Lipoprotein particle concentration measured by nuclear magnetic resonance spectroscopy is associated with gestational age at delivery: a prospective cohort study

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Objective To estimate the association between lipoprotein particle concentrations in pregnancy and gestational age at delivery.

Design Prospective cohort study.

Setting The study was conducted in the USA at the University of North Carolina.

Population We assessed 715 women enrolled in the Pregnancy, Infection, and Nutrition study from 2001 to 2005.

Methods Fasting blood was collected at two time points (<20 and 24–29 weeks of gestation). Nuclear magnetic resonance (NMR) quantified lipoprotein particle concentrations [low-density lipoprotein (LDL), high-density lipoprotein (HDL), very-low density lipoprotein (VLDL)] and 10 subclasses of lipoproteins. Concentrations were assessed as continuous measures, with the exception of medium HDL which was classified as any or no detectable level, given its distribution. Cox proportional hazards models estimated hazard ratios (HR) for gestational age at delivery adjusting for covariates.

Main outcome measures Gestational age at delivery, preterm birth (<37 weeks of gestation), and spontaneous preterm birth.

Results At <20 weeks of gestation, three lipoproteins were associated with later gestational ages at delivery [large LDL_{NMR} (HR 0.78, 95% CI 0.64–0.96), total $VLDL_{NMR}$ (HR 0.77, 95% CI 0.61–0.98), and small $VLDL_{NMR}$ (HR 0.78, 95% CI 0.62–0.98], whereas large $VLDL_{NMR}$ (HR 1.19, 95% CI 1.01–1.41) was associated with a greater hazard of earlier delivery. At 24–28 weeks of gestation, average $VLDL_{NMR}$ (HR 1.25, 95% CI 1.03–1.51) and a detectable level of medium HDL_{NMR} (HR 1.90, 95% CI 1.19–3.02) were associated with earlier gestational ages at delivery.

Conclusion In this sample of pregnant women, particle concentrations of $VLDL_{NMR}$, LDL_{NMR}, IDL_{NMR}, and HDL_{NMR} were each independently associated with gestational age at delivery for all deliveries or spontaneous deliveries <37 weeks of gestation. These findings may help formulate hypotheses for future studies of the complex relationship between maternal lipoproteins and preterm birth.

Keywords Cholesterol, dyslipidaemia, gestational age at delivery, lipoproteins, nuclear magnetic resonance spectroscopy, preterm birth.

Tweetable abstract Nuclear magnetic resonance spectroscopy may identify lipoprotein particles associated with preterm delivery.

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Introduction

Maternal metabolic changes in pregnancy increase fat storage and subsequent mobilization of these stores to support the growing fetus. In the second trimester, increasing estrogen, insulin, and insulin resistance increases maternal serum fatty acids and lipoproteins. The relationship between aberrations in lipid metabolism and adverse

cardiovascular and metabolic outcomes in adults is well described. In pregnant women, exaggerations in lipoproteins are associated with adverse pregnancy outcomes such as pre-eclampsia and gestational diabetes.[1,2] These pregnancy complications are known risk factors for future metabolic diseases.[3,4] Maternal dysmetabolic conditions may also enhance cardiovascular disease susceptibility in offspring.[5,6]

Preterm birth is the leading cause of morbidity and mortality among neonates without congenital anomalies in the USA.[7,8] Epidemiologic studies have demonstrated complex associations between maternal metabolic disease, preterm birth, and maternal risk of cardiovascular complications later in life.[9–16] The mechanism linking metabolic exposures, pregnancy complications, and subsequent cardiovascular outcomes is not well understood, however. Previous studies associating maternal dyslipidaemia with preterm birth risk vary by timing of sample collection, whether or not the patient was fasting, lipids measured, and the technique used to measure lipid concentrations. They also rely on traditional chemical techniques for measuring lipid concentrations that measure the cholesterol content of a person's low-density (LDL-C) and high-density (HDL-C) lipoprotein particles, but not the absolute number of lipoprotein particles. Previous studies have thus associated lipid concentrations and not lipoprotein particle concentrations with pregnancy outcomes.

Nuclear magnetic resonance (NMR) spectroscopy is an alternative method of measuring lipoproteins that relies on the characteristic NMR signals of lipoprotein particles to quantify particle size and concentration.[17] This is clinically relevant because chemically measured lipoprotein lipid levels and NMR-measured lipoprotein particle numbers are not comparable. Two patients with the same lipid concentration may have vastly different concentrations of particles in their serum, and thus different risk profiles.[18] Studies in non-pregnant patients have demonstrated that lipid particle concentrations measured by NMR have significant and independent associations with cardiovascular disease events, regardless of lipid concentrations.[19–21] NMR has been used in hundreds of studies in non-pregnant populations and has yielded new insights into the roles of particle subtypes in health outcomes. To date, only one study has reported the use of NMR to associate maternal dyslipidaemia with preterm bith; however, this study was limited by the fact that it was a case–control study of women with a history of preterm birth, used samples from non-fasting patients, and had a different primary outcome.[22] In addition, the investigators only had one sample for each patient and were not able to assess changes in lipids at two time points in pregnancy. The objective of our study was to use NMR to determine whether lipoprotein particle concentrations at two points in pregnancy were associated with gestational age at delivery, specifically preterm birth and spontaneous preterm birth, in a cohort of pregnant women.

Methods

Study population

The Pregnancy, Infection, and Nutrition Study (PIN) was a large, multiphase, cohort study, the primary aim of which was to identify aetiologic factors for preterm delivery. The third phase of the study (PIN3) recruited women from prenatal clinics at the University of North Carolina before 20 weeks of gestation from 2001 to 2005 ($n = 2006$). Women attended two research clinic visits (at <20 and 24–29 weeks of gestation), providing a fasting blood sample at each visit, completed two telephone interviews (at 17–22 and 27–30 weeks of gestation), and self-administered questionnaires at each clinic visit. Medical charts were abstracted following delivery. Women were excluded from participating in PIN3 if they were non-English speaking, <16 years of age, carrying multiple gestations, not planning to continue care or deliver at the study hospital, or did not have a telephone for completing the interviews. The study protocols were approved by the Institutional Review Board at the University of North Carolina at Chapel Hill.

Of those eligible, 967 (63%) women agreed to participate in the two research clinic visits and provide fasting blood samples, and 755 (78.1%) consented to and had at least one blood sample drawn. We further excluded women missing data on gestational age at delivery ($n = 3$) and those with a history of diabetes ($n = 37$). The final sample included 715 women who provided at least one blood sample for analysis: 662 (92.6%) had two blood samples and 53 (7.4%) had one blood sample drawn during pregnancy.

Age, race, Hispanic ethnicity, education, and income were reported during the first telephone interview; the average number of cigarettes smoked in the first 6 months of pregnancy was reported during the second phone interview. Pre-pregnancy body mass index (BMI) was calculated from self-reported weight and measured height, and was categorised according to the recommendations of the Institute of Medicine (2009).[23] Gestational age was determined using both early ultrasound and last menstrual period. In the PIN study, 96% of participants had an ultrasound performed <22 weeks of gestation that was used to confirm the gestational age. Spontaneous preterm birth was based on clinical determination.

Lipoprotein particle analysis

Lipoproteins were measured by NMR Lipoprofile®-II autoanalyser (formerly of Liposcience, Inc., Raleigh, NC, USA; now Laboratory Corporation of America, Burlington, NC, USA). This technology allowed for the assessment of each participant's lipoprotein subclass particle size (in

nanometres) and concentration (particle nanomoles/l or μmol/l), and included total LDL_{NMR}, HDL_{NMR}, and $VLDL_{NMR}$, and subclasses by particle size. It also provided calculated values for mean $VLDL_{NMR}$, LDL_{NMR}, and HDL_{NMR} particle size, as well as total triglyceride levels. The laboratory determined the thresholds for particle size that were used to categorise the lipoprotein particle size and concentration measurements from each plasma sample into 16 subclasses, yielding up to 32 total assessments per participant.

Lipoprotein particle concentrations and sizes were assessed as continuous variables. Most women (70%) had no detectable medium HDL_{NMR} concentration at either time point and, as such, medium HDL_{NMR} was categorised as any or no detectable concentration.

Statistical analysis

Descriptive analyses were conducted to compare maternal and infant characteristics by preterm birth status. The associations between gestational age at delivery and lipoprotein particle concentrations, scaled to one standard deviation (1 SD), were assessed using adjusted and unadjusted Cox proportional hazard models, with gestational age as the time scale. Time-varying coefficients were employed to estimate separate hazard ratios (HRs) and 95% confidence intervals (95% CIs) during the time period before and after 37 weeks of gestation, with HRs of delivery before 37 weeks of gestation being the target of inference. Adjustments were made for covariates identified a priori from the literature as being associated with either preterm birth or dyslipidaemia, including age, race, education, income, pre-pregnancy BMI, and smoking. Each lipoprotein subclass was examined separately, adjusting for other lipoprotein subclasses and triglycerides, to estimate the direct effect of lipoproteins on preterm birth, independent of other lipids. For example, LDL and VLDL models adjusted for total HDL and triglyceride concentrations, whereas HDL models adjusted for total LDL and triglyceride concentrations. We considered lipoprotein particle concentrations measured at <20 weeks of gestation and those measured at 24–29 weeks of gestation separately. We also assessed the absolute change in lipoprotein particle concentrations between the two time points for all lipoproteins, controlling for baseline concentrations, with the exception of medium HDL_{NMR}. We performed each of these analyses including all preterm births (defined as live births <37 weeks of gestation) and for spontaneous preterm births only.

We multiply imputed missing lipid data for those with only one blood sample (n = 53 women) or missing covariate data (n = 122) using Markov chain Monte Carlo methods (n = 50 imputations). Analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA).

Results

Women in our analytic sample were predominantly white, married, and had greater than a high school education. In addition, 32.2% had an income <185% of the poverty line, 21.0% were obese, and 10.3% smoked cigarettes <20 weeks of gestation. Birth outcomes included 100 (14.0%) preterm births and among those, 54 (7.6%) were spontaneous preterm births. Demographic information and characteristics of the sample, stratified by preterm birth outcomes, are presented in Table 1. Preterm deliveries were more common among black women, women with lower education and incomes, as well as among women with a BMI indicative of underweight and overweight, compared with full-term deliveries.

Mean particle concentrations and average particle sizes stratified by preterm birth status and timing of blood draw are listed in Table 2. Most lipids increase over the two time points regardless of preterm birth status, with the exception of medium HDL_{NMR}. In addition, particle size changes were much smaller in magnitude compared with particle concentration changes.

All deliveries (preterm and term)

Adjusted HRs for the association between lipoprotein particle concentrations and timing of delivery <37 weeks of gestation are shown in Table 3 (unadjusted results are shown in Table S1). At <20 weeks of gestation, a 1-SD change in large LDL_{NMR} particle concentrations (HR 0.78, 95% CI 0.64–0.96), and total (HR 0.77, 95% CI 0.61–0.98) and small (HR 0.78, 95% CI 0.62–0.98) $VLDL_{NMR}$ particle concentrations, were associated with older gestational ages at delivery. At this same time point, a 1-SD change in large $VLDL_{NMR}$ particle concentrations was associated with an increased risk of earlier delivery (HR 1.19, 95% CI 1.01–1.41). At 24–28 weeks of gestation, these associations were not observed; however, the presence of a detectable level of medium HDL_{NMR} (HR 1.90, 95% CI 1.19–3.02) and a 1-SD change in average $VLDL_{NMR}$ particle size (HR 1.25, 95% CI 1.03–1.51) were associated with an increased hazard of earlier delivery at this time point. A 1-SD change in total LDL_{NMR} from <20 to 24–28 weeks of gestation was associated with an HR of 0.76 (95% CI 0.60–0.97), corresponding to older gestational ages at delivery.

The other time-varying coefficient in the Cox model described associations between lipoprotein particle concentrations and timing of term births. All 95% CIs for these variables included the null value of 1, indicating that lipid levels were unrelated to gestational age at delivery among term births (data not shown).

Table 1. Characteristics of pregnant women by preterm birth status (n = 715), PIN study, 2001–2005

	Full-term deliveries		All preterm deliveries		*P*	Spontaneous preterm deliveries only	
Overall, *n* (%)	615	86.0	100	14.0	<0.0001	54	7.2
Gestational age (mean, SD)	39.2	1.2	33.4	3.9	<0.0001	32.9	4.3
Maternal age (mean, SD)	28.8	5.5	28	6.5	0.28	26.8	6.4
Maternal age, *n* (%)							
≤24 years	144	23.4	28	28.0	0.06	19	35.2
25–29 years	172	28.0	32	32.0		14	25.9
30–34 years	213	34.6	21	21.0		14	25.9
35+ years	86	14.0	19	19.0		7	8.1
Maternal race & Hispanic ethnicity, *n* (%)							
Non-Hispanic white	461	75.0	62	62.0	0.002	35	64.8
Non-Hispanic black	103	16.8	32	32.0		16	29.6
Non-Hispanic other	50	8.1	6	6.0		3	5.6
Hispanic	0	0.0	0	0.0		0	0.0
Education, *n* (%)							
Less than high school	42	6.8	10	10.0	0.0002	5	9.3
High school graduate	77	12.5	26	26.0		18	33.3
Some college	122	19.8	24	24.0		11	20.4
College graduate	374	60.8	40	40.0		20	37.0
Income (% of poverty), *n* (%)							
<185%	113	18.4	32	32.0	0.002	21	38.9
185–350%	142	23.1	16	16.0		5	9.3
>350%	334	54.3	42	42.0		22	40.7
Pre-pregnancy body mass index, *n* (%)							
Underweight	26	4.2	8	8.0	0.02	6	11.1
Normal weight	342	55.6	41	41.0		23	42.6
Overweight	118	19.2	28	28.0		14	25.9
Obese	129	21.0	21	21.0		11	20.4
Prenatal cigarette smoking, *n* (%)*	61	9.9	13	13.0	0.15	8	14.8
Any MVPA, *n* (%)**	402	65.4	56	56.0	0.11	27	50.0
Nulliparous, *n* (%)	305	49.6	43	43.0	0.26	22	40.7
Delivered a low-birthweight infant, *n* (%)	9	1.5	60	60.0	<0.0001	34	63.0
Delivered an SGA infant, *n* (%)	33	5.4	9	9.0	0.11	1	1.9

SD, standard deviation; MVPA, moderate to vigorous physical activity; SGA, small-for-gestational age. Data were missing for the following: race and Hispanic ethnicity (n = 1); income (n = 36); pre-pregnancy body mass index (n = 2); prenatal cigarette smoking (n = 51); MVPA (n = 3); delivered a low-birthweight infant (n = 3); delivered an SGA infant (n = 99).

*Cigarette smoking in the first 6 months.

**MVPA at the first phone interview (17–22 weeks of gestation).

Spontaneous deliveries (preterm and term)

Adjusted HRs for the association between lipoprotein particle concentrations and timing of spontaneous preterm birth <37 weeks of gestation are shown in Table 4 (unadjusted results are shown in Table S2). At <20 weeks of gestation, a 1-SD change in large LDL_{NMR} was associated with older gestational ages of spontaneous delivery (HR 0.73, 95% CI 0.55–0.97). At 24–28 weeks of gestation, a 1-SD change in total LDL_{NMR} (HR 0.67, 95% CI 0.48–0.94) and IDL_{NMR} (HR 0.68, 95% CI 0.47–0.98) particle concentrations were also associated with older gestational ages. The presence of a detectable level of medium HDL_{NMR} at 24–29 weeks of gestation was associated with an increased risk of earlier spontaneous delivery (HR 1.94, 95% CI 1.01–3.75). A 1-SD change in total LDL_{NMR} from <20 to 24–28 weeks of gestation was associated with a hazard ratio for spontaneous delivery of 0.59 (95% CI 0.41–0.85), and thus older gestational ages.

All 95% CIs for associations between lipoprotein particle concentrations and timing of spontaneous delivery for term births included the null value of 1, indicating that lipid levels were unrelated to gestational age at delivery among term births (data not shown).

Table 2. Lipoprotein particle concentrations and average particle size, by timing of blood draw; PIN study, 2001–2005 (n = 715)

	Full-term deliveries				All preterm deliveries			
	<20 weeks		24–28 weeks		<20 weeks		24–28 weeks	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LDL_{NMR}								
Total	1120.05	363.97	1374.57	464.84	1154.41	456.93	1328.50	497.20
IDL	38.73	46.16	81.46	70.97	38.67	49.29	73.97	74.87
Large	669.71	239.73	845.36	304.56	598.03	237.91	793.54	313.57
Medium	82.83	81.95	87.84	97.86	106.37	101.06	98.04	108.48
Small	328.77	321.69	359.89	395.71	411.32	394.15	362.93	411.22
HDL_{NMR}								
Total	32.78	5.30	33.30	5.62	33.65	5.95	34.02	6.66
Large	11.98	3.10	12.74	2.90	11.66	3.50	12.87	3.23
Medium	0.81	1.73	0.33	1.15	1.00	2.08	0.68	1.80
Small	19.98	4.51	20.23	4.90	20.98	5.18	20.46	5.63
$VLDL_{NMR}$								
Total	58.91	34.31	71.91	43.20	56.28	33.23	68.01	44.43
Large	1.54	2.28	2.87	3.25	2.22	3.70	3.53	4.70
Medium	27.52	20.53	34.52	23.52	27.60	19.35	32.74	23.82
Small	29.84	18.42	34.50	24.73	26.45	19.52	31.73	25.27
Average particle size								
LDL_{NMR}	21.85	0.74	21.92	0.75	21.66	0.86	21.89	0.82
HDL_{NMR}	9.70	0.37	9.77	0.37	9.64	0.40	9.76	0.40
$VLDL_{NMR}$	49.15	8.08	51.18	8.26	50.33	7.33	53.40	10.02
Total cholesterol	203.12	35.86	244.49	46.64	198.02	37.55	235.76	49.77
Triglycerides	121.05	51.01	166.63	64.76	131.55	70.72	175.28	82.15

Concentrations were measured in particle nanomoles/l for ($VLDL_{NMR}$ and HDL_{NMR}) and μmoles/l (for HDL_{NMR}); particle size was measured in nanometres.

Discussion

Main findings

In this sample of pregnant women from the PIN cohort, we used NMR to perform advanced measurements of lipoprotein particle concentrations at two time points in pregnancy. NMR was further able to characterise subclasses of these particles that were associated with gestational age at delivery. We found that particle concentrations of $VLDL_{NMR}$, LDL_{NMR}, IDL_{NMR}, and HDL_{NMR} were each independently associated with gestational age at delivery for all deliveries or for spontaneous deliveries <37 weeks of gestation. These associations were dynamic and dependent upon the time point in pregnancy at which they were assessed, such that lipoprotein particle concentrations at <20 weeks of gestation had different associations with timing of delivery than those measured at 24–28 weeks of gestation. Higher particle concentrations of these lipoproteins at <20 weeks of gestation (small $VLDL_{NMR}$, total $VLDL_{NMR}$, and large LDL_{NMR}) were associated with older gestational ages at delivery, but these associations were not observed at 24–28 weeks of gestation. Medium HDL_{NMR} was associated with earlier delivery at 24–28 weeks of gestation. Similar associations were observed with spontaneous delivery for large LDL_{NMR} (<20 weeks of gestation) and medium HDL_{NMR} (24–28 weeks of gestation), with additional associations observed with older gestational ages of spontaneous deliveries for total LDL and IDL. Medium HDL_{NMR} is a unique particle, as most women did not have a detectable level. When treated as a dichotomous variable, we found that any detectable concentration of medium HDL_{NMR} was associated with earlier deliveries among all preterm and spontaneous preterm deliveries.

Strengths and limitations

This study builds on prior studies by examining the association between lipoprotein particle concentrations and gestational age at delivery using an advanced method of lipoprotein measurement. The strengths of this study include the large prospective cohort design, medical records and birth outcomes readily available, and abstracted by trained study personnel in a standardised manner, and the collection of fasting blood samples at two time points in

Table 3. Adjusted hazard ratios* for the association between lipids and gestational age at delivery for births <37 weeks of gestation, PIN study, 2001–2005 (n = 715)

	<20 weeks		24–28 weeks		Change from <20 to 24–28 weeks	
	HR	95% CI	HR	95% CI	HR	95% CI
LDL_{NMR}						
Total	1.04	(0.85–1.27)	0.86	(0.69–1.07)	**0.76**	**(0.60–0.97)**
IDL	0.95	(0.77–1.17)	0.84	(0.66–1.06)	0.84	(0.66–1.08)
Large	**0.78**	**(0.64–0.96)**	0.82	(0.66–1.01)	0.97	(0.77–1.23)
Medium	1.21	(1.00–1.45)	1.10	(0.90–1.34)	0.95	(0.76–1.20)
Small	1.18	(0.97–1.42)	0.99	(0.81–1.22)	0.82	(0.66–1.03)
HDL_{NMR}						
Total	1.18	(0.96–1.44)	1.06	(0.86–1.30)	0.90	(0.71–1.15)
Large	0.97	(0.79–1.18)	0.98	(0.80–1.21)	1.01	(0.79–1.29)
Medium**	1.16	(0.77–1.74)	**1.90**	**(1.19–3.02)**		
Small	1.19	(0.97–1.45)	1.00	(0.81–1.23)	0.80	(0.62–1.02)
$VLDL_{NMR}$						
Total	**0.77**	**(0.61–0.98)**	0.88	(0.70–1.12)	1.03	(0.81–1.30)
Large	**1.19**	**(1.01–1.41)**	1.15	(0.95–1.38)	1.05	(0.86–1.27)
Medium	0.82	(0.64–1.04)	0.87	(0.69–1.11)	0.93	(0.72–1.20)
Small	**0.78**	**(0.62–0.98)**	0.89	(0.71–1.11)	1.02	(0.82–1.28)
Average Particle Size LDL_{NMR}	0.84	(0.69–1.02)	0.94	(0.77–1.15)	1.11	(0.88–1.40)
Average Particle Size HDL_{NMR}	0.91	(0.75–1.11)	1.01	(0.82–1.24)	1.16	(0.92–1.47)
Average Particle Size $VLDL_{NMR}$	1.14	(0.96–1.36)	**1.25**	**(1.03–1.51)**	1.23	(0.98–1.55)

Entries in bold indicate statistically significant estimates ($P < 0.05$).

*Adjusted for measured triglyceride concentration, measured total HDL concentration (LDL and VLDL models), measured total LDL concentration (HDL models), maternal age, race (white as reference), number of years of education, household income expressed as percentage of the federal poverty line, pre-pregnancy body mass index (normal weight as reference), and smoking.

**Medium HDL_{NMR} is categorised as any or no detectable concentration; the change in medium HDL_{NMR} from <20 to 24–28 weeks of gestation was not assessed.

pregnancy. The use of NMR to characterise lipoprotein particle concentrations has only been reported once in a study of pregnant women, and represents a novel way to investigate the association between dyslipidaemia and adverse pregnancy outcomes.

There are limitations. Some measures (e.g. pre-pregnancy BMI, income, and smoking) were self-reported and are subject to misclassification from recall. Confounding by unmeasured factors may have affected the associations that were observed. The generalisability of this study may be limited because the women were recruited from one clinic in North Carolina, and probably do not represent the general population. In addition, the women in our analytic sample were not necessarily representative of the original pregnancy cohort because of exclusion criteria, and thus selection bias may affect our results.

Interpretation

Previous studies have demonstrated associations between lipid concentrations and both spontaneous and indicated preterm birth; however, these studies did not assess lipid particle concentrations. In studies of non-pregnant populations, lipid concentration and particle concentration are not equivalent in their association with outcomes (i.e. cardiovascular disease risk).[19] Thus, we sought to identify specific particles and particle concentrations that were associated with gestational age at delivery among women in our study.

In addition, previous studies varied in their methodology, such as the collection of non-fasting samples, assessment of different lipids, the technology used to measure concentrations, and outcome definitions. These differences make direct comparison with our results difficult. Prior studies have demonstrated conflicting results. For example, Catov et al.[13] reported that mean concentrations of total cholesterol (from non-fasting samples) at <15 weeks of gestation were higher among women with preterm birth <34 weeks of gestation, compared with those with term births. More recently, Mudd et al.[24] reported increased odds of preterm birth among women with low total

Table 4. Adjusted hazard ratios* for the association between lipids and timing of delivery among spontaneous deliveries (<37 weeks of gestation) only

	<20 weeks		24–28 weeks		Change from <20 to 9 24–28 weeks	
	HR	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
LDL_{NMR}						
Total	0.93	(0.70–1.24)	**0.67**	**(0.48–0.94)**	**0.59**	**(0.41–0.85)**
IDL	0.89	(0.66–1.20)	**0.68**	**(0.47–0.98)**	0.69	(0.47–1.01)
Large	**0.73**	**(0.55–0.97)**	0.75	(0.56–1.00)	0.92	(0.67–1.27)
Medium	1.21	(0.95–1.56)	1.06	(0.80–1.40)	0.90	(0.65–1.23)
Small	1.11	(0.85–1.45)	0.86	(0.64–1.17)	0.72	(0.52–1.00)
HDL_{NMR}						
Total	1.14	(0.87–1.50)	1.14	(0.86–1.51)	1.07	(0.77–1.48)
Large	0.97	(0.74–1.27)	0.97	(0.73–1.29)	0.99	(0.70–1.39)
Medium**	1.09	(0.62–1.91)	**1.94**	**(1.01–3.75)**		
Small	1.16	(0.88–1.52)	1.08	(0.82–1.43)	0.96	(0.69–1.33)
$VLDL_{NMR}$						
Total	0.76	(0.55–1.04)	0.95	(0.70–1.29)	1.15	(0.84–1.57)
Large	1.16	(0.93–1.46)	1.12	(0.87–1.44)	1.05	(0.80–1.36)
Medium	0.84	(0.62–1.14)	0.96	(0.71–1.30)	1.04	(0.75–1.43)
Small	0.74	(0.54–1.00)	0.91	(0.67–1.23)	1.09	(0.81–1.47)
Average Particle Size LDL_{NMR}	0.85	(0.65–1.10)	0.99	(0.75–1.32)	1.19	(0.86–1.64)
Average Particle Size HDL_{NMR}	0.94	(0.72–1.23)	1.03	(0.78–1.36)	1.14	(0.82–1.58)
Average Particle Size $VLDL_{NMR}$	1.17	(0.93–1.47)	1.24	(0.95–1.62)	1.20	(0.87–1.67)

Entries in bold indicate statistically significant estimates ($P < 0.05$).

*Adjusted for measured triglyceride concentration, measured total HDL concentration (LDL and VLDL models), measured total LDL concentration (HDL models), maternal age, race (white as reference), number of years of education, household income expressed as a percentage of the federal poverty line, pre-pregnancy body mass index (normal weight as reference), and smoking.

**Medium HDL_{NMR} is categorised as any or no detectable concentration; the change in medium HDL_{NMR} from <20 to 24–28 weeks of gestation was not assessed.

cholesterol, low HDL-C, and low LDL-C levels from non-fasting samples at 15–27 weeks of gestation.

Similar to our observation that average $VLDL_{NMR}$ (24–28 weeks of gestation) was associated with earlier gestational age at delivery, Thorp et al.[22] observed an increased odds of recurrent preterm birth (<35 weeks of gestation) per nanometre increase in average $VLDL_{NMR}$ particle size (OR 1.04, 95% CI 1.01–1.08). Our finding that medium HDL $_{NMR}$ was associated with an increased risk of delivery before 37 weeks of gestation is also similar to the observation that medium HDL $_{NMR}$ was associated with an increased odds of recurrent preterm birth in the Thorp et al. study. In contrast to the Thorp et al. study (in which statistically signicant findings were only associated with an increased odds of recurrent preterm birth), we found several additional particles that were associated with a decreased risk of delivery <37 weeks of gestation. Similar to our study, Thorp et al. reported a median medium HDL_{NMR} concentration of 0.1 among preterm birth cases and 0.0 among controls, suggesting that the majority of women in their study did not have a detectable medium HDL_{NMR} concentration as well. Direct comparison of results with the Thorp et al. study is limited by the fact that their study design (nested case–control), study population (patients with a history of a prior preterm birth), and primary outcome (delivery <35 weeks) were different than the present study. In addition, in the Thorp et al. study all of the patients were exposed to 17-hydroxyprogesterone supplementation, and a portion of the patients were exposed to omega-3 fatty acid supplementation, which may explain some differences in the results.

Conclusion

The association between maternal dyslipidaemia and adverse pregnancy outcomes has been previously described in other studies, but there is great variability in the associations found and the manner in which the exposures and outcomes are defined. The mechanisms behind these associations have not been elucidated. Advanced measurements of lipid subclasses and lipoprotein particle concentrations using NMR represent an opportunity to investigate these

associations more closely, and may provide insight into the mechanism. Our study confirms the association between dyslipidaemia and preterm delivery, and expands the literature by identifying specific lipoprotein subclasses associated with gestational age at delivery in our sample. These findings may help formulate hypotheses for future studes of the complex relationship between maternal lipoproteins and preterm birth. Additional prospective studies using advanced measurements of lipoproteins are necessary to confirm these findings and may further elucidate underlying mechanisms.

Disclosure of interests

Full disclosure of interests form available to view online as supporting information.

Contribution of authorship

The Pregnancy, Infection, and Nutrition study was conceived, planned, and carried out by AMSR, AH, DS, and JT. The analysis of the data was performed by RN, MG, CV, and TM. The manuscript was written by MG and CV, with revisions and final approval from TM, AMSR, AH, DS, RN, and JT.

Details of ethics approval

The original Pregnancy, Infection, and Nutrition study was approved by the Institutional Review Board (IRB) at the University of North Carolina (UNC) at the time that the study was performed. The present study used a de-identified data set and was determined to be exempt by the UNC IRB.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Unadjusted hazard ratios for the association between lipids and gestational age at delivery for births <37 weeks, PIN Study, 2001–2005 (n = 715).

Table S2. Unadjusted hazard ratios for the association between lipids and timing of delivery among spontaneous deliveries (<37 weeks of gestation) only.

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