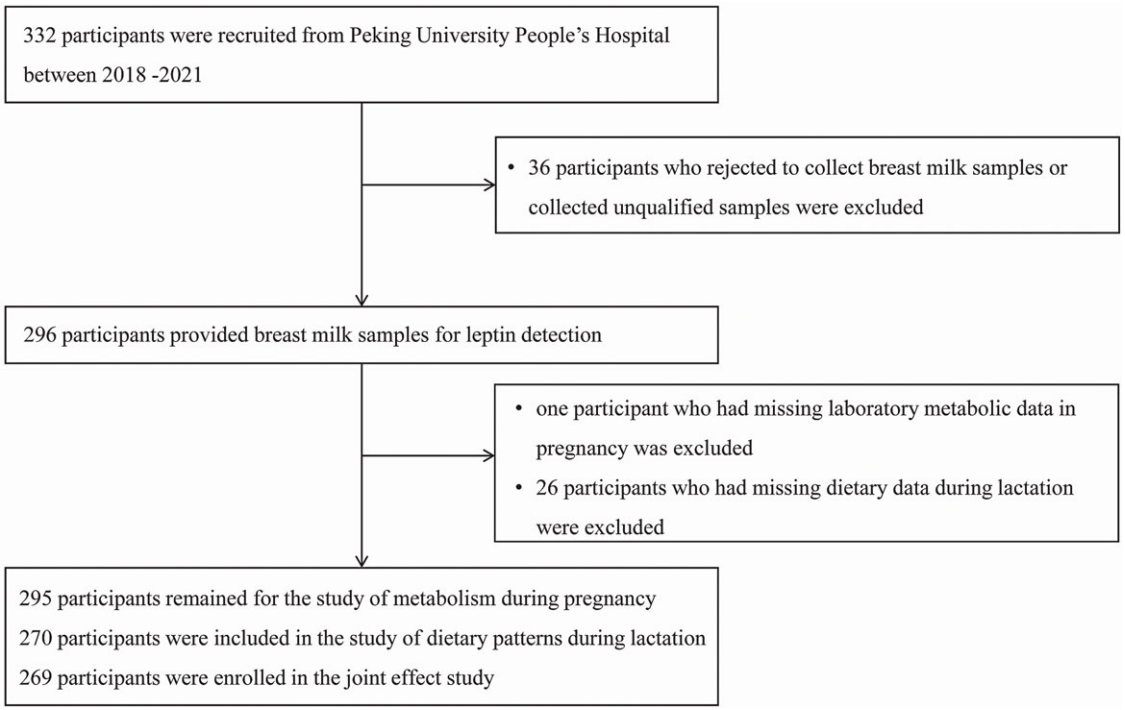

Fig. 1. Flow chart of this study.

Dietary assessment during lactation

We assessed dietary intake when participants visited the hospital and collected breast milk samples at around 42 d postpartum. A semi-quantitative FFQ was used to collect dietary intakes between delivery and the last day before the hospital visit[31]. Professional investigators gathered dietary information during lactation through face-to-face interviews. We also used food pictures and models to help participants recall their diet. The daily intakes of food items were calculated by consumption frequency (times per day/week/month) and detailed intake (g/ml). According to the latest China Food Composition[32–34], we calculated the daily intakes of energy and twenty-one nutrients. We further calculated energy-adjusted nutrient daily intake using the residual method. According to the industry standard of food composition data expression in China[35], we reclassified fifteen food groups under the regulation of food composition data expression, including cereal, tuber, soyabean, vegetable, fungi and algae, fruit, nut and seed, meat and poultry, milk, egg, fish and shrimp, snack, beverage, oil and condiment. PCA was used to derive dietary patterns from participants' dietary data.

Diet balance index (DBI) is a diet quality index designed by the Dietary Guidelines for Chinese Residents, and the latest revision is the DBI-16[36,37]. DBI-16 comprises fourteen subgroups of eight components, including cereal, vegetable and fruit, dairy products and soyabean, animal food (meat and poultry, fish and shrimp, egg), empty energy food (cooking oil, alcoholic beverage), condiment (addible sugar, salt), diet variety and drinking water. According to the dietary guidelines, there are eleven energy intake levels for calculating DBI-16 scores. We determined the scoring criteria for the energy requirement of lactating women at different physical activity levels. The scoring criteria are detailed elsewhere[37]. Three indicators were used to evaluate diet quality by DBI-16: high bound score (HBS), low bound score (LBS) and diet quality distance (DQD). HBS was used to assess the degree of diet intake excess, LBS was used to determine the degree of diet intake insufficiency and DQD was used to evaluate the degree of diet imbalance. Each indicator was subdivided into four levels, including (1) almost no diet problem; (2) low level; (3) moderate level and (4) high level (online Supplementary Table 1).

Collection and detection of breast milk samples

Breast milk samples were collected in the morning (09.00–11.00) by complete expression of breast on one side. Most milk samples were collected by hand expression on participants' preferences. After transportation at low temperature, samples were divided into aliquots and stored at −80°C freezers until analysis.

To avoid fat interference, skimmed milk was prepared by centrifugation (3000 ***g*** for 20 min at 4°C). The fat layer and bottom sediment were discarded. ELISA kits (Bioss bsk11027) were used to detect leptin concentrations in breast milk samples. The inter-assay and intra-assay coefficients of variability in the kits were less than 10 %. The limit of detection was 15 pg/ml. Breast milk samples were diluted two times before detection. Each standard and each sample was tested in duplicate wells. Four-parameter logistic curve fitting was used to calculate breast milk leptin.

Covariate data

Demographic variables, such as age, ethnicity, pre-pregnancy BMI, parity, gestational age, mode of delivery and gestational diseases, were retrospectively extracted from maternal medical

records. Physical activity levels were evaluated by the metabolic equivalent of tasks using the International Physical Activity Questionnaire – Short Form[38]. Trained investigators gathered the times and frequency of physical activity in the past 7 d via the questionnaire. Calculations of metabolic equivalent of tasks and classification criteria were shown elsewhere[39].

Statistical analysis

Categorical variables were described using frequency and proportion. The Shapiro–Wilk test was used to determine the normality of continuous variables. Mean values and standard deviations were used to present data obeyed normal distribution, and median (inter-quartile range) was used to describe skewness distributed variables. Differences in demographic characteristics were assessed by the Mann–Whitney U test or Kruskal–Wallis test. Because leptin concentration in breast milk obeyed a skewness distribution, we transformed the variable into a natural logarithm for subsequent regression analysis. The variance inflation factor was used to measure the multicollinearity in the regression models (online Supplementary Table 2).

When analysing the association between biomarkers in pregnancy and human milk leptin, we used Spearman correlation analyses to clarify multicollinearity among twenty-three metabolic profiles. Least absolute shrinkage and selection operator (LASSO) regression was used to select metabolic profiles associated with breast milk leptin. As a regularisation technique, the LASSO regression model can include all twenty-three biomarkers and uses shrinkage to minimise multicollinearity among variables. To avoid overfitting, only metabolic profiles correlated with milk leptin were retained in the model, and the regression coefficients of other unimportant profiles were set to zero value. All twenty-three metabolic profiles were standardised before LASSO regression analysis. The optimal LASSO tuning parameter (λ) was determined by minimising the root mean squared error using 10-fold cross-validation. We rerun this cross-validation ten times to ensure stable selection[40]. Multiple linear model was fitted to determine the effect estimate of selected metabolic biomarkers on leptin concentrations in human milk. Selected metabolic biomarkers were transformed into natural logarithmic transformation in the regression. The directed acyclic graph was used to identify confounding variables[41] (online Supplementary Fig. 1).

To derive lactating participants' dietary patterns, we conducted a PCA with varimax rotation using standardised daily intakes of fifteen food groups. The numbers of dietary patterns were determined by eigenvalue above 1·5, scree plot and interpretability[42]. We named dietary patterns using major food groups with the absolute value of component loading ≥ 0·3[43]. Furthermore, scores of dietary patterns were calculated by daily diet intakes and the principal component score matrix. Spearman correlation analyses were used to determine relationships between food groups/nutrients and scores of dietary patterns. For subsequent analysis, dietary pattern scores were divided into quartiles. We also utilised DBI-16 indicators (namely HBS, LBS and DQD) to evaluate dietary quality during lactation. We integrated the frequency of moderate and high level because of the small number of participants in high level diet problems. Multiple linear regressions were respectively performed to describe the effects of dietary patterns/quality during lactation on human milk leptin. According to directed acyclic graph, we adjusted the following variables: age, ethnicity, pre-pregnancy BMI, parity, gestational week, mode of delivery, gestational diabetes, gestational hypertension and physical activity level during lactation (online Supplementary Fig. 1).

To determine whether metabolic biomarkers in pregnancy and dietary patterns/quality during lactation had a joint effect on breast milk leptin, we further fitted a LASSO regression model using twenty-three metabolic profiles, PCA-derived food pattern scores and DBI-16 indicator scores. All independent variables were standardised before LASSO analysis. Similarly, we also established a multiple linear regression model to explore the effect estimate of selected variables. Confounders that influenced metabolism and diet during lactation at the same time were adjusted in the model (online Supplementary Fig. 1). Partial regression coefficients (β) and 95 % CI were used to present the effect estimate on breast milk leptin. P values < 0·05 were considered statistically significance. R packages '*glmnet*', '*psych*' were used for LASSO and PCA analyses, respectively. All statistical analyses were performed using R (version 4.1.2; R Development Core Team) and StataMP (version 16.0; Stata Corp.).

Results

Characteristics of participants in this study

A total of 295 subjects provided their breast milk samples and laboratory metabolic data. The median concentration of human milk leptin was 272·75 (inter-quartile range: 125·61–601·92) pg/ml. Table 1 shows the demographic characteristics of the participants. Most women's age was between 30 and 34 years (n 137/295, 46·4 %). Most participants had the same ethnicity (n 277/295, 93·9 %), normal pre-pregnancy BMI (18·5–23·9 kg/m^2; n 199/295, 67·5 %) and were primipara (n 220/295, 74·6 %). The majority of women were term delivery (37–42 weeks; n 284/295, 96·3 %). Most participants were vaginal delivery (n 185/295, 62·7 %), not suffering from gestational diabetes or gestational hypertension. Leptin concentrations showed significant difference in maternal age (P = 0·004), pre-pregnancy BMI (P < 0·001), mode of delivery (P < 0·001) and gestational hypertension (P = 0·019) (Table 1). Additional characteristics, such as metabolic profiles in pregnancy and dietary intakes during lactation, are presented in online Supplementary Tables 3–4.

Association between maternal metabolism and breast milk leptin

Given that significant correlations were found among twenty-three metabolic profiles in pregnancy (online Supplementary Fig. 2), LASSO regression was used to select metabolic variables associated with breast milk leptin (Fig. 2). We observed nine metabolic variables (GLU, ALB, GGT, urea, lactate dehydrogenase, total protein, creatine kinase, aspartate aminotransferase, LDL-cholesterol) were correlated with leptin in human milk. The importance of selected metabolic variables of LASSO

Table 1. Characteristics of participants in the study
(Numbers and percentages; medians and inter-quartile ranges)

Characteristics	*n*	%	Leptin (pg/ml)*		*P*†
			Median	IQR	
Age (years)					0·004
< 25	3	1·0	868·52	127·78–1182·44	
25–29	89	30·2	162·32	72·76–498·92	
30–34	137	46·4	291·92	144·81–526·57	
> 34	66	22·4	363·45	176·89–701·60	
Ethnicity					0·060
Han	277	93·9	270·10	122·35–537·91	
Others	18	6·1	445·43	237·15–731·24	
Pre-pregnancy BMI (kg/m^2)					< 0·001
< 18·5	37	12·5	163·56	57·82–331·96	
18·5–23·9	199	67·5	260·48	121·29–493·75	
≥ 24	59	20·0	615·44	271·31–873·90	
Parity					0·896
Primiparous	220	74·6	272·24	119·58–648·86	
Multiparous	75	25·4	289·32	156·87–470·70	
Gestational week (weeks)					0·296
< 37	6	2·0	139·13	85·71–176·89	
37–42	284	96·3	275·17	126·09–615·82	
> 42	5	1·7	276·18	127·78–443·15	
Mode of delivery					<0·001
Vaginal	185	62·7	248·17	106·08–463·15	
Caesarean	110	37·3	382·90	162·32–811·17	
Gestational diabetes					0·670
Yes	51	17·3	277·30	137·89–638·92	
No	244	82·7	272·24	122·06–588·15	
Gestational hypertension					0·019
Yes	27	9·2	536·98	217·79–722·75	
No	268	90·8	266·56	119·58–530·95	
Physical activity level during lactation‡					0·915
Low	150	55·6	254·33	121·29–525·03	
Moderate	113	41·8	305·95	122·35–543·67	
High	7	2·6	253·83	90·60–731·57	

*Values are median (IQR).
† Differences between variables were performed by Mann–Whitney U test (ethnicity, parity, mode of delivery, gestational diabetes, gestational hypertension) or Kruskal–Wallis test (age, pre-pregnancy BMI, gestational age, lactational physical activity level).
‡ Missing data: *n* 25 for physical activity level during lactation.

regression is presented in online Supplementary Fig. 3. After adjusted confounding variables, serum GLU ($\beta = 1{\cdot}61$, $P = 0{\cdot}009$) and GGT ($\beta = 0{\cdot}32$, $P = 0{\cdot}047$) were positively associated with breast milk leptin, and serum ALB ($\beta = -2{\cdot}96$, $P = 0{\cdot}044$) in pregnancy was negatively correlated with human milk leptin via the multiple linear model fitted by selected metabolic biomarkers (Table 2).

Correlation between maternal dietary patterns and human milk leptin

Two major dietary patterns were derived by PCA, accounting for 22 % of the total variance (online Supplementary Fig. 4). PCA pattern 1 (eigenvalue = 1·75, explained variance = 12 %) was labelled as the 'Meat-Cereal-Vegetable' pattern, characterised by higher intakes of meat and poultry, fish and shrimp, cereal and vegetables (Fig. 3). PCA pattern 2 (eigenvalue = 1·53, explained variance = 10 %) was labelled as the 'Nut-Soyabean' pattern, which featured relatively higher intakes of nut, soyabean, condiment, snack, beverage and oil (Fig. 3). Nutrition characteristics of the above two dietary patterns are shown in online Supplementary Tables 5–6. According to DBI-16 indicators, most participants were without excess diet intakes (80·7 %) and had insufficient diet intake problems (poor level: 46·7 %, moderate/high level: 34·4 %). Nearly 60·4 % of participants were under a low level of diet imbalance (online Supplementary Table 7). The intakes of egg, meat and poultry tended to excess, but cereal, vegetable, fruit, dairy products, soyabean, fish, shrimp and diet variety still had insufficient consumption among participants (online Supplementary Fig. 5).

Both dietary patterns and DBI-16 indices were correlated with human milk leptin during lactation (Table 3). 'Nut-Soyabean' pattern scores were associated with higher leptin concentrations in human milk (quartile 4 *v.* quartile 1: $\beta = 0{\cdot}39$, $P = 0{\cdot}032$). There was no significant difference between the 'Meat-Vegetable-Cereal' pattern and breast milk leptin. Interestingly, all dietary quality indicators had some specific subgroups correlated with the decrease in human milk leptin. For HBS scores, the low level of diet intake excess might significantly decrease leptin concentrations in breast milk compared with suitable diet intake ($\beta = -0{\cdot}41$, $P = 0{\cdot}023$). However, when considering LBS scores, the moderate/high level of diet insufficiency was related to lower leptin concentrations in breast milk (*v.* almost no diet problem: $\beta = -0{\cdot}51$, $P = 0{\cdot}031$). According to

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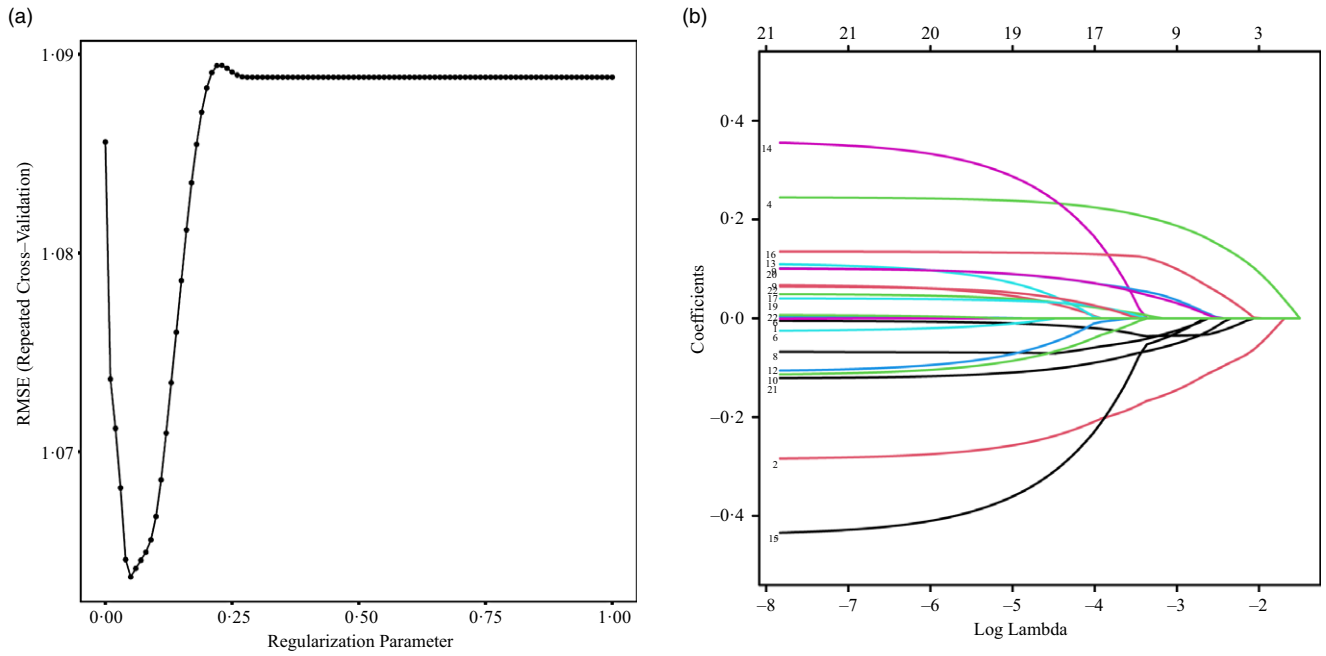

Fig. 2. LASSO regression between twenty-three metabolic biomarkers of pregnant women and breast milk leptin*. * Selection of tuning parameter (λ) in the LASSO regression using repeated cross-validation. (a) Coefficient routes of twenty-three candidate variables in the LASSO model. (b) The optimal λ was set as 0·05. LASSO, least absolute shrinkage and selection operator.

Table 2. Associations between selected metabolic profiles in pregnancy and breast milk leptin* (β-coefficients; 95 % confidence intervals)

	Unadjusted model			Adjusted model†		
Selected metabolic profiles	β	95 % CI	*P*	β	95 % CI	*P*
TP (g/l)	−1·01	−4·39, 2·36	0·555	−1·55	−4·80, 1·70	0·349
ALB (g/l)	−3·80	−6·74, −0·86	0·012	−2·96	−5·83, −0·09	0·044
GLU (mmol/l)	2·29	1·07, 3·50	< 0·001	1·61	0·41, 2·82	0·009
LDL-cholesterol (mmol/l)	−0·37	−0·97, 0·22	0·218	−0·40	−0·97, 0·17	0·169
AST (U/l)	−0·37	−0·77, 0·03	0·073	−0·24	−0·64, 0·16	0·236
GGT (U/l)	0·48	0·17, 0·79	0·002	0·32	0·00, 0·64	0·047
LDH (U/l)	0·67	−0·17, 1·50	0·117	0·33	−0·49, 1·15	0·424
CK (U/l)	0·25	−0·06, 0·57	0·111	0·28	−0·02, 0·58	0·065
Urea (mmol/l)	−0·43	−0·99, 0·13	0·131	−0·37	−0·91, 0·17	0·174

TP, total protein; ALB, albumin; GLU, glucose; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; LDH, lactate dehydrogenase; CK, creatine kinase.
*We performed a natural logarithmic transformation for breast milk leptin and selected profiles. A total of 295 participants were included in this analysis.
† According to DAG, models are adjusted for age, ethnicity, pre-pregnancy BMI and parity.

DQD scores, each level of diet imbalance was associated with lower breast milk leptin (low level *v.* no diet problem: $\beta = -0{\cdot}62$, $P = 0{\cdot}003$; moderate/high level *v.* no diet problem: $\beta = -0{\cdot}66$, $P = 0{\cdot}007$).

Joint effects of maternal metabolism in pregnancy and dietary patterns during lactation on breast milk leptin

We further observed the joint effects of metabolic biomarkers in pregnancy and dietary patterns during lactation by LASSO regression. Specifically, twenty-eight predictors (containing twenty-three metabolic profiles, two PCA-derived patterns and three DBI-16 indicators) were included in the model. Eight variables were selected as major predictors. We observed that both maternal metabolic profiles in pregnancy (including GLU, ALB, GGT, total protein, lactate dehydrogenase, urea, creatine kinase) and dietary patterns during lactation (namely DQD) were selected as major variables correlated with leptin concentrations (Fig. 4). Although GLU, ALB and GGT in pregnancy had higher importance among the selected eight variables (online Supplementary Fig. 6), only DQD was significantly associated with breast milk leptin (Table 4). Compared with participants who reached diet balance during lactation, participants who had any level of diet imbalance inclined to have a significantly lower leptin concentration in breast milk after adjusted confounding variables (Table 4).

Discussion

We explored the effect of maternal metabolism in pregnancy and dietary patterns during lactation on breast milk leptin in a

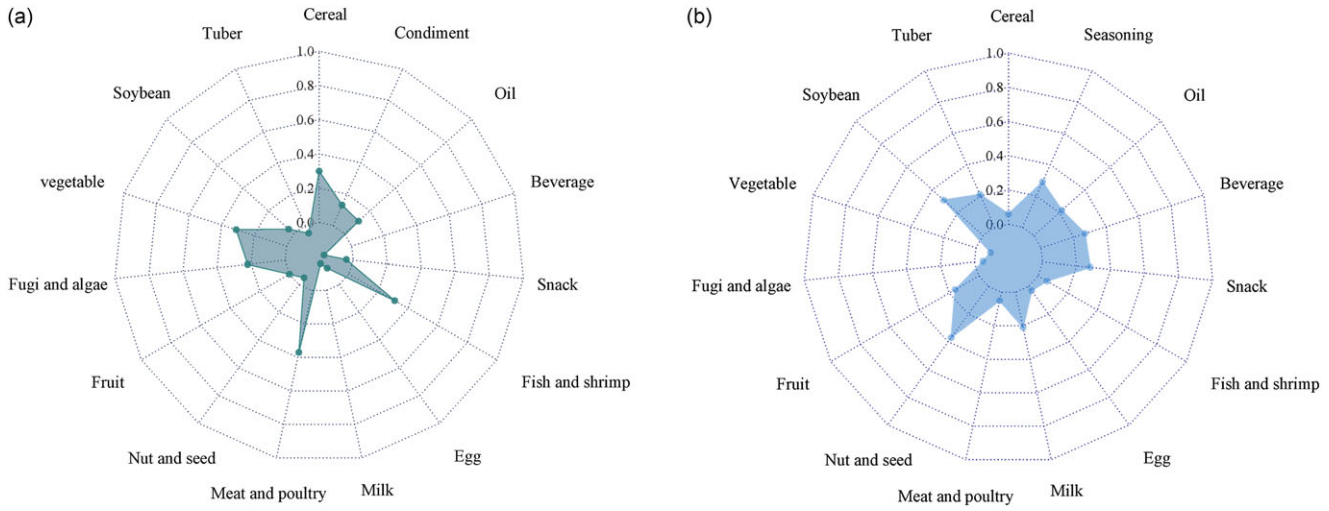

Fig. 3. Component loading for PCA-derived dietary patterns during lactation. (a) 'Meat-Vegetable-Cereal' pattern. (b) 'Nut-Soyabean' pattern. PCA, principal component analysis.

Table 3. Associations between dietary patterns during lactation and breast milk leptin*
(β-coefficients; 95 % confidence intervals)

		Unadjusted model			Adjusted model†		
Dietary patterns	*n*	β	95 % CI	*P*	β	95 % CI	*P*
'Meat-Vegetable-Cereal' pattern							
Q1	67						
Q2	68	−0·03	−0·40, 0·35	0·890	−0·06	−0·42, 0·30	0·744
Q3	68	−0·20	−0·58, 0·17	0·286	−0·27	−0·63, 0·10	0·150
Q4	67	0·07	−0·31, 0·44	0·720	−0·03	−0·40, 0·33	0·855
'Nut-Soyabean' pattern							
Q1	67						
Q2	68	0·24	−0·13, 0·61	0·197	0·22	−0·14, 0·58	0·236
Q3	68	−0·07	−0·44, 0·30	0·701	−0·11	−0·47, 0·25	0·546
Q4	67	0·35	−0·02, 0·72	0·063	0·39	0·03, 0·75	0·032
HBS							
Almost no diet problem	218						
Low level	41	−0·32	−0·69, 0·05	0·089	−0·41	−0·76, −0·06	0·023
Moderate/high level	11	0·15	−0·52, 0·82	0·662	0·25	−0·39, 0·89	0·438
LBS							
Almost no diet problem	51						
Low level	126	−0·23	−0·59, 0·13	0·206	−0·36	−0·76, 0·03	0·074
Moderate/high level	93	−0·29	−0·66, 0·09	0·136	−0·51	−0·96, −0·05	0·031
DQD							
Almost no diet problem	41						
Low level	163	−0·51	−0·88, −0·13	0·008	−0·62	−1·02, −0·21	0·003
Moderate/high level	66	−0·48	−0·90, −0·05	0·029	−0·66	−1·14, −0·18	0·007

Q, quartile; HBS, high bound score; LBS, low bound score; DQD, diet quality distance.
*We performed a natural logarithmic transformation for leptin concentrations in breast milk. A total of 270 participants were included in this analysis.
† Models are adjusted for age, ethnicity, pre-pregnancy BMI, parity, gestational age, mode of delivery, gestational diabetes, gestational hypertension and physical activity level during lactation.

retrospective cohort. Specifically, GLU, GGT and ALB in pregnant women presented significant correlations with breast milk leptin. Moreover, maternal dietary patterns might affect leptin concentrations in breast milk during lactation. We further found that lactating mothers' diet balance was a major influencing factor in higher breast leptin concentrations through the joint effect of metabolism in pregnancy and dietary patterns during lactation. In addition, we observed several maternal characteristics were associated with breast milk.

Consistent with Chan *et al.*[5], maternal age and pre-pregnancy BMI were correlated with breast milk leptin in this study. In the current results, we observed that participants with obese BMI had a higher level of breast milk leptin; it might be explained by the maternal fat reserves, which could affect adiposity tissue to produce leptin in circulation[44]. We also found that breast milk leptin might be affected by the mode of delivery, while previous study had not observed this phenomenon[16,45]. Given that delivery mode was chosen by clinical indication instead of the

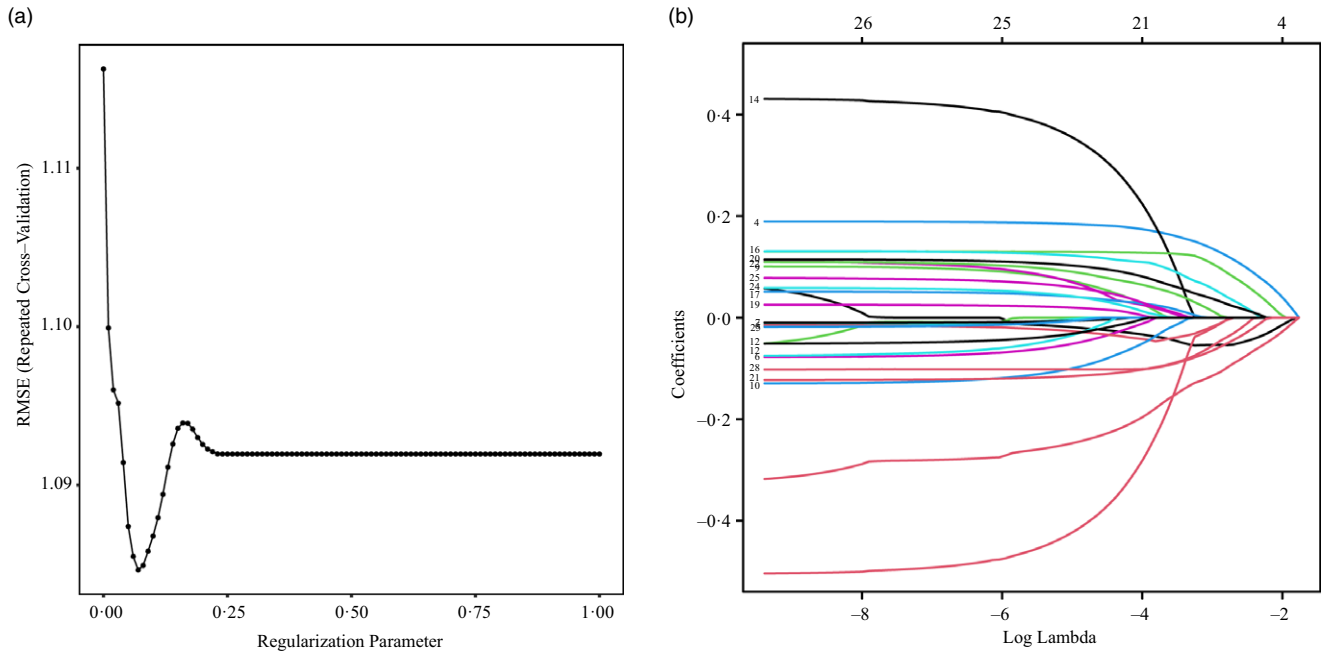

Fig. 4. LASSO regression between twenty-eight candidate variables and breast milk leptin considering joint effect of metabolism in pregnancy and dietary patterns during lactation*. *Selection of tuning parameter (λ) in the LASSO regression using repeated cross-validation. (a) Coefficient routes of twenty-eight candidate variables in the LASSO model. (b) The optimal λ was set as 0·07. LASSO, least absolute shrinkage and selection operator.

Table 4. Associations between selected variables and breast milk leptin considering joint effect of metabolism in pregnancy and dietary patterns during lactation*
(β-coefficients; 95 % confidence intervals)

	Unadjusted model			Adjusted model†		
Selected variables	β	95 % CI	*P*	β	95 % CI	*P*
Metabolic profiles in pregnancy						
TP (g/l)	−1·66	−5·06, 1·73	0·336	−2·30	−5·56, 0·96	0·166
ALB (g/l)	−3·06	−6·09, −0·02	0·048	−2·39	−5·34, 0·56	0·112
GLU (mmol/l)	1·84	0·51, 3·18	0·007	1·29	−0·02, 2·60	0·054
GGT (U/l)	0·42	0·11, 0·73	0·008	0·24	−0·08, 0·55	0·141
LDH (U/l)	0·58	−0·28, 1·43	0·184	0·32	−0·5, 1·15	0·443
CK (U/l)	0·22	−0·10, 0·55	0·178	0·25	−0·06, 0·56	0·120
Urea (mmol/l)	−0·50	−1·08, 0·08	0·089	−0·40	−0·97, 0·16	0·159
Dietary patterns during lactation						
DQD						
No diet problem						
Low level	−0·46	−0·82, −0·10	0·013	−0·46	−0·82, −0·11	0·011
Moderate/high level	−0·43	−0·84, −0·02	0·042	−0·43	−0·83, −0·03	0·035

TP, total protein; ALB, albumin; GLU, glucose; GGT, γ-glutamyl transferase; LDH, lactate dehydrogenase; CK, creatine kinase; DQD, diet quality distance.
*We performed a natural logarithmic transformation for leptin concentrations in breast milk and selected metabolic profiles in pregnancy. A total of 269 participants were included in this analysis.
† Models are adjusted for age, ethnicity, pre-pregnancy BMI and parity.

participants' purpose, participants with caesarean section were subjected to have gestational diseases or other obstetric problems. Although limited research with a small sample size observed human milk leptin might not change with gestational hypertension[16], the present study found that gestational hypertension was associated with higher human milk leptin in this study. The difference in population and sample size might explain this reverse result. Previous research showed that pregnant women with pre-eclampsia had higher serum leptin levels[46]. The current study implicated that mothers with gestational hypertension might transport more leptin from blood to breast milk. Since lactation involves complex metabolic mechanisms[47], the impact of delivery mode and gestational hypertension might explain by the difference in maternal metabolism.

There is still a lack of research exploring the association between pregnant women's metabolism and human milk composition. In this study, we used twenty-three metabolic profiles to comprehensively describe maternal metabolism in pregnancy, all of which are commonly used in routine prenatal examinations. Through LASSO regression of twenty-three metabolic biomarkers, we found GLU and GGT were correlated with higher breast milk leptin, while ALB was associated with lower

leptin concentrations in human milk. The above associations are biologically plausible. As an important indicator of nutritional status, serum ALB decrease indicated insufficient dietary intake and malnutrition[48,49]. Blood leptin concentrations were associated with lower food intake on energy regulation[50]. Similarly, blood leptin compensatory increased to maintain GLU homoeostasis when blood GLU increased[51]. Previous studies suggested that human milk leptin might originate from synthesis with the mammary gland and transport from maternal blood leptin[52–54]. Therefore, the association between serum ALB, GLU and breast milk leptin could be explained via blood leptin. Several studies showed breast-feeding was associated with a lower risk of non-alcoholic fatty liver disease in women's later life[55,56]. The association between pregnant women's GGT and human milk leptin further emphasised the effect of liver function in pregnancy on long-term breast milk conduction.

We firstly investigated the effect of lactating participants' dietary patterns on breast milk leptin. Fung *et al.*[57] found a significant positive correlation between plasma leptin and the Western pattern, which was characterised by higher intakes of red meats and high-fat dairy products. It is likely that the correlation between the 'Nut-Soyabean' pattern and higher leptin could be explained by characteristics of the dietary pattern, which was rich in energy and fat. Based on the results of DBI-16 indicators, excess diet intakes (HBS), insufficient diet intakes (LBS) and diet imbalance (DQD) were all associated with lower leptin concentrations in breast milk. The above results emphasised the importance of maternal diet balance during lactation on breast milk leptin.

Interestingly, when evaluating the joint effects of maternal metabolism in pregnancy and dietary patterns during lactation on breast milk leptin, only diet imbalance (DQD) was significantly associated with breast milk leptin decrease. Appropriate food intake might change the leptin concentrations in human milk during lactation rather than the amount of food. Our results further highlight the importance of maternal diet balance during lactation for human milk components. However, there were still a considerable number of participants in the condition of diet imbalance in this study. More health education and dietary guidance should be considered regarding public health services for the postpartum population.

The first strength of our study was that we first evaluated the effects of pregnant women's metabolism and lactating participants' dietary patterns on specific breast milk components. Moreover, we employed multiple measures to evaluate both independent and joint effects of metabolism and dietary patterns on breast milk leptin. Third, breast milk samples in this study were collected at concentrated periods, avoiding interference with lactational stages. There were still several limitations in this study. Although the FFQ could assess long-term dietary status, dietary information collected by FFQ had recall bias. To minimise this restriction, participants' dietary information during lactation was gathered by professional investigators through face-to-face interviews. Moreover, we only evaluated pregnant women's metabolism at one time point, and participants in this study were recruited from a single centre. More nationwide prospective cohorts are needed to explore maternal metabolism at different stages of pregnancy and enhance the extrapolation of the above results. Additionally, future studies need to elucidate the underlying mechanism of the association of human milk leptin with maternal metabolism in pregnancy and dietary patterns during lactation. Further studies exploring the effect of interactions and some potential mediators such as maternal metabolism during lactation, maternal blood leptin and gestational weight gain might be helpful to clarify the mechanism.

In conclusion, we found that both maternal metabolic biomarkers in pregnancy and dietary patterns during lactation were associated with leptin concentrations in breast milk. Considering the joint effect of metabolism in pregnancy and lactating participants' diet on breast milk leptin, diet balance during lactation may exert a significant influence on breast milk leptin. Our research first found an association between lactating mothers' diet balance and human milk leptin. More prospective cohort and mechanism research should be conducted in the future.

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The authors declare no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114523000600

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