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Relationships among maternal nutrient intake and placental biomarkers during the 1st trimester in low-income women

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Abstract

Purpose—Pre-eclampsia is a multi-system disorder caused by inadequate placentation in early pregnancy; however, little is known about the influence of nutrient intake on placental development during the crucial 1st trimester. The objective of this study was to examine the relationships between nutrient intake and the raw values and ratios of angiogenic [placental growth factor (PlGF)] and antiangiogenic [soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng)] placental biomarkers in the 1st trimester.

Methods—A cross-sectional study of low-income, pregnant women ($n = 118$). Average nutrient intake was calculated from three 24-h dietary recalls. Biomarker values were adjusted for gestational age and nutrients were adjusted for energy.

Results—The angiogenic to antiangiogenic ratio [PIGF/(sFlt-1 $\times$ sEng)] was positively related to intake of vitamin D ($r = 0.24$), vitamin B₂ ($r = 0.25$), B₁₂ ($r = 0.20$), dietary folate equivalents ($r = 0.19$), iron ($r = 0.19$), and zinc ($r = 0.19$) and negatively related to trans fats ($r = -0.24$). Principal component analysis revealed that a vitamin/mineral factor [$t(112) = 2.58, p = 0.011$] and trans fats factor [$t(112) = -2.03, p = 0.045$] were significant predictors of the PIGF/(sFlt-1 $\times$ sEng) ratio. The vitamin and mineral factor was a significant predictor of sFlt-1 [$t(122) = 2.29, p = 0.024$].

Conclusions—Expression of placental biomarkers in the early weeks of pregnancy may be influenced by intake of nutrients. Understanding the influence of maternal nutrient intake and placental development in the 1st trimester may provide the opportunity to avert the development or blunt the severity of preeclampsia.

Keywords

Preeclampsia; Vitamin D; Trans fats; Pregnancy; Low income; Placental growth factor; Soluble fms-like tyrosine kinase-1; Soluble endoglin

Introduction

Pre-eclampsia (PE) is a multi-system disorder that is linked to inadequate placental angiogenesis in the 1st trimester of pregnancy. PE is associated not only with adverse pregnancy outcomes, such as preterm birth, but also with the development of cardiovascular disease and related mortality in women 20 years after a pre-eclamptic pregnancy [1, 2]. Several biomarkers [vascular endothelial growth factor (VEGF), placental growth factor (PIGF) [3, 4], and soluble fms-like tyrosine kinase (sVEGFR-1, also known as sFlt-1), and soluble endoglin (sEng)] have been identified as potential indicators of adequate placental angiogenesis and establishment of adequate uteroplacental perfusion [5, 6]. VEGF (A isoform), a glycoprotein, is a potent angiogenic factor in the placenta that stimulates endothelial cell production and increases vascular endothelial permeability [6–8]. VEGF is found in the cytotrophoblasts that surround the maternal spiral arteries and contributes to vascular formation within the placenta. PIGF, another glycoprotein, has a similar structure as VEGF and is found in placental syncytiotrophoblasts with direct contact with maternal circulation and, like VEGF, stimulates vascular endothelial proliferation [4, 6–8]. sFlt-1, the VEGF receptor, is produced by the placenta and acts as a potent antagonist to the angiogenic actions of VEGF and PIGF by binding with the respective receptors, thus inhibiting the interaction of VEGF and PIGF on the endothelium and leading to endothelial dysfunction [9]. Endoglin, another glycoprotein, is also a potent anti-angiogenic factor that inhibits endothelial function and induces hypertension, proteinuria, and fetal growth restriction in animal models [10, 11]. Disproportionate levels of these biomarkers, even in the 1st trimester, are noted in pre-eclampsia [9–15], such that lower levels of PIGF and higher levels of sEng and sFlt-1 are noted in the first trimester in women who later developed PE than women who remained normotensive during pregnancy [16–18]. Alterations in the PIGF/sFlt-1 $\times$ sEng ratio were more predictive of PE than use of the isolated biomarkers [19].

Little is known about the role that maternal nutritional status plays on expression of angiogenic-related placental biomarkers in the 1st trimester and the subsequent onset of PE. The onset of PE has been connected with an inadequate intake of foods rich in antioxidants, polyunsaturated fatty acids (PUFA), particularly omega-3 fatty acids, and vitamin D [20–24]. Low income women are more likely to consume diets with inadequate intake of these

nutrients due partly to an inadequate understanding of nutritional requirements and limited ability to purchase healthy foods compared with their wealthier counterparts [25]. Less than recommended intakes of iron, folate, Vitamin D, and fatty acids have been observed in pregnant women [26–31]; thus, many pregnant women do not consume foods that meet the requirements for achieving adequate nutritional status. Thus, low-income women may be at higher risk for development of a pregnancy-onset hypertensive disorder that may signal the onset of cardiovascular disease later in life.

A pregnant woman's nutritional status, particularly in the 1st trimester of pregnancy, also has a profound effect on pregnancy outcomes [32–34]. During the early weeks of pregnancy, successful placental implantation is influenced by the genetic structure of the mother and fetus and the maternal supply of nutrients [32–35]. Poor maternal nutritional status in the 1st trimester alters the intrauterine hormonal environment and reduces the available nutrient supply that adversely affects placental development, thus predisposing women to pre-eclampsia (PE) [34–36], and can adversely affect infant birth weight at delivery regardless of gestational weight gain or maternal nutritional status in the 2nd and 3rd trimesters of pregnancy [34, 36]. Furthermore, data from the Dutch Famine Study signaled the critical nature of maternal nutrition in pregnancy [37] on the child's subsequent health. Inadequate placentation in the 1st trimester can result in a diminished supply of nutrients to the fetus throughout pregnancy, thus inhibiting proper fetal growth and leading to intrauterine growth restriction and infant low birthweight (LBW) [38]. Emerging evidence strongly suggests that inadequate nutrition during this critical period of fetal development may alter the epigenetic intrauterine environment and lead to "re-programming" of the fetal phenotype, resulting in permanent physiological changes and predisposing the child to coronary heart disease, altered insulin metabolism, obesity, and hypertension as an adult [39–44]. To date, however, little research exists that examines the relationship between nutritional status and measures of placental biomarkers in the 1st trimester of pregnancy.

While there has been a plethora of research seeking to identify biomarkers that may predict preeclampsia, the effect of maternal dietary intake on these placental biomarkers in early pregnancy remains unclear. Maternal dietary intake may provide essential nutrients (antioxidants, vitamin D, fatty acids) that may blunt or heighten mediating biological processes, such as oxidative stress and a systemic inflammatory response, that may lead to disproportionate levels of placental biomarkers [22]. To date, however, little research exists that examines the relationship between maternal nutritional status and measures of placental biomarkers in pregnancy. The purpose of this study was to explore the relationships between maternal nutrient intake and levels of placental biomarkers during the first trimester of pregnancy.

Method

Design

An observational design was used in a sample of low-income women who were recruited from five clinics in Central Texas that provide low-cost pregnancy testing to uninsured and underinsured women.

Procedures

The Full Board of the university Institutional Review Board approved all study procedures prior to data collection. Women interested in learning more about the study completed a recruitment card at the clinics and were subsequently screened by phone to determine their eligibility. Women were eligible if they were 16 years of age and older, received confirmation of pregnancy, and were in the first trimester of pregnancy (≤ 14 weeks from the

last menstrual date), and explained the study procedures. Women were excluded if they had a pre-existing health condition including Type 1 or Type 2 diabetes, hypertensive disorders, positive HIV status, renal disease, heart disease, diagnosed eating disorder, milk or gluten allergies, or other conditions that require special diets.

Women who passed the telephone screening then complete initial data collection at the Family Wellness Center, located in Austin, Texas and operated by the University of Texas at Austin's School of Nursing. Women aged eighteen and older completed written informed consent prior to study procedures. For participants 16 and 17 years of age, parental consent would be ascertained prior to the appointment and child assent forms were completed during the visit. During the data collection visit, participants completed a demographic form, had their height and weight measured, had blood drawn, and were trained on portion estimation using the Food Amount Booklet followed by a 24-h dietary recall using the Nutrition Data System for Research (NDSR) created by the Nutrition Coordinating Center at the University of Minnesota. Over the next 2 weeks, two additional non-consecutive dietary recalls were conducted via telephone, along with questions following the Rhodes Index of Nausea and Vomiting. Once data collection was complete, each participant received feedback comparing recommendations provided by MyPyramid.gov to her average dietary intake along with individualized suggestions for meeting the dietary recommendations. Women were compensated in the form of cash and gift cards for their time and participation in the study.

Serum analysis

Placental biomarkers were assessed using individual serum levels of VEGF, PlGF, sFlt-1, and sEng. A 20-ml volume of blood was obtained from a peripheral vein using two Vacutainer™ serum separator tubes (SST). The SST's were inverted 8–10 times and allowed to sit at room temperature for 30 min. After the clot formed, the tubes were centrifuged for 20 min. Then, the serum was pipetted into a polypropylene 1.7 cc microtubes and placed in a –70°C freezer until analyzed in batches. The Biobehavioral Laboratory at the University of Texas at Austin, School of Nursing conducted the analysis using sensitive enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc., Minneapolis, MN, USA). The minimal detectable dose was less than 5 pg/ml of VEGF, less than 7 pg/ml for PlGF, less than 3.5 pg/ml for sFlt-1, and less than 100 ng/ml for sEng. All assays were performed in duplicate using a Spectramax M2e plate reader (Molecular Devices, Sunnyvale, CA, USA). Inter- and intra-assay variance was less than 12% for each biomarker.

Data analysis

Descriptive statistics were calculated on sample characteristic information and nutrient intake. Nutrient data were based on the average from the three dietary recalls obtained from each participant that was adjusted for energy intake. Raw values for levels of VEGF, sFlt-1, PlGF, and sEng were used in this analysis. A ratio of angiogenic PlGF to antiangiogenic sFlt-1 and sEng ($\text{PlGF/sFlt-1} \times \text{sEng}$) was calculated and values were adjusted for gestational age. Unadjusted and adjusted ratios were used in this analysis. Non-parametric correlation (Spearman's rho) statistics were used because data from placental markers were nonnormally distributed.

Analysis of the relationship between nutrients and placental biomarkers was conducted in two phases. The goal of the first phase was to reduce the number of nutrients by grouping together co-occurring nutrients which would otherwise produce multicollinearity in regression models that were used in the second phase of the study. Principle component analysis (PCA) with a varimax rotation was conducted to identify highly correlated nutrients. In addition to reducing the number of independent variables and increasing the *N*-

to-number of predictor ratio, using the principle components rather than averages of variables that load on a putative principle component has the advantage of reducing multicollinearity among independent variables because the principle components are orthogonal to each other [45]. We required that items have a loading of at least 0.3 to be included in the second phase of the analysis. PCA scores for each participant were computed to serve as independent variables in the second phase of the analysis. The second phase of the analysis examined the relationship between the principle components derived in the first phase of analysis and four outcomes: sEng, VEGFR, and PlGF and the PlGF/sFlt-1 $\times$ sEng ratio. Robust regression was implemented to reduce the influence of outliers as preliminary analysis of the data indicated that there were outliers in both the independent and dependent variables in the model [46]. Robust regression is also robust to heteroskedastic errors. Nevertheless, we examined plots of predicted values by residuals to examine the distribution of the error term as well as assessing the normality of the residuals using QQ plots. Descriptive and correlational analyses were conducted using SPSS (v.17), and principal component analysis (PCA) was conducted using Stata 11.1.

Results

Of the 132 women who completed initial data collection procedures, 118 women who completed all dietary data points were included in this analysis. Reasons for removal for analysis were miscarriage ($n = 3$), inability to contact ($n = 9$), and outliers ($3 \pm SD$) on psychosocial variables ($n = 4$; results reported elsewhere). Table 1 presents data concerning socio-demographic and lifestyle variables. Average age was 25 years ($SD = 5.3$; range = 16–40 years old) and average weeks gestation at the time of serum collection was 9 weeks ($SD = 2.2$; range = 4.5–13 weeks).

Table 2 presents data concerning nutrient intake. More than half of the women consumed foods that provided less than the recommended amount of vitamins A, D, E, folate, calcium, magnesium, iron, zinc, and DHA. Women consumed foods that provided more than the recommended amounts of kilocalories from fat. All but one woman consumed foods that resulted in an omega-6/omega-3 ratio higher than recommended.

Table 3 presents data regarding the relationship between nutrient intake and unadjusted levels of, sFlt-1 (VEGFR), PlGF, and sEng. While most of the nutrient had a non-significant relationship to the placental biomarkers, it is notable that many of the nutrients had a significantly negative relationship with sEng, such that lower levels of antiangiogenic endoglin were associated with higher levels of vitamin D, B₁₂, riboflavin, niacin folate, calcium, iron, and zinc.

Table 4 presents data on the relationship between nutrient intake, adjusted for energy intake, and the PlGF/sFlt-1 $\times$ sEng ratio reported with the unadjusted values, and with the ratio value adjusted for gestational age. Vitamin D and riboflavin were significantly positively related to the unadjusted angiogenic ratio while levels of trans fatty acids had a significantly negative relationship to the unadjusted angiogenic ratio and these relationships become stronger when the ratio was adjusted for gestational age. Vitamin B₁₂, dietary folate equivalents, iron, and zinc had a significantly positive relationship only with the adjusted PlGF/sFlt-1 $\times$ sEng ratio.

Results of the principal component analysis are presented in Table 5. Vitamins and minerals loaded more strongly on factor 1 and omega 6 and omega 3 fatty acids loaded on factor 2. Factor 3 was comprised of trans fatty acids and essential fatty acids loaded on factor 4. Arachidonic acid cross-loaded on the vitamin and mineral factor, and omega-6/omega-3 fatty acid factors, but more strongly on the latter factor.

Prior to conducting regression analysis, we adjusted the values of the placental biomarkers for gestational age and examined predicted-by-residual plots and QQ plots for all models. Because there appeared to be heteroscedasticity in the residuals, we log transformed the dependent variables; after the transformation, plots exhibited homoskedastic error. QQ plots of residuals fit a straight line indicated normality of error terms. Table 6 presents the results of the regression analysis. The four nutrient factors explained 4% of the variance in log-transformed values of PIGF (adjusted for gestational age) with the trans fatty acid factor exhibiting a marginally significant influence. The four nutrient factors explained 6% of the variance in log-transformed values of sFlt-1 with the vitamin, and mineral factor was a significant predictor. The four nutrient factors explained 4% of the variance in log-transformed sEng levels with the essential fatty acids factor demonstrating a marginally significant influence. The vitamin and mineral factor, and trans fatty acid factor each explained a significant proportion of the variance in the PIGF/sFlt-1 $\times$ sEng ratio (8.2 and 6.3%, respectively). These two factors, when combined, explained 10.8% of the variance in the PIGF/sFlt-1 $\times$ sEng ratio and were significant predictors.

Discussion

The literature describing the relationships between nutrient intake and placental biomarkers is scant and conflicting. For example, 1,25(OH)₂D₃ levels, the bioactive form of vitamin D, have been related to elevated levels of vascular endothelial growth factor (VEGF) in osteoblastic cells [47, 48], in vascular smooth muscle [49], and aortic smooth muscle [50]. In contrast, 1,25(OH)₂D₃ levels was found to inhibit VEGF production in human lumbar annulus cells [51], human cancer cells [52], and in mouse models [53]. Recent studies in India comparing placental and umbilical cord tissues from preeclamptic and normotensive women noted that levels of soluble fms-like tyrosine kinase-1 (sFlt-1) were inversely related to levels of docosahexaenoic acid (DHA) and other omega-3 fatty acids [54, 55]. No studies were found that addressed the interaction of nutrients with placental growth factor or endoglin during pregnancy.

To our knowledge, this is the first study to explore the relationships among nutrients and placental biomarkers that may be useful in predicting preeclampsia in sample of low-income women. The expression of placental biomarkers in the early weeks of pregnancy may be associated with maternal nutrient intake status. Some vitamins (D and B₂) and minerals (iron, and folate) may foster placental angiogenesis, whereas consumption of trans fatty acids may hamper placental angiogenesis. Maternal food consumption provides the essential nutrients (vitamin D, essential fatty acids) that may blunt or heighten a physiological response to altered placental development [20]. If inadequate, nutritional status may be unable to modify the inflammatory response, promoting inadequate placentation and subsequent onset of pre-eclampsia. In particular, inadequate intake of polyunsaturated fatty acids (PUFA), particularly omega-3 fatty acids, and vitamin D has been linked to PE [38, 56–59]. Insufficient intake of these nutrients and of calcium, iron, and folate has been noted in low-income women [16] and this increases their risk for PE [26–31, 60, 61].

The ability of the nutrient factors to account for only a small amount of explained variance in either the isolated biomarkers or ratios the placental biomarkers is not unexpected. Large meta-analyses have reported no effect of vitamin E or C supplementation for reducing the risk of PE [62, 63]. The complex interactions among nutrients may obscure the ability to identify an isolated nutrient that may promote or inhibit early placental development and reduce the risk of developing PE.

This study has several limitations that must be considered. The observational study design prevents establishing a definitive influence of maternal nutrient intake on placental

biomarkers. Furthermore, participants were tested only during their first trimester and birth outcomes were not known and thus the actual development of preeclampsia is unknown. Low-income women may have dietary intake patterns that vary from their wealthier counterparts and may limit the ability to generalize the results. The study sample is relatively small, which may limit our ability to find small differences in nutrient intake that may influence the placental biomarkers. Also, participants may have realized that they had poor diets and chose to be in the study to learn how to improve their dietary intake during pregnancy. Conversely, participants who self-selected into this study may have been more aware of the importance of good nutrition to promote healthy birth outcomes or may have gained useful nutritional knowledge that may have altered their diets during the remainder of the pregnancy. Last, the women included in this study were mainly of low-income and unpartnered, with Hispanic women included at a higher proportion than in the general US population; therefore, the results may not be generalizable to the overall population of pregnant women. The current study has several strengths. The study used validated, standardized measures for assessing nutrient intake and placental biomarkers. Also, the current study identifies maternal intake of nutrients that may influence the expression of placental biomarkers during the critical 1st trimester of pregnancy.

More research is needed to explore the relationships between maternal nutrient intake and placental development in the 1st trimester. Learning more about the critical importance of maternal nutritional status in the 1st trimester provides the opportunity for primary prevention of cardiovascular disease in women later in life by improving nutritional status during pregnancy that may avert the onset or blunt the severity of preeclampsia. The results could justify increased access to preconceptional and/or interconceptional health care or prenatal care very early in pregnancy for all women by emphasizing the importance of nutrition in the early weeks of pregnancy.

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Table 1Sample characteristics ($n = 118$)

Variable	<i>n</i>	%
Race		
Anglo	45	38.1
African-American	15	12.7
Hispanic	55	46.6
Other	3	2.5
Marital status		
Married	30	25.4
Single	88	74.6
Socioeconomic status		
Minor professional	20	16.9
Clerical/sales/skilled workers	42	35.6
Semi-skilled workers	40	33.9
Unskilled/service workers	16	13.6
Body mass index		
Underweight	9	7.6
Normal	49	41.5
Overweight	29	24.6
Obese	31	26.3
Intended pregnancy		
Unintended	77	65.3
Intended	41	34.7
Smoking during pregnancy		
Yes	11	9.3
No	107	90.7

Table 2

Nutrient intake

Nutrient	Pregnancy requirement	Sample statistics		Out of range in sample (number lower than norm unless otherwise specified)
		Mean	SD	
Macronutrients				
Carbohydrates (g/day)	175	280.15	102.75	14 11.9%
Protein (g/day)	71	75.08	27.04	57 48.3%
Fiber (g/day)	28	17.09	7.37	111 94.1%
Fat	20–35% of all energy (kcal)/day	32.63	5.53	Low: 1, High: 27 (23.7%)
Vitamins				
A (µg/day)	770	817.43	612.09	73 61.9%
C (mg/day)	85	104.20	94.45	57 48.3%
D (µg/day)	5	4.51	3.69	84 71.2%
E (mg/day)	15	8.51	4.61	112 95.0%
Thiamin (mg/day)	1.4	1.79	0.71	40 33.9%
Riboflavin (mg/day)	1.4	2.08	0.93	26 22.0%
Niacin (mg/day)	18	22.22	8.98	44 37.3%
B ₆ (mg/day)	1.9	1.92	0.97	69 58.5%
Folate (µg/day)	600	471.34	287.26	97 82.2%
B ₁₂ (µg/day)	2.6	4.99	3.49	26 22.0%
Minerals				
Calcium (mg/day)	1,100	966.35	411.22	82 69.5%
Phosphorus (mg/day)	700	1222.32	433.17	12 10.2%
Magnesium (mg/day)	350	268.38	95.36	94 79.7%
Iron (mg/day)	27	16.57	7.12	106 89.8%
Zinc (mg/day)	11	10.91	5.07	74 62.7%
Copper (mg/day)	1	1.25	0.49	43 36.4%
Sodium (g/day)	1.5	3.73	1.30	1 0.8%
Potassium (g/day)	4.7	2.38	0.83	117 99.2%
Fatty acids				
Linoleic [LA (g/day)]	13 (precursor to arachidonic acid)	16.02	6.81	40 33.9%

Nutrient	Pregnancy requirement	Sample statistics		Out of range in sample (number lower than norm unless otherwise specified)
		Mean	SD	
α -Linolenic [ALA (g/day)]	1.4 (precursor to EPA and DHA)	1.72	0.81	43
EPA	250–500 mg [combined (EPA + DPA)]	49.21	99.80	Low: 114 (96.6%) High: 2 (1.7%)
DPA				
DHA (mg/day)	300	65.35	125.32	113
O6/O3 ratio	1:1 to 4:1	9.92	3.44	High: 117
				High: 99.2%

Table 3

Spearman rho correlations between average nutrient intake (adjusted for energy intake) and unadjusted placental biomarkers

	VEGF	VEGFR	PIGF	sEng
Vitamin D (μg)	-0.108	-0.198 *	-0.099	-0.244 **
Thiamin (mg)	-0.259 **	-0.119	-0.091	-0.177
Riboflavin (mg)	-0.169	-0.183 *	-0.057	-0.313 ***
Niacin (mg)	-0.181 *	-0.133	-0.054	-0.183 *
Vitamin B ₁₂ (μg)	-0.147	-0.154	-0.112	-0.335 ***
Total folate (μg)	-0.132	-0.194 *	-0.128	-0.206 *
Dietary folate equivalents (μg)	-0.144	-0.210 *	-0.142	-0.244 **
Calcium (mg)	-0.131	-0.074	-0.014	-0.201 *
Iron (mg)	-0.186 *	-0.188 *	-0.180 *	-0.300 ***
Zinc (mg)	-0.166	-0.176	-0.106	-0.239 **
Total trans-isomer fats (g)	0.084	0.143	0.062	0.172

*
 $p < 0.05$,

**
 $p < 0.01$,

 $p < 0.001$

Table 4

Spearman rho correlations of energy-adjusted nutrient intake with unadjusted and adjusted PIGF/Sft-1 $\times$ sEng ratios

Nutrient ^a	PIGF/sFIt-1 $\times$ sEng unadjusted	PIGF/sFIt-1 $\times$ sEng adjusted for gestational age
Vitamin D (μ g)	0.189 [*]	0.242 ^{**}
Thiamin (mg)	0.067	0.103
Riboflavin (mg)	0.202 [*]	0.246 ^{**}
Niacin (mg)	0.116	0.146
Vitamin B ₁₂ (mg)	0.151	0.195 [*]
Total folate (μ g)	0.139	0.179
Dietary folate equivalents (μ g)	0.151	0.193 [*]
Calcium (mg)	0.129	0.137
Iron (mg)	0.135	0.189 [*]
Zinc (mg)	-0.176	0.192 [*]
Total trans-isomer fats (g)	-0.184 [*]	-0.236 ^{**}

^aNutrients adjusted for energy intake

*
 $p < 0.05$;

**
 $p < 0.01$

Table 5

Principal component factor pattern of energy-adjusted nutrient averages

Nutrient ^a	Vitamins and minerals	Fatty acids	Transfats	Essential fatty acids
Vitamin D	0.338	0.309		
Vitamin B ₂	0.495			
Iron	0.515			
Dietary folate equivalents	0.517			
Arachidonic acid	-0.313	0.424		
Eicosapentaenoic acid		0.580		
Docosahexaenoic acid		0.615		
Transoctadecenoic acid (elaidic acid)			0.684	
Transoctadecadienoic acid (elaidic acid)			0.688	
Linoleic acid				0.682
α -Linolenic acid				0.707

^a Nutrient values have been adjusted for energy intake

Table 6Regression analysis of nutrient factors on PIGF, sFlt-1, sEng and PIGF/sFlt-1 $\times$ sEng ratio (adjusted for gestational age)

Placental biomarker (ratio)	R ²	F	Prob > F	t	p
PIGF	0.04	0.85 (df 4, 112)	0.48		
Factor					
Vitamins and minerals				-0.85	0.397
Fatty acids				0.73	0.464
Transfatty acids				-1.73	0.086
Essential fatty acids				-0.63	0.527
sFlt-1	0.06	2.39 (df 4, 112)	0.055		
Factor					
Vitamins and minerals				-2.29	0.024
Fatty acids				0.84	0.402
Transfatty acids				0.82	0.414
Essential fatty acids				0.11	0.910
sEng	0.04	1.54 (df 4, 112)	0.194		
Factor					
Vitamins and minerals				0.45	0.653
Fatty acids				-0.32	0.750
Transfatty acids				-1.44	0.153
Essential fatty acids				1.91	0.059
PIGF/sFlt-1 $\times$ sEng	0.108	5.28 (df 4, 112)	0.0006		
Factor					
Vitamins and minerals				2.58	0.011
Fatty acids				0.22	0.827
Transfatty acids				-2.03	0.045
Essential fatty acids				-0.09	0.930