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Original Contribution

Nonesterified Fatty Acids and Spontaneous Preterm Birth: A Factor Analysis for Identification of Risk Patterns

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We considered that accumulation of nonesterified (free) fatty acids (NEFAs) in the first trimester of pregnancy would mark women at excess risk of spontaneous preterm birth (sPTB) and examined the interplay between NEFAs, lipids, and other markers to explore pathways to sPTB. In a case-control study nested in the Pregnancy Exposures and Preeclampsia Prevention Study (Pittsburgh, Pennsylvania, 1997–2001), we assayed NEFA levels in nonfasting serum collected at a mean gestational week of 9.4 (range, 4–20 weeks) in 115 women with sPTB (<37 weeks) and 222 women with births occurring at ≥37 weeks. C-reactive protein, total cholesterol, low-density lipoprotein and high-density lipoprotein (HDL) cholesterol, triglycerides, and uric acid were also measured. Polytomous logistic regression models were used to evaluate tertiles of NEFA levels and sPTB at <34 weeks and 34–36 weeks; factor analysis was used to characterize patterns of biomarkers. Women with NEFA levels in the highest tertile versus the lowest were 2.02 (95% confidence interval: 1.13, 3.48) times more likely to have sPTB, after adjustment for covariates. Risk of sPTB before 34 weeks was particularly high among women with high NEFA levels (odds ratio = 3.73, 95% confidence interval: 1.33, 10.44). Six biomarker patterns were identified, and 2 were associated with sPTB: 1) increasing NEFA and HDL cholesterol levels and 2) family history of gestational hypertension. NEFA levels early in pregnancy were independently associated with sPTB, particularly before 34 weeks. We also detected a novel risk pattern suggesting that NEFAs together with HDL cholesterol may be related to sPTB.

factor analysis; inflammation; nonesterified fatty acids; pregnancy; prematurity; preterm birth; risk factors

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFAs, nonesterified (free) fatty acids; OR, odds ratio; sPTB, spontaneous preterm birth.

Preterm birth is the primary determinant of neonatal morbidity and mortality, and although the rate has decreased slightly during the past few years, preterm birth occurs in 12% of all US births each year (1). Infants born before 37 weeks of gestation are more likely to suffer from lifelong disability and early death than are infants born at term. There are also persistent racial disparities, with black women having rates twice those of white women. We have previously demonstrated that elevated maternal total cholesterol or triglyceride levels early in gestation, independent of inflammation (as assessed by C-reactive protein (CRP) level), were associated with excess risk of spontaneous preterm birth (sPTB) (2). These findings were corroborated in other large US pregnancy cohorts (3, 4), and we and others have demonstrated that high total cholesterol or triglyceride levels before pregnancy are associated with excess risk of preterm birth (5, 6). Mechanisms that may link higher atherogenic lipid levels to sPTB are not understood, although inflammation is one possibility.

Chen and Scholl (7) reported that excess third-trimester levels of nonesterified (free) fatty acids (NEFAs) were also associated with preterm birth. Serum NEFA levels usually begin to increase late in the second trimester (later than levels of lipoprotein-associated triglycerides or cholesterol) and become roughly double prepregnancy levels by late pregnancy (8). Increased blood levels of human placental lactogen contribute to the increase in NEFA levels through its direct lipolytic action on adipose tissue, making NEFAs available to

meet the growth needs of the fetus (9). In excess, NEFAs are proinflammatory, are markers of tissue damage, and directly perturb membranes. They also serve as a substrate for oxidative processes, generating a broad range of reactive oxidative species (10). Thus, the accumulation of NEFAs could mark elevated risk for sPTB or may provide a mechanistic link between elevated lipid levels and sPTB. In the current study, we investigated this hypothesis by assessing concentrations of NEFAs before 21 weeks of gestation in women with and without sPTB. We also investigated the interplay between NEFAs, lipids, inflammatory markers, and maternal characteristics assessed in early pregnancy, using factor analysis to explore potential pathways leading to sPTB.

METHODS

The Pregnancy Exposures and Preeclampsia Prevention (PEPP) Study was a prospective study of pregnant women enrolled at <16 weeks' gestation and followed through the postpartum visit. Women were recruited from clinics and private practices in Pittsburgh, Pennsylvania, from 1997 to 2001. The study was approved by the University of Pittsburgh Institutional Review Board, and all participants provided written informed consent. Of the 2,211 women enrolled, we excluded women with conditions associated with increased risk of preterm birth (preexisting hypertension or diabetes ($n = 46$); preeclampsia or gestational hypertension ($n = 166$); small size for gestational age (<10th percentile; $n = 85$); multiple gestation ($n = 38$); or a positive toxicology screen ($n = 46$)). We also excluded women with incomplete diagnostic information ($n = 161$) and subsequent births to the same women in the cohort ($n = 106$). Of the 1,563 eligible women with otherwise uncomplicated pregnancies, 116 delivered preterm (<37 weeks' gestation). To describe associations detected in the first half of pregnancy, we defined cases as all women from this group with spontaneous onset of labor or preterm premature rupture of membranes with a first blood specimen drawn at <21 weeks' gestation ($n = 115$). Controls (2:1 ratio) were randomly selected (using a random number assignment) from women with uncomplicated pregnancies who delivered at ≥37 weeks' gestation with a first blood sample drawn at <21 weeks ($n = 222$).

Maternal nonfasting blood samples were collected at the first prenatal visit for the majority of participants (89%) and at the next prenatal visit for the remaining participants (mean gestational age = 10.2 weeks (standard deviation, 3.9); range, 4–20 weeks). Samples were collected in 10-mL sterile, red-topped Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey), and the time from collection to processing and freezing was recorded. The blood was allowed to clot by leaving it undisturbed at room temperature for 30 minutes. The samples were centrifuged at 2,000 × g at 4°C for 20 minutes. Serum aliquots were stored at −80°C until assayed; they were thawed only immediately prior to assay and were kept chilled. Median sit time for the samples analyzed in this study was 1.0 hour (interquartile range, 0.8–1.4 hours). In addition, there was no correlation between NEFAs and the sit time (Spearman's $r = -0.00676$, $P = 0.90$). There was no systematic difference in results by year of sample collection, suggesting that freezing did not affect the results. NEFA levels were quantitatively determined by means of an enzymatic colorimetric method developed by Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The coefficient of variation for variation between runs in our laboratory was 6.5%.

To characterize inflammation, which has been associated with sPTB (11), CRP concentration was measured by high-sensitivity enzyme-linked immunosorbent assay. The detection limit was 0.2 μg/mL, with intra- and interassay variabilities in our laboratory of 4.5% and 7%, respectively. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured in duplicate by means of a colorimetric technique using commercial kits from Pointe Scientific, Inc. (Canton, Michigan); the average coefficients of variation for variation between runs in our laboratory ranged from 5.3% to 8.4%. Low-density lipoprotein (LDL) cholesterol level was estimated using the Friedewald equation, and no triglyceride concentrations were greater than 400 mg/dL, as is required for this equation to be valid (12). While we could not assess insulin resistance in nonfasting serum samples, uric acid level has been associated with metabolic syndrome and insulin resistance (13). Thus, we measured uric acid levels using a uricase-based colorimetric assay from Pointe Scientific, Inc. (Kit U7581-120; lower detection limit = 1 mg/dL). The coefficient of variance assessed in our laboratory was 9.0%.

Gestational age at delivery was based on the date of the last menstrual period and ultrasonography when available; at our hospital during this time period, 60% of deliveries had early dating ultrasound performed. When the 2 dates (last menstrual period and ultrasonography) differed by less than 2 weeks, gestational age was based on the last menstrual period; when they differed by more than 2 weeks, dating was based upon ultrasound. To describe the severity of preterm status, we categorized preterm births as delivery at 34–36 completed weeks' gestation ($n = 82$) or delivery at less than 34 completed weeks ($n = 33$). Women underwent a structured interview at their first prenatal visit. Reported prepregnancy weight and measured height were used to calculate prepregnancy body mass index (BMI; weight (kg)/height $(m)^2$). The self-reported covariates considered were maternal age at delivery, education (less than high school for women older than 19 years who did not complete high school vs. high school or greater), periconceptional multivitamin use (multivitamin use during the 6 months prior to the first prenatal visit), smoking during pregnancy (any smoking since suspected pregnancy), family history of gestational hypertension (mother or sister with a pregnancy complicated by preeclampsia or nonproteinuric gestational hypertension), marital status (married or living with a partner), and race. Because of the small number of women who reported their race as other than black or white ($n = 4$), results are reported for black women versus nonblack women. Information on gestational diabetes was not abstracted from the medical record.

Maternal characteristics were summarized according to tertile of early-gestation NEFA levels. Many markers were nonnormally distributed, so the Kruskal-Wallis test and χ^2 test were used to evaluate the significance of differences in characteristics between tertiles for continuous and categorical

variables, respectively. Spearman correlation coefficients were also calculated to quantify the relationship between each pair of biomarkers as well as relationships among prepregnancy BMI, age, and gestational age at blood draw. We used 2 approaches to evaluate the association between NEFAs assessed early in gestation and sPTB risk. The first approach explored the association between tertiles of NEFA and sPTB using logistic regression, accounting for maternal factors that have been previously linked to sPTB (age, race, prepregnancy BMI, family history of gestational hypertension, periconceptional multivitamin use, and smoking). Polytomous logistic regression was used to estimate the risks of sPTB at 34–36 weeks and <34 weeks associated with NEFA tertiles. Women with term births were the referent group. Given the association between NEFAs and adiposity, we examined whether the association between NEFAs and sPTB was modified by prepregnancy BMI.

It is unknown how a variety of early-gestation biomarkers may cluster and potentially characterize patterns of risk for sPTB, so our second data-driven approach analyzed biomarkers and maternal characteristics using factor analysis. Factor analysis identifies correlations between multiple variables and creates a summary composite measure (or factor) which potentially relates to a common underlying mechanism. Factors are first created to be independent; however, loosening that restriction allows us to explore different rotations of the factors to find those that are the most interpretable. Many rotations provided similar results; we present the results from orthogonal varimax rotation, which minimizes the number of variables in each factor that contribute significantly to the value of the factor. Because factor analysis is also a data reduction technique, we used 6 of the 15 factors, explaining 61.5% of the variation in the original variables. Polytomous regression was then used to quantify the contribution of a 1-standard-deviation change in each of the 6 factors (modeled together) to risk of sPTB.

Pregnancies complicated by preeclampsia and gestational hypertension were excluded by design, but because these conditions are also likely to be associated with elevations in first-trimester NEFA levels, this may distort the distribution of biomarkers across the population and potentially exaggerate differences between persons with and without sPTB. We conducted sensitivity analyses to address this possibility. The overall rate of preeclampsia and gestational hypertension in the original cohort was 8% (166/2,075), and we identified 43 women in whom all markers except CRP and uric acid were measured early in gestation. In order to reflect the prevalence of preeclampsia and gestational hypertension in our source cohort, we created 10 simulated data sets that were enriched with 19 randomly selected cases. CRP and uric acid levels were imputed based on the mean and standard deviation from our study participants and log-transformed as appropriate, which resulted in concentrations that were very similar to the distribution of these markers evaluated in a separate set of women who developed preeclampsia. We then replicated all analyses in these 10 simulated data sets.

SAS, version 9.3 (SAS Institute, Inc., Cary, North Carolina), was used for all analyses, and nominal *P* values are provided for descriptive analyses.

RESULTS

This cohort of mostly nulliparous women, 34% of whom were black, had a median age of 23 years at delivery and a median prepregnancy BMI of 24.7 (Table 1). NEFA levels were measured, on average, at a median of 9.4 weeks' gestation (range, 4–20 weeks). The mean NEFA concentration was 0.28 mmol/L (standard deviation, 0.16; range, 0.04–0.95), and tertiles were defined within the study population at <0.19, 0.19–<0.33, and ≥0.33 mmol/L. Total cholesterol and LDL cholesterol levels varied significantly by tertile. Although levels of both total cholesterol and LDL cholesterol increased as NEFA tertile increased (Table 1), there were only modest correlations between NEFA and total cholesterol levels ($r = 0.14$, $P < 0.05$) and NEFA and LDL cholesterol levels ($r = 0.14$, $P < 0.05$) in these early-gestation samples (Table 2). NEFA levels were unrelated to gestational age at blood draw, CRP, or any other biomarkers in these samples, which were collected before 21 weeks' gestation.

Women with NEFA levels in the highest tertile had higher rates of preterm birth than those in the lowest tertile (Table 3). After accounting for maternal age, race, prepregnancy BMI, family history of gestational hypertension, periconceptional multivitamin use, and smoking, women with NEFA levels in the highest tertile were 2.02 (95% confidence interval (CI): 1.13, 3.48) times more likely to deliver at <37 weeks than were their counterparts with NEFAs in the lowest tertile. While there were no differences in rates of sPTB at 34–36 weeks according to early-pregnancy NEFA tertile, rates of sPTB at <34 weeks were significantly higher among women with NEFA levels in the highest tertile compared with the lowest (adjusted odds ratio (OR) = 3.73, 95% CI: 1.33, 10.44). There was no evidence of effect modification by prepregnancy overweight status ($P_{\text{interaction}} = 0.47$).

We then clustered 14 maternal characteristics and biomarkers using factor analysis to identify patterns of risk (Table 4). To interpret the factors, we identified the variables that had a factor loading magnitude of >0.50 and hence dominated the factor score. Factor 1, explaining 16% of total variance, clustered the adiposity characteristics. Factor 2, explaining 14% of the variance, clustered the socioeconomic characteristics. Factor 3, explaining 10% of the variance, clustered together black race and nonuse of periconceptional multivitamins. Factor 4, explaining 8% of total variance, clustered together smoking during pregnancy and less than a high school education. None of the first 4 factors were associated with sPTB. Factor 5 clustered NEFA levels with HDL cholesterol. Despite explaining only 7.5% of the variation in the risk factors, each standard-deviation increase in this NEFA–HDL cholesterol cluster (which increased as both NEFA and HDL cholesterol levels increased) was associated with excess risks of sPTB at 34–36 weeks and <34 weeks (OR = 1.5 (95% CI: 1.1, 1.9) and OR = 1.9 (95% CI: 1.3, 2.8), respectively). Factor 6, explaining 7% of the variance, accounted for family history of gestational hypertension, and a 1-standard-deviation increase was associated with sPTB at <34 weeks (OR = 1.7, 95% CI: 1.2, 2.3). We then further explored the NEFA–HDL cholesterol association. These biomarkers were not correlated ($r = 0.06$, $P = 0.29$), nor was there evidence of multiplicative interaction ($P = 0.17$ and $P = 0.74$ for tertiles 2 and 3, respectively).

Table 1. Characteristics of Participants According to Tertile of Nonesterified (Free) Fatty Acid Level, Pregnancy Exposures and Preeclampsia Prevention Study, 1997–2001

	Tertile of NEFA Level[a]								*P* Value
	Total (*n* = 337)		Low (*n* = 111)		Medium (*n* = 113)		High (*n* = 113)		
	%	Median (IQR[b])	%	Median (IQR)	%	Median (IQR)	%	Median (IQR)	
Age at delivery, years		23.0 (20.0–29.0)		23.0 (20.0–32.0)		23.0 (20.0–27.0)		24.0 (20.0–28.0)	0.17
Prepregnancy body mass index[c]		24.7 (21.7–29.7)		23.7 (20.9–28.7)		25.5 (22.2–30.4)		24.8 (21.4–30.9)	0.22
Black race	34.4		34.5		36.9		31.9		0.73
Less than high school education	9.8		9.7		8.1		11.5		0.69
Family history of preeclampsia	7.4		8		8.1		6.2		0.83
Smoking during pregnancy	33.8		27.4		40.5		33.6		0.12
Married or living with a partner	50.3		56.6		50		44.2		0.18
Nulliparous	56.1		57.5		54.1		56.6		0.86
Periconceptional multivitamin use	44.5		52.2		37.8		43.4		0.09
Gestational age at blood draw, weeks		9.4 (7.3–12.3)		10.3 (7.7–12.9)		8.9 (6.9–12.9)		8.9 (7.1–11.7)	0.09
Cholesterol concentration, mg/dL									
Total cholesterol		189.7 (169.4–219.2)		185.5 (165.9–209.6)		189.7 (169.1–212.7)		198.3 (176.0–234.7)	0.04
LDL cholesterol		106.1 (87.9–127.5)		101.7 (83.4–121.8)		104.8 (87.6–126.5)		112.9 (95.1–134.7)	0.04
HDL cholesterol		63.6 (54.1–77.0)		62.1 (53.1–76.8)		66.2 (56.1–75.5)		64.4 (54.3–78.0)	0.68
Triglyceride concentration, mg/dL		89.1 (67.5–123.6)		89.9 (71.6–123.3)		88.7 (69.2–127.2)		87.2 (63.9–121.8)	0.90
C-reactive protein concentration, μg/mL		2.7 (0.9–6.0)		2.6 (1.1–4.9)		2.6 (1.0–5.6)		3.05 (0.9–6.6)	0.76
Uric acid concentration, mg/dL		3.3 (2.6–4.0)		3.1 (2.5–3.9)		3.3 (2.8, 4.0)		3.3 (2.6, 4.1)	0.19

Abbreviations: HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; NEFA, nonesterified (free) fatty acid.

[a] Lowest tertile, <0.19 mmol/L; middle tertile, 0.19–<0.33 mmol/L; highest tertile, ≥0.33 mmol/L.

[b] 25th–75th percentiles.

[c] Weight (kg)/height $(m)^2$.

Table 2. Spearman Coefficients for Correlations Between Biomarkers Associated with Spontaneous Preterm Birth and Maternal Characteristics, Pregnancy Exposures and Preeclampsia Prevention Study, 1997–2001

Biomarker	Biomarker									
	BMI	Age	GW of BD	TC	Triglycerides	HDL-C	LDL-C	CRP	Uric Acid	NEFAs
BMI	1.00	0.09	−0.06	0.09	0.18[a]	−0.16[a]	0.14[a]	0.44[a]	0.35[a]	0.06
Age		1.00	−0.03	0.13[a]	0.09	0.07	0.08	0.19[a]	0.06	−0.02
GW of BD			1.00	0.48[a]	0.47[a]	0.33[a]	0.26[a]	0.31[a]	−0.05	−0.10
TC				1.00	0.44[a]	0.36[a]	0.83[a]	0.26[a]	0.16[a]	0.14[a]
Triglycerides					1.00	−0.05	0.28[a]	0.40[a]	0.26[a]	0.02
HDL-C						1.00	−0.10	−0.05	−0.13[a]	0.03
LDL-C							1.00	0.22[a]	0.17[a]	0.14[a]
CRP								1.00	0.22[a]	0.02
Uric acid									1.00	0.08
NEFAs										1.00

Abbreviations: BD, blood draw; BMI, body mass index; CRP, C-reactive protein; GW, gestational week; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NEFAs, nonesterified (free) fatty acids; TC, total cholesterol.

[a] $P < 0.05$.

We conducted a sensitivity analysis to ensure that the exclusion of women with preeclampsia or gestational hypertension did not distort the distribution of biomarkers and influence our results. Ten simulated data sets included randomly selected cases of women with preeclampsia or gestational hypertension in which all markers were measured ($n = 5$) or imputed ($n = 2$). In all simulations, NEFA levels measured before 21 weeks' gestation were associated with sPTB, especially cases with delivery before 34 weeks. Seven of the 10 simulations identified a new factor that combined increasing triglyceride levels with LDL cholesterol, which was associated with sPTB at 34–36 weeks. Consistent with our main findings, however, all runs identified a factor comprised of NEFAs and HDL cholesterol in which increasing concentrations were associated with sPTB. As in our main findings, estimates relating this factor to sPTB at <34 weeks were higher than those for sPTB at 34–36 weeks.

Table 3. Risk of Spontaneous Preterm Birth According to Tertile of Early-Gestation NEFA Level, Pregnancy Exposures and Preeclampsia Prevention Study, 1997–2001

sPTB Outcome and NEFA Tertile[a]	No. of sPTBs	%	Crude OR	Adjusted OR[b]	95% CI
sPTB at <37 weeks ($n = 115$)					
Tertile 1	33	29.2	1.0	1.0	Referent
Tertile 2	33	29.7	1.03	1.12	0.61, 2.05
Tertile 3	49	43.4	1.86	2.02	1.13, 3.48
sPTB at 34–36 weeks ($n = 82$)					
Tertile 1	26	23.0	1.0	1.0	Referent
Tertile 2	24	21.6	0.9	1.17	0.53, 2.00
Tertile 3	32	28.3	1.53	1.03	0.90, 3.27
sPTB at <34 weeks ($n = 33$)					
Tertile 1	7	6.2	1.0	1.0	Referent
Tertile 2	9	8.1	1.32	1.47	0.48, 4.53
Tertile 3	17	15.0	3.04	3.73	1.33, 10.44

Abbreviations: CI, confidence interval; NEFA, nonesterified (free) fatty acid; OR, odds ratio; sPTB, spontaneous preterm birth.

[a] Lowest tertile, <0.19 mmol/L; middle tertile, 0.19–<0.33 mmol/L; highest tertile, ≥0.33 mmol/L.

[b] Adjusted for maternal age, race, prepregnancy body mass index, family history of preeclampsia, periconceptional multivitamin use, and smoking.

DISCUSSION

Our results indicate that higher levels of NEFAs assessed at gestational week 9 (interquartile range, 7–12 weeks) are associated with excess risk of sPTB, particularly births occurring before 34 weeks' gestation. When combined with a panel of biomarkers and maternal risk factors, NEFAs clustered with HDL cholesterol, and increasing concentrations of both were associated with elevated risks of both moderate (34–36 weeks) and earlier (<34 weeks) sPTB.

NEFAs are proinflammatory, are markers of tissue damage, and directly perturb membranes. They also serve as a substrate for oxidative processes generating products, such as lipid radicals and aldehydes, that have potent physiological effects. Higher NEFA levels have been associated with preeclampsia, the leading cause of indicated preterm births (14, 15), and one possible explanation is that higher NEFA levels in early gestation could be a marker of a proinflammatory, proatherogenic phenotype that could play a role in a cascade that triggers preterm labor or premature membrane rupture. This possibility is consistent with a recent observation that

Table 4. Results of Factor Analysis Conducted to Identify Patterns of Risk for Spontaneous Preterm Birth, Pregnancy Exposures and Preeclampsia Prevention Study, 1997–2001

Factor and Dominant Characteristic(s)	% of Variance Explained	sPTB at 34–36 Weeks		sPTB at <34 Weeks	
		OR[a]	95% CI	OR[a]	95% CI
Factor 1					
Prepregnancy body mass index	15.7	1.1	0.9, 1.5	1.0	0.6, 1.4
C-reactive protein					
Uric acid level					
Triglyceride level					
Factor 2					
Maternal age	13.9	1.0	0.8, 1.3	0.8	0.5, 1.1
Married or living with partner					
Nulliparity					
Factor 3					
Black race	9.5	1.0	0.7, 1.2	1.3	0.9, 1.9
No multivitamin use					
Factor 4					
Less than high school education	7.8	1.1	0.9, 1.4	1.4	1.0, 2.0
Smoking					
Factor 5					
NEFA level	7.5	1.5	1.1, 1.9	1.9	1.3, 2.8
HDL cholesterol level					
Factor 6					
Family history of preeclampsia	7.1	1.0	0.7, 1.3	1.7	1.2, 2.3

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; NEFA, nonesterified (free) fatty acid; OR, odds ratio; sPTB, spontaneous preterm birth.

[a] OR per 1-standard-deviation change in the characteristic, or for the presence of the characteristic compared with absence.

elevated prepregnancy BMI is a risk factor for early sPTB (16). Within the first half of pregnancy, during which the samples in our study were collected, NEFA concentrations were unrelated to increasing week of gestation. In contrast, levels of all of the other lipids and CRP increased as the gestational week of sample collection increased. Thus, the NEFA concentrations in our study may have reflected prepregnancy levels, although we were unable to directly evaluate this possibility. In addition, although NEFA levels were related to concentrations of total cholesterol and LDL cholesterol, they were not correlated with CRP, the only inflammatory marker included. Thus, the possibility that NEFAs may mark a predisposing atherogenic, proinflammatory phenotype warrants additional study that better characterizes the inflammatory signature. Our results are consistent with those of the only other study relating NEFA levels (in the third trimester) to preterm birth risk (7). Notably, the risk of sPTB associated with NEFAs measured at 30 weeks' gestation in that study was increased 2-fold—remarkably similar to the estimate we detected in serum collected at a mean gestational age of 9 weeks.

Inflammation is a central component of many human reproductive processes, including ovulation, implantation, and parturition (17). Excess inflammation has been proposed as a cause of preterm birth, particularly early preterm birth. Increased inflammatory markers have been detected in maternal serum, amniotic and cervical fluid, umbilical venous plasma, and umbilical cord blood in mothers and infants delivered preterm. However, levels of individual cytokines evaluated at midgestation have not been prognostic of preterm birth (18). We speculate that the proinflammatory effect of NEFAs, along with their oxidative properties, might antedate conception, possibly interfere with placentation, and contribute to at least a portion of sPTBs. Alternatively, it is possible that higher NEFA levels may be a marker for other causal processes such as placental injury. Future mechanistic studies are needed to directly evaluate these possibilities.

The clusters of biomarkers and maternal characteristics identified in our study indicated that although many factors commonly associated with sPTB were indeed correlated with each other, after we accounted for these correlations only the clusters dominated by NEFAs, HDL cholesterol, and family history of gestational hypertension appeared to be importantly related to sPTB. The NEFA–HDL cholesterol association is novel and may appear surprising, because although increased NEFA levels have deleterious biologic effects, increasing HDL concentrations protect against atherogenesis. An emerging body of evidence, however, has suggested that

dysfunctional HDL cholesterol metabolism may be related to inflammation-related outcomes such as atherosclerosis (19, 20). The composition and function of HDL cholesterol change in the setting of inflammation, such that its antioxidant role, largely due to paraoxonase 1 (21), is transformed to promote oxidation and the classic HDL reverse cholesterol transport function is impeded. HDL cholesterol levels and function are altered in persons with immune-related conditions such as rheumatoid arthritis, lupus, and multiple sclerosis, and animal models suggest a role of dysfunctional HDL cholesterol metabolism in female infertility (22). How HDL cholesterol function may be altered during pregnancy and perhaps be associated with risk of sPTB is, to our knowledge, unknown.

Our findings must be considered in light of limitations. We analyzed levels of NEFA and other biomarkers at a single time point during the first half of pregnancy. The trajectory of change across the entire duration of gestation may also be informative, and this warrants additional study. In addition, although our pregnancy samples were taken in nonfasting persons, adults in modern society spend many of their waking hours in the nonfasting state, and recent studies suggest that nonfasting levels of fasting-sensitive lipids (especially triglycerides and NEFAs) may predict adverse nonpregnancy outcomes (such as cardiovascular events) better than or similarly to fasting levels (23). Furthermore, postprandial responses, such as those relating to glucose and triglyceride metabolism, may trigger a number of proatherosclerotic and prothrombotic processes, including inflammation, oxidative stress, and vasoconstriction (23). In addition, differences in postprandial status at the time of venipuncture are unlikely to occur systematically between women who later develop sPTB and controls. By design, our study excluded women with preeclampsia and gestational hypertension, and although our sensitivity analysis suggested that this did not bias our findings, our results should be replicated. Strengths of our study include the prospectively assembled cohort from which our nested cases and controls were selected, measurement of multiple biomarkers, and the use of innovative analytical approaches to consider the correlation and interplay of these factors, which may be important in the pathophysiology of sPTB.

In summary, our study indicates that women with NEFA levels in the highest tertile during the first half of pregnancy have an excess risk of sPTB, particularly delivery before 34 weeks. We also detected a novel pattern of risk suggesting that higher NEFA levels in conjunction with higher HDL cholesterol levels may be associated with sPTB.

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