Title
Association of Spontaneous Preterm Delivery and Future Maternal Cardiovascular Risk.

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Publication Date
2017

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Association of Spontaneous Preterm Delivery and Future Maternal Cardiovascular Disease Risk

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy in Nursing

by

Margo Beth Minissian

2017

ABSTRACT OF THE DISSERTATION

Association of Spontaneous Preterm Delivery and Future Maternal Cardiovascular Disease Risk

by

Margo Beth Minissian

Doctor of Philosophy in Nursing

University of California, Los Angeles, 2017

Professor Lynn V. Doering, Chair

Background: Although many atherosclerotic cardiovascular disease (ASCVD) risk factors are well established, less is known about a woman's cardiovascular response to pregnancy, which appears to be an early marker of future maternal CVD risk. Adverse pregnancy outcomes (APO) including spontaneous preterm delivery (sPTD) has been associated with up to a three-fold increased risk of maternal ASCVD later in life. However, little is known about the association between changes in vascular function associated with APOs and future maternal CVD risk.[1]

Methods: We completed a case controlled, matched study, "Is Spontaneous Preterm Delivery Associated with Clustering of Future Maternal Cardiovascular Risk MarkErs?" (SPACE)

enrolled 20 women with sPTD (gestation ≤34 weeks) and 20 control women (gestation ≥39 weeks) matched for age (±5 years), parity, race/ethnicity and route of delivery collected between 24-72 hours postpartum. Vascular function was measured by augmentation index corrected for heart rate 75 bpm (AIx75), central pulse pressure (CPP), and pulse wave velocity (PWV). Linear regression models were used to analyze the data.

Results: The mean age at enrollment for sPTD and controls was 33 ± 6 years and 32 ± 6 years respectively. Women with sPTD had significantly lower AIx75 compared to controls (24.10 ± 16.10 % vs 39.90 ± 15.2 %, p=0.001), respectively. In addition, CPP was significantly lower in sPTD compared to controls (29.1±5 mmHg vs. 34.6 ±6 mmHg, respectively, p=0.004). Furthermore, women with sPTD and chorioamnionitis both clinically and/or on pathology report (n=8) had significantly lower AIx75 than matched controls (13.5 ±13.7% vs 39.9 ± 15.2% vs, p=0.001) respectively, while women with sPTD and no signs or symptoms of chorioamnionitis had a trend toward lower AIx75 than matched controls (26.6± 15.9 % vs. 39.9 ± 15.2 %, p=0.065). There was no difference between sPTD and controls in PWV (5.12 ± 1.62 m/s vs 5.58 ± 1.51m/s, p=0.1219), respectively.

The dissertation of Margo Beth Minissian is approved.

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List of Acronyms

Name	Acronym
Johns Hopkins Nursing Evidence-based Practice Rating Scale	JHNEBP
Coronary Artery Disease	CAD
Total Cholesterol	TC
High Density Lipoprotein	HDL
Low Density Lipoprotein	LDL
Triglycerides	TRG
C-Reactive Protein	CRP
Interleukin-6	IL-6
Ischemic Heart Disease	IHD
Spontaneous Preterm Delivery	sPTD
Small for Gestational Age	SGA
Beats per minute	BPM
Pulse wave velocity	PWV
Augmentation index corrected for heart rate	AIx75
Pulse Pressure	PP
Pulse Wave Analysis	PWA
Spontaneous preterm delivery	sPTD
Atherosclerotic Heart Disease	ASCVD
Premature rupture of membranes	PROM
Adverse Pregnancy Outcome	APO
Ischemic Heart Disease	IHD
Blood Pressure	BP

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Acknowledgements

Research reported in this study was supported by the National Institutes of Health under the award number: F31NR015725. CTSI support UL1TR000124 and UL1TR001881-01, and the Preventive Cardiovascular Nurses Association through the American Nurses Foundation (#5362). Additional support was provided by the Cedars-Sinai Department of Obstetrics and Gynecology, the Cedars-Sinai Brawerman Nursing Institute, Department of Nursing Research and the Cedars-Sinai Barbra Streisand Women's Heart Center.

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Chapter 1: Association of Spontaneous Preterm Delivery and Future Maternal Cardiovascular Disease Risk: A Literature Review

Introduction

Atherosclerotic cardiovascular disease (ASCVD) is the single largest killer of women[2] and more women than men die each year despite advancement in life-saving therapies.[3] Women who experience pregnancy complications -- including placenta disorders, preeclampsia, gestational hypertension, intrauterine growth restriction, and gestational diabetes -- are at increased risk of future ASCVD risk.[1, 4] Adverse pregnancy outcomes (APO) such as preterm delivery and small for gestational age (SGA), have been recently identified as conferring increase risk of future ASCVD risk.[1, 4, 5] Drastic hemodynamic changes occur in a woman's body during and after pregnancy.[6] Increases in cardiac output, heart rate and systolic blood pressure during pregnancy mimics a normal response to exercise seen on a treadmill stress test.[7, 8] At the time of delivery and immediately postpartum, sudden decreases in cardiac output, heart rate and blood pressure are observed, similar to those cardiovascular changes seen during the post recovery period during a treadmill stress test.[6, 7] Therefore, pregnancy may potentially serve as a window into future maternal ASCVD risk.

Preterm delivery is a common APO that affects 9.6% of women who give birth in the United States.[9] There are two primary types of premature delivery: spontaneous or medically indicated. Approximately 75% of preterm deliveries are the result of spontaneous preterm delivery (sPTD).[10] Spontaneous preterm delivery includes preterm labor which leads to delivery or preterm spontaneous rupture of membranes (PROM).[10] On the contrary, medically indicated preterm delivery can result from induction of preterm labor or from preterm cesarean delivery for

maternal or fetal indications.[10] Medically indicated preterm delivery has been associated with arterial stiffness and up to an eight-fold increased future maternal ASCVD risk.[1] Less is known about the association of sPTD with subsequent future maternal ASCVD risk. Recent literature has suggested that an otherwise healthy woman who is unable to carry her baby full term has failed her first physiological "stress test".[4] This manuscript will review research which highlights potential mechanistic pathways that might explain why women who experience deliver sPTD are at increased risk for ASCVD later in life.

Magnitude of the Problem

Researchers have reported associations between adverse APOs and later ASCVD risk in women. In a recent meta-analysis, Heida et al. concluded that sPTD is an independent risk factor for the development of ischemic heart disease (IHD), stroke and overall ASCVD.[11] They noted that there is much heterogeneity amongst current papers that have been published to date on this topic. In this review, we aim to discern specific mechanistic pathways which likely associate sPTD related to subsequent ASCVD. This manuscript will focus on three key areas that pertain to sPTD and future maternal ASCVD risk. These three areas of focus are: 1) traditional cardiovascular risk markers 2) inflammatory biomarkers, and 3) specific forms of vascular dysfunction such as endothelial function and arterial stiffness and the mechanisms by which these states may be associated with sPTD.

The literature search for this review was conducted in Pub Med and included key word searches: preterm delivery; maternal ASCVD; maternal cardiac risk; preterm birth; spontaneous preterm delivery; endothelial dysfunction; vascular dysfunction; arterial stiffness; inflammation; spontaneous preterm labor; cardiovascular risk factors; pregnancy complications; maternal risk of ischemia. This review included original research articles, including case control studies,

retrospective cohort studies and prospective cohort studies from 2000-2016. Each manuscript was rated with the Johns Hopkins Nursing Evidence-Based Practice Rating Scale (JHNEBP). This instrument was specifically developed to evaluate the strength and quality of research evidence. Strength and quality are each rated on three-point categorical scales, with higher scores indicating better evidence. [12] A list of abbreviations is provided in Table 1.

Association of Pregnancy Complications and Later Maternal Risk of ASCVD

Previous literature has documented the association between pregnancy complications such as preeclampsia, gestational hypertension, SGA and gestational diabetes with subsequent increased maternal ASCVD risk (Table 2).[13-18] In a landmark population based cohort study, researchers reported that mothers with preterm delivery experienced a 2.95- fold increased risk of ASCVD death compared to controls over a 25 year follow-up period.[1] Women with preeclampsia were divided into two groups based on whether the mother delivered term or preterm. Women with term preeclampsia were at a 1.65-fold increased risk of ASCVD death compared to women without preeclampsia. Of note, in a similar comparison, women with preterm delivery and preeclampsia experienced an 8.12-fold higher risk of ASCVD death, independent of lifestyle or other socioeconomic variations.[1]

There is consistency in the literature regarding gestational hypertension, preeclampsia and SGA infants related to increased maternal ASCVD complications. [1, 13, 19, 20] Using a retrospective cohort design over a 15 year period, Ray et al. evaluated over 1 million women free of ASCVD for presence of maternal placental syndrome (including preeclampsia, gestational hypertension, placental abruption, and placental infarction) before their first obstetrical delivery. They were assessed for presence of ASCVD beginning 90 days after delivery. Risk of ASCVD was twice as high in women with maternal placental syndrome compared to those without the

condition. Other factors such as maternal tobacco use in combination with placental syndrome also increased the risk of ASCVD.[19] Bonamy et al. identified the risk of ASCVD increased with preterm delivery and SGA infants. Compared to mothers who delivered at term, mothers who had preterm delivery complicated by SGA had higher risk of subsequent ASCVD ($p<.001$). [20] Mothers of very SGA infants experienced a hazard ratio increase from 1.38 to 3.40. The earlier the delivery, the more significant the SGA therefore contributing to higher maternal ASCVD risk.[21]

Association of Preterm Delivery and Later Maternal ASCVD/IHD Risk

All types of preterm delivery (including medically indicated and sPTD) have been added to the list of putative factors associated with increased maternal ASCVD risk (Table 2).[21] Otherwise healthy women who present with premature rupture of membranes (PROM) or an early delivery (less than a gestational age of 37 weeks) have up to a three-fold increased risk of ASCVD-related death later in life.[1] More recent reports demonstrate increased rates of morbidity, IHD, hospitalizations and atherosclerosis in all cause preterm delivery (Table 3).[22] From 2000-2016 eleven studies[20, 23-29] examined ASCVD and IHD death rates to be two-three fold higher for women with all preterm delivery compared to women who deliver at term.[22] Over time, recent studies have further demonstrated increased maternal ASCVD risk in as little as five years postpartum.[30] To further phenotype preterm delivery and IHD risk, Hastie et al.[27] looked for associations between medically indicated preterm delivery compared to sPTD and later maternal IHD risk.[27] In this retrospective cohort study, the investigators demonstrated independent associations between preterm delivery and both IHD death and events. Both sPTD and medically indicated preterm delivery had strong associations with IHD, likely resulting from SGA or preeclampsia which both result from placental dysfunction. [27] Women with medically

indicated preterm delivery tend to be older, more likely to have APOs such as hypertension and preeclampsia, and more likely to deliver SGA infants. On the other hand, women with sPTD tended to be younger, less likely to have preeclampsia, more likely to deliver higher birthweight infants and more likely to be socioeconomically deprived.[27] As the age at first IHD event decreased, the association between preterm delivery and an IHD event increased. Kessous et al.[31] also investigated the relationship of preterm delivery with later increased risk for cardiovascular morbidity. At 10-year follow up, patients with preterm delivery had higher rates of simple and complex cardiovascular events and higher rates of total cardiovascular-related hospitalizations.[31]

In conclusion, women who deliver prematurely for medical indication such as hypertension in pregnancy and preeclampsia experience the highest rates of ASCVD related death.[1, 32,30,5] These women tend to be older and deliver SGA infants.[27] Women who experience sPTD are also at increased risk for ASCVD related death and should be considered a CV risk marker in addition to currently known APOs.[4, 27, 31] Women with sPTD tend to be younger, have larger infants and are socioeconomically deprived.[27] Finally, for all preterm delivery there is an inverse relationship between severity of preterm delivery and maternal risk.[20]

Traditional Cardiovascular Risk Markers in Preterm Delivery

In the 2011 Prevention of Cardiovascular Disease in Women Update, APOs such as hypertension in pregnancy, preeclampsia and gestational diabetes were characterized as newer cardiovascular risk factors which help predict future maternal ASCVD.[3] More recently, preterm delivery has been identified as a risk marker for future maternal ASCVD. Less understood is the impact of known traditional risk markers such as cholesterol and inflammation in the setting of sPTD and subsequent future maternal ASCVD risk. These risk factors include total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TRG), and

C-reactive protein (CRP) which are measured for ASCVD risk prevention traditionally in non-pregnant, mid-life women. These important risk factors may potentially play a role in the earlier identification of women with sPTD and increased future maternal ASCVD risk.

Cholesterol. In uncomplicated pregnancy, a steady rise occurs in major lipoprotein lipids, and these levels nadir near term delivery.[33] The lipid changes provide fuel for fetal development and promote a state of insulin resistance for the mother as pregnancy progresses. In uncomplicated pregnancies, neither TC nor TRG exceeds 250 mg/dL at any time during pregnancy.[33, 34] Eleven studies have investigated the relationship of cholesterol levels to preterm delivery and sPTD (Table 4). Mudd et al. found an increased incidence of sPTD among women with higher TC, elevated LDL and higher TRG. Interestingly, they found an association of lower levels of total cholesterol, lower levels of HDL and lower LDL in the medically indicated preterm delivery cohort. [35] Magnussen et al. [36] prospectively evaluated data as part of the Norwegian population study known as the Nord-Trøndelag Health Study from the Medical Birth Registry of Norway. After adjusting for hypertensive disorders in pregnancy, such as preeclampsia, they found a positive association with preterm delivery and pre-pregnancy dyslipidemia. TRG above 1.6mm/L were associated with a 60% higher risk of preterm delivery although lower levels of TRG (less than 0.7mm/L) were not associated with preterm delivery. Furthermore, higher levels of TC, TRG and glucose correlated with increased severity of preterm delivery.[36] These are similar findings to Mudd et al., although Magnussen et al. included women with impaired glucose, a known maternal ASCVD risk factor. The general theme of these studies is that dyslipidemia is prevalent in women who experience preterm delivery either prior, during, or after pregnancy.

Metabolic changes including impaired cholesterol levels pre-pregnancy and during pregnancy have been associated with increased sPTD risk.[37-39] Catov et al.[37] reported that women with dyslipidemia in early pregnancy were 2.8 times more likely to deliver prior to 34 weeks after adjusting for race, body mass index (BMI), education and family history of hypertensive disorders of pregnancy.[37] There were a significant linear trends for both TC and LDL as dyslipidemia progressed and sPTD severity increased.[37] In a subset of patients from the Coronary Artery Risk Development in Young Adults study, investigators reported a U – shaped relationship curve between pre-pregnancy cholesterol and preterm birth risk. Pre-pregnancy TRG in the highest quartile (>195 mg/dl) was associated with preterm delivery <34 weeks in non-hypertensive women. Preterm birth risk was also seen in the lowest quartile (TC <156 mg/dl). These rates were independent of ethnicity, age, parity, body mass index (BMI), hypertension during pregnancy, physical activity, and years from measurement to birth.[40] Dyslipidemias including elevations in TC, TRG, LDL and reduced HDL in women with preterm birth either prior, during or after pregnancy has been well documented. [37, 38, 41-43]

Measurements designed to assess overall cardiovascular risk have been used to evaluate long-term effects of preterm delivery on subsequent ASCVD risk. The Avon Longitudinal Study of Parents and Children in the United Kingdom [44] used the Framingham risk score to evaluate the relationship of preterm delivery < 37 weeks and other pregnancy-related factors (i.e. gestational diabetes, hypertensive disorders of pregnancy, intrauterine growth restriction) to 10-year ASCVD risk. They reported that all preterm delivery was associated with higher systolic blood pressure but not with 10-year ASCVD risk.[44] Of note, a weakness of this study was the use of a risk score that was developed for use in individuals over the age of 30.[45,46]

Past cardiac risk scores such as Framingham have under identified childbearing women at potential risk for ASCVD. In more recent years, APOs have been identified as ASCVD risk markers. Traditional cardiac risk factors, including metabolic conditions, appear to be in associated with sPTD however the association is not clear. Early identification of traditional risk markers including APOs paired with risk factor modification may help improve a women's cardiovascular health trajectory.

Inflammatory Biomarkers and Preterm Delivery

C-Reactive Protein

Serum C-reactive protein (CRP) is an inflammatory biomarker, acute-phase protein secreted by the liver in response to inflammation. CRP has been associated with systemic inflammation leading to increased ASCVD risk in women[47, 48] however, has conflicting data regarding it's utility during pregnancy for detecting infection.[49] More recently, there is increasing evidence that parturition itself is an inflammatory process therefore questioning the utility of this widely used cardiovascular risk marker.[10] There are many causes of inflammation in pregnancy, a common cause is infection. Infections such as chorioamnionitis account for 10% of all preterm births,[10] although the mechanisms behind its implications for preterm birth are not fully understood.[10, 50] Table 5 summarizes studies of relationship of preterm delivery to CRP and other key inflammatory markers.

C-Reactive Protein in Pregnancy. By the third trimester in uncomplicated pregnancies, CRP is elevated compared to non-pregnancy levels (4.5 mg/L vs. 3mg/L).[51] Pitiphat et al.[52] investigated CRP in preterm women and their matched controls.[52] They found an association among presence of infection, elevated CRP and subsequent development of sPTD.[52] Other

studies confirmed the association of elevated CRP with increased risk of preterm delivery.[53, 54] Stratified analyses indicated that elevated CRP was associated with an increased risk of sPTD (OR=2.15, 95%CI: 0.85-5.42), medically indicated preterm delivery (OR=3.29, 95%CI: 0.98-11.02), and very preterm (OR= 20.6, 95%CI: 2.53-168.03), but not with preterm PROM (OR=1.48, 95%CI: 0.56-3.86).[53] These findings indicate that the higher CRP values are associated preterm delivery. Catov et al.[38] investigated CRP, cholesterol and their associated with sPTD. They concluded that early onset inflammation and dyslipidemia during pregnancy were independently associated with sPTD from 34 to 37 weeks. Interestingly the presence of both conditions increased risk of sPTD at < 34 weeks 6.4-fold (95% CI 1.7, 24.1). Half of women with early pregnancy inflammation had elevated CRP late in gestation, and both early pregnancy inflammation and dyslipidemia were independently related to the risk of sPTD at < 34 weeks.

C-Reactive Protein in the Postpartum Period. Studies in this area have yielded mixed results. In contrast to prior studies in which CRP was studied during pregnancy, Hastie et al. completed a retrospective cohort study to compare later maternal CRP (mean period =13 years after delivery) among women who had previous sPTD, those who had medically indicated PTD, and those with term births. After adjusting for potential cofounders, investigators reported an elevation in CRP for the medically indicated preterm group compared to term births, but not for the sPTD group.[55] CRP levels in women with sPTD over time were not significantly higher than those of women with term deliveries. Recently, investigators have indicated that increased vascular resistance is associated with higher levels of CRP, earlier preterm delivery, and poorer neonatal outcomes.[56]

In summary, CRP has been associated with increased risk of sPTD. Women with higher CRP have earlier preterm deliveries, are potentially at risk for dyslipidemias and increased vascular stiffness. More research is needed to further assess the role of CRP in sPTD in relationship to other factors, such as infections and vascular stiffness.

Interleukin-6 and sPTD

Interleukin-6 is an inflammatory biomarker and cytokine of interest in further detecting ASCVD risk in healthy populations.[47] IL-6 is considered an upstream biomarker from CRP, and is the primary cytokine leading to hepatic CRP production. [47] There is mixed data on the utility of IL-6 in sPTD.[57]

Cytokine IL-6 has been well studied in healthy non-pregnant women and may play an important role in risk stratification for preterm delivery and future maternal ASCVD risk. [48] Dulay et al. [58] evaluated IL-6 and CRP as potential early predictor biomarkers for preterm delivery. They found that maternal blood IL-6 and CRP levels were higher in women with subclinical intra-amniotic infection compared to time matched controls. However, the investigators noted there was overlap with confidence intervals leading to difficulty in interpretation of these results clinically. A systematic review further suggested that IL-6 and CRP are measurable in mid-trimester cervicovaginal fluid, but not in maternal blood samples, suggesting a localized, not systemic inflammatory process. [57] In a sample of 40 women with sPTD and 50 term controls, Bartha et al. evaluated a clustering of risk factors including ASCVD risk markers as well as both systemic inflammation and endothelial dysfunction in women with sPTD. [43] They reported that BMI and TC were the best predictors of sPTD. Overall, cholesterol fractions and myeloperoxidase were lower in the sPTD group than the control group. This confirms what many others have seen in regards to lower HDL in this population. [5, 21, 35, 42,43]

Conversely, IL-6 and the ratio to total cholesterol/HDL-C were significantly increased and correlated with each other. [43]

Together, these papers begin to cluster together traditional risk markers and inflammation, which might paint a clearer clinical picture of future maternal ASCVD risk. Looking upstream into the inflammatory cascade from CRP to IL-6 shows promise in atherosclerosis prevention in non-pregnant populations. [47] Building on the data presented, improved understanding of the relationship of traditional markers to inflammatory biomarkers may inform earlier identification of ASCVD risk in women earlier in their health trajectory, such as those who have experienced sPTD.

Vascular Function in Preterm Delivery

Many hemodynamic changes occur in a woman's body during and after pregnancy.[6] Increases in cardiac output, heart rate and systolic blood pressure during pregnancy mimic a normal response to exercise seen on a treadmill stress test.[7, 8] At the time of delivery and immediately postpartum, sudden decreases in cardiac output, heart rate and blood pressure are observed and continue into the postpartum period up until 6 months postpartum.[6]

A growing body of work supports the hypothesis that a women's response to pregnancy, as evidenced by whether or not she is able to carry full term, may serve as her first physiological "stress test." [4] Several studies indicate that an abnormal vascular response to pregnancy and subsequent delivery may be an early warning sign for development of future maternal ASCVD (Table 6).[4, 25, 41, 59] Novel non-invasive methods to assess arterial stiffness, including pulse wave analysis (PWA) and augmentation index (AI), during normal pregnancy[60-63] and preeclampsia[64-

[67] are now available to extend the initial work in this area through testing vascular function after sPTD compared to term delivery.

Preeclampsia: Abnormal vascular function, traditionally characterized by increased arterial stiffness,[68] has been well characterized in the preeclampsia literature.[64-67] Preeclampsia, a multisystem organ disease caused by the placenta, is commonly characterized by hypertension and proteinuria. Women with medically indicated preterm delivery are often hypertensive or experiencing severe features of preeclampsia resulting in provider initiated delivery. These patients have been shown to have significant increases in AI compared to women with a normal pregnancy.[69, 70 71] Importantly, increased AI associated with preeclampsia remains significantly elevated at 7 weeks postpartum.[72]

Preterm Delivery and ASCVD Risk: In an initial report of the use of non-invasive pulse wave velocity (PWV), PWA, and AI in the context of sPTD, Khalil et al.[73] studied arterial stiffness in women with all preterm delivery compared to controls at a single time point. In this prospective study, they examined arterial stiffness in women at 11 to 13 weeks' gestation and compared three cohorts: 1) medically indicated preterm delivery, 2) sPTD, and 3) term deliveries. They found that women with medically indicated preterm delivery had significantly higher arterial stiffness than women with sPTD and term delivery.[73] Interestingly, they saw a decreased systemic arterial response to sPTD <34 weeks.[73] There is limited data assessing differences in vascular stiffness in women with sPTD during pregnancy and in the postpartum period. A potential hypothesis for Khalil's finding may be that sPTD has a different vascular response than other APOs indicating an alternative mechanistic pathway which warrants additional inquiry. Catov et al.[74] evaluated women with a history of preterm delivery 4-12 years postpartum and found that women compared to controls had no difference in PWV or flow

mediated dilation. This data is in congruence that women with preterm delivery are less likely to have arterial stiffness and more likely have an alternative vascular mechanistic pathway.

Conclusions

This literature review provided this a basis for future study of mechanisms potentially contributing to sPTD and its association to future maternal ASCVD risk. Overall, the literature suggests that women who experience sPTD are at increased risk for future IHD and ASCVD cardiac-related hospitalizations.[20, 31] Preeclampsia and hypertension during pregnancy are established as important risk factors for future ASCVD risk. However, a growing body of evidence suggests that APOs, such as preterm delivery, may be important for identification of women at risk for development of ASCVD later in life. Immediate research goals for further development of this science should aim at: 1) identifying mechanistic pathway differences of specific presentations of sPTD (e.g., PROM vs spontaneous delivery), and 2) improving the understanding of vascular physiology in pregnancy and during delivery in the context of preterm delivery. Longer range future research may lead to early identification of women whose birth experiences put them at high risk for future ASCVD. For example, a better understanding of pregnancy/birth-related vascular function and inflammatory biomarkers, together with improved adherence to traditional risk factor management, may yield improved targeting of women at high risk for future ASCVD. Targeting of such women would lay the groundwork for bio-behavioral studies to promote awareness, lifestyle changes and development of preventive interventions. In addition to traditional ASCVD risk factors,

Table 1. Comparison of Findings in Medically Indicated Preterm Delivery and sPTD

Topics	Medically Induced Preterm Delivery	sPTD	All Preterm Delivery
Atherosclerotic Cardiovascular Disease (ASCVD) Outcomes	1. Fraser et al. [44] saw an increased Framingham risk score in women with preeclampsia and diabetes compared to women without, 18 years after pregnancy. 2. Cain et al.[30] evaluated women and girls with placental syndrome including preeclampsia, preterm birth or SGA are at increased risk of subsequent ASCVD in short term follow up. 3. Perng et al. [5] showed that preterm birth is related to greater ASCVD risk by 3 years postpartum, as indicated by higher SBP and lower HDL. 4. Lykke et al. [75] Fetal growth is a marker of subsequent risk for premature death, cardiovascular disease, and diabetes mellitus in the mother. 5. Ray et al. [19] Risk of ASCVD was highest in women with placental syndrome with poor fetal growth or intrauterine fetal death. 6. Irgens et al. [1] Risk of death from cardiovascular causes among women with preeclampsia and a	1. Kessous et al. [31] showed an association increased cardiac hospitalization remained significant for sPTD vs medical indicated preterm delivery and for early (<34 weeks) and late (34-36 weeks) preterm.	1. Bonamy et al. [20] evaluated all types of preterm birth showing an inverse relationship between severity of preterm delivery and maternal risk. 2. Pell et al.[29] found that women who experience SGA births, preterm delivery and previous spontaneous abortion have a 7 fold increased risk of cardiovascular related stroke. 3. Irgens et al. [1] Increase in cardiovascular causes was 2.95-fold in preterm delivery.

Topics	Medically Induced Preterm Delivery	sPTD	All Preterm Delivery
	preterm delivery was 8.12-fold higher than term delivery.		
Ischemic Heart Disease (IHD) Outcomes	1. Cain et al. [30] IHD and death increased in medically indicated preterm delivery had a stronger association, likely resulting from SGA or preeclampsia which both result from placental dysfunction.	2. Hastie et al. [27] Associations were greater for medically indicated than sPTD although independent association was seen between sPTD and IHD.	1. Nardi et al. found that mothers who had all cause preterm delivery were at an increased risk for IHD.
Traditional ASCVD risk factors	1. Wang et al. showed that women who subsequently developed APOs had higher levels of TC, TG, LDL and lower levels of HDL during early pregnancy. 2. Mudd et al. [35] Extremely low TC, HDLc, and LDLc were associated with a modest increase in risk of medically indicated preterm delivery, whereas high TC, LDLc and TG modestly increased the risk of sPTD. 3. Lei et al. [76] Pregnant women with a cluster of metabolic risk factors are more likely to have adverse pregnancy outcomes.	1. Catov et al. [25] showed pre-pregnancy cholesterol in the highest quartile (>195 mg/dl) were associated with preterm delivery <34 weeks in women who did not have hypertension or pre-eclampsia. 2. Bartha et al. [43] found a combination of low maternal BMI, low cholesterol levels, and high total cholesterol is present in women with sPTD. 3. Catov et al. [37]found TC and TRG elevated prior to 15 weeks gestation were associated with a 2.8 fold increased risk of sPTD.	1. Catov et al. [74] 4-12 years postpartum women with a prior preterm vs. term birth had higher blood pressure, on average, and a more atherogenic lipid profile. 2. Magnussen et al.[42] Unfavorable pre-pregnancy levels of TRG, TC, HDL and glucose were associated with increased risk of preterm delivery and shorter gestational length. 3. Catov et al. [39] Up to 25 years postpartum, elevated blood pressure and central adiposity were individual metabolic

Topics	Medically Induced Preterm Delivery	sPTD	All Preterm Delivery
			syndrome significantly elevated in preterm compared with term births.

Table 2. Association of Preterm Delivery and Later Maternal CVD/IHD Risk Studies

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
Bonamy et al. [20]	They examined associations between gestational age and low birth weight and first occurrence of maternal ASCVD (hospitalization, or death with ASCVD, cerebrovascular event, heart failure).	N 923,686 Women of Swedish origin. Investigated large-scale record linkages of nationwide health registries in Sweden.	Quantitative, prospective observational study N 923,686 Studied assoc. between gestational length, fetal growth, maternal hospitalization and death from ASCVD.	This studied showed that there is an inverse association between severity of preterm delivery and subsequent risks of maternal hospitalization or death from ASCVD. ASCVD is 39% higher in women delivering moderately preterm infants and more than doubled in women delivery very and extremely preterm infants. This large study shows an inverse association between severity of preterm delivery and maternal subsequent risks.	Large observational study. Rating: Level II, A
2. Cain et al. [30]	They identified short term risk within 5 years of first pregnancy of ASCVD among women who had maternal placental	Nulliparous 302,686 women and girls 15-49 years of age with no history	Retrospective cohort study with 4.9 years follow up. Compared risk of subsequent ASCVD with women who did	Women and girls with >1 placental syndrome had the highest ASCVD risk (hazard ratio, 1.43; 95% confidence interval, 1.20-1.70), followed by those with eclampsia/ preeclampsia along (hazard	Large observational study. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
	syndrome, preterm birth of SGA infant.	of ASCVD risk factors. Florida residents.	and did not have placental syndromes. Risk was assessed among women with placental syndrome and preterm birth or SGA infant with those women who did not have APO.	ratio, 1.42; 95% confidence interval, 1.14-1.76). When placental syndrome was combined with preterm birth and/ or SGA, the adjusted risk of ASCVD increased 45% (95% confidence interval, 1.24-1.71). Women and girls with placental syndrome, preterm birth or SGA are at increased risk of subsequent ASCVD in short term follow up.	
3. Catov et al. [25]	The purpose of the study is to consider if dyslipidemia may predispose women to both PTD and ASCVD and to evaluate if PTD was related to pre-pregnancy dyslipidemia.	1,010 women (49% black descent) enrolled in a multicenter prospective Coronary Artery Risk Development in Young Adults study with at least one singleton birth during 20	Quantitative Multicenter, longitudinal, observational. Secondary analysis of the CARDIA study. Participants had a singleton birth during 20 years of follow up.	Pre-pregnancy cholesterol in the highest quartile (>195 mg/dl) were associated with PTD <34 weeks in women who did not have hypertension or pre-eclampsia (odds ratio 3.80; 95% CI 1.07, 7.57). Preterm birth risk was also seen in the lowest quartile compared to the second quartile (<156 vs. 156-171 mg/dl) had an increased risk of PTD in the 34 less than 37 weeks group (odds ratio	Good quality secondary analysis, large observational study. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
		years follow-up. Recruitment from 4 sites: Birmingham, AL; Chicago, IL; Minneapolis, MN; Oakland, CA.		1.86; 95% CI 1.10, 3.15) and PTD less than 34 weeks (odds ratio 3.04; 1.35, 6.81).	
4. Fraser et al. [44]	They studied associations of pregnancy diabetes mellitus, hypertensive disorders of pregnancy, preterm delivery, and size for gestational age with calculated 10-year ASCVD risk (based on the Framingham score) and a wide range of cardiovascular risk factors measured 18 years after pregnancy.	The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective population-based birth cohort study. 13 617 women, singleton. Based in Avon England.	Prospective population-based birth cohort study. All measures taken 18 years after pregnancy, BMI, Waist circumference, SBP, DBP, glucose, insulin, proinsulin, TRG, HDL, LDL, CRP, non-smokers. Calculation of a 10-year risk of ASCVD based on the Framingham risk score.	Gestational diabetes mellitus was positively associated with fasting glucose and insulin, even after adjustment for potential confounders, whereas hypertensive disorders of pregnancy were associated with body mass index, waist circumference, blood pressure, lipids, and insulin. Large for gestational age was associated with greater waist circumference and glucose concentrations, whereas small for gestational age and preterm delivery were associated with higher blood pressure. The association with	Large prospective study with extended follow up. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
				the calculated 10-year ASCVD risk based on the Framingham prediction score was odds ratio 1.31 (95% confidence interval, 1.11–1.53) for preeclampsia and 1.26 (95% confidence interval, 0.95–1.68) for gestational diabetes mellitus compared with women without preeclampsia and without gestational diabetes mellitus, respectively.	
5. Kessous et al.[31]	The purpose of this study was to investigate whether a history of preterm delivery poses a risk for subsequent maternal long-term cardiovascular morbidity.	47,908 women, 5,992 of them delivered prematurely. Follow up for 10 years.	A population based study in Israel compared the incidence of cardiovascular morbidity in a cohort of women who delivered preterm <37 weeks compared to term deliveries. 1988-1989 with follow up until 2010.	At 10 year follow up, patients with preterm delivery had higher rates of simple and complex cardiovascular events and higher rates of total cardiovascular-related hospitalizations. A linear association was found between the number of previous preterm delivery and future risk for cardiovascular hospitalizations (5.5% for >2 preterm deliveries; 5% for 1 preterm delivery vs 3.5% in	Large observational study with 10 year follow up. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
				the comparison group (p=<.001). The association remained significant for sPTD vs medical indicated preterm delivery and for early (<34 weeks) and late (34-36 weeks) preterm. After controlling for labor induction, diabetes, preeclampsia, obesity, preterm delivery was associated independently with cardiovascular hospitalizations (adjusted hazard ratio, 1.4; 95% CI, 1.2-1.6).	
6. Lykke et al. [75]	They investigated the relation between the standardized birthweight by gestational age and gender and the ponderal index and the mother's subsequent mortality and	Women with a first singleton delivery in Denmark from 1978 to 2007. Population. 782 287 women followed for 14.6 years	Cox proportional hazard models. The primary exposures were variation in the standardized birthweight and ponderal index. The endpoints were	The risk-profile for the standardized birthweight and subsequent maternal death had a nadir between –0.5 and –1 SD (HR 0.91; 95%CI 0.83–1.00) and increased with decreasing fetal growth peaking at <–3 SD (HR 2.75; 95%CI 2.37–3.19) compared to the median. The risk-profile	Large observational study with 14.6 years follow up. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
	cardiovascular morbidity.	yielding 11 600 945 person-years.	subsequent maternal death, hypertension, ischemic heart disease, stroke, thrombosis, and diabetes mellitus.	for subsequent diabetes mellitus by standardized ponderal index had a nadir between +0.5 to +1 SD (HR 0.82; 95% CI 0.76–0.89) rising with increasing fetal growth and peaking at >+3SD (HR 17.8; 95% CI 15.0–21.0). The risk-profiles for standardized ponderal index paralleled those for birthweight, but with smaller risk estimates. Adjusting for other pregnancy complications diminished the estimates. The fetal growth is a marker of subsequent risk for premature death, cardiovascular disease, and diabetes mellitus in the mother.	
7. Nardi et al.[77]	The aim of this screening study was to assess the association between preterm delivery of a first child	109 women who delivered preterm, 395 controls.	Quantitative, prospective study IHD questionnaires were sent out between 1990-	Univariate and multivariate conditional logistic regressions models used to estimate HR.	Smaller prospective study interesting ASCVD

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
	and IHD, and the effect of major ASCVD factors on this association.	E3N French prospective study. 98,997 women born between 1925-1950.	1991which included information about hospitalizations and myocardial infarction (MI).	Mothers who had PTD were at an increased risk for IHD [multivariate hazard ratio 2.09 (95% CI 1.07-4.09)].	outcome data. Rating: Level II, B
8. Pell et al. [29]	The aim of this study was to determine whether mothers who deliver low birth weight infants or who suffer related pregnancy complications are also at increased risk.	119,668 women who delivered singleton first live births in Scotland. Recruitment 1981-1985	Quantitative Maternity hospital data base in Scotland collected data on live births > 500g and > 24 weeks of age.	Statistical significance of differences in case mix was assessed using Mann-Whitney U, x^2 and x^2 for trend tests for continuous, categorical, and ordinal data respectively. Women who experience SGA births, preterm delivery and previous spontaneous abortion have a 7 fold increased risk of cardiovascular related stroke (adjusted HR= 7.03, 95% CI: 2.24, 22.06)	Interesting study showing correlations of PTD and cerebrovascular disease. Large observational study. Rating: Level II, A

Table 3. The Association of Traditional Cardiovascular Risk Markers in Preterm Delivery Studies.

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
1. Bartha et al. [43]	The purpose of this study was to evaluate the relationships between selected metabolic ASCVD risk factors and markers of both systemic inflammation and endothelial dysfunction in women with sPTD.	90 pregnant women: 40 pregnant with sPTD compared to 50 case controls during gestation. The study was conducted in Spain.	Quantitative, prospective, case control study at a university tertiary referral center. Maternal serum triglycerides, HDL-C, LDL-C, TC, glycemia, insulinemia, homeostasis model assessment (HOMA) leptin, TNF alpha, IL-6, sVCAM, sICAM, selectin, and MPO measured.	Body mass index (BMI) (21.72 – 2.99 vs. 23.56 – 3.80, p = 0.01), all cholesterol fractions (HDL-C 53.44 – 18.22 vs. 68.32 – 18.38, p = 0.0003; LDL-C 125.71 – 35.56 vs. 142.15 – 36.07, p = 0.03, and total cholesterol 219.55 – 32.29 vs. 240.38 – 40.01, p = 0.009) and MPO (3.07 – 0.63 vs. 3.48 – 0.32, p = 0.0009) were significantly lower in women with sPTD. Serum levels of IL-6 (0.61 – 0.46 vs. 0.33 – 0.46, p = 0.007) and the ratio of total cholesterol/HDL-C (4.52 – 1.48 vs. 3.77 – 1.37, p = 0.01) were significantly increased and correlated each other (r = 0.21, p = 0.04).	Case control, prospective, looks at risk factors and inflammation, endothelial function. Rating: Level II, B

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
				A combination of low maternal BMI, low cholesterol levels, and high total cholesterol/HDL-C ratio is present in women with sPTD and is related to inflammation.	
1. Catov et al. [74]	This study considered women with prior preterm birth would have evidence of subclinical atherosclerosis, endothelial dysfunction, and arterial stiffness.	The Women and Infant Study of Healthy Hearts (WISH) cohort study 702 women either delivered preterm (n=181) or term (n=306). Women who delivered SGA were excluded (n=190). Delivered between 1997-2002 in Pittsburg, PA.	Four to 12 years after pregnancy, blood pressure and fasting lipids were analyzed, and women underwent evaluation, following standardized protocols, of carotid intima-media thickness (IMT), brachial flow mediated dilation (FMD), and pulse wave velocity (PWV). Women with prior preterm (<37 weeks, n=181) or term births (>=37	Women with a prior preterm vs. term birth had higher blood pressure, on average, and a more atherogenic lipid profile. They also had marginally higher IMT (0.579 standard error [SE] 0.005—vs. 0.567 [0.004] mm, p=0.06), adjusted for body size, demographics, and smoking. IMT differences were greater among those with non-preeclamptic-indicated preterm birth (0.034mm, p=0.05) and PTB<34 weeks (0.024mm, p=0.04) compared to those with term births. These differences appeared to be explained in part by the atherogenic lipid	Larger prospective, observational study. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
			weeks, n=306) were compared.	elevations in women with preterm birth. Women with prior preterm birth <34 weeks tended to have lower FMD, but results were not statistically significant. PWV did not differ according to preterm birth.	
2. Catov et al. [37]	The purpose of the study is to determine if women who delivered infants before 34 weeks had higher concentrations of TC, LDL, and lower HDL before 15 weeks gestation compared to women who delivered >37 weeks.	23 women delivered prior to 34 weeks, 67 delivered between 34 and less than 37 weeks. Women recruited from clinics and private practices from 1997-2001.	Quantitative Nested case control study of women with sPTD, TC, HDL, TRG, and LDL. Lipid concentrations and gestational changes, as well as risk of sPTD. Linear contrasts evaluated lipid concentration trends. Multinomial logistic regression was used to estimate the risk of preterm birth.	TC and TRG elevated prior to 15 weeks gestation were associated with a 2.8 fold increased risk of sPTD. Overweight women who delivered less than 34 weeks had particularly elevated early pregnancy concentrations of TC and LDL. There was a reduced TRG response in women who delivered <34 weeks. No difference in HDL between groups.	Relevant study to topic. Good study design. Rating: Level II, B

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
3. Catov et al. [40]	The purpose of the study is to consider if dyslipidemia may predispose women to both PTD and ASCVD and to evaluate if PTD was related to pre-pregnancy dyslipidemia.	1,010 women (49% black descent) enrolled in a multicenter prospective Coronary Artery Risk Development in Young Adults study with at least one singleton birth during 20 years follow-up. Recruitment from 4 sites: Birmingham, AL; Chicago, IL; Minneapolis,	Quantitative Multicenter, longitudinal, observational. Secondary analysis of the CARDIA study. Participants had a singleton birth during 20 years of follow up.	Pre-pregnancy cholesterol in the highest quartile (>195 mg/dl) were associated with preterm birth < 34 weeks in women who did not have hypertension or pre-eclampsia (odds ratio 3.80; 95% CI 1.07, 7.57). Preterm birth risk was also seen in the lowest quartile compared to the second quartile (<156 vs. 156-171 mg/dl) had an increased risk of preterm birth 34- 37 weeks group (odds ratio 1.86; 95% CI 1.10, 3.15) and preterm birth < 34 weeks (odds ratio 3.04; 1.35, 6.81).	Large, observational study. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
		MN; Oakland, CA.			
4. Catov et al. [21]	To estimate whether women who deliver small babies due to preterm birth or growth restriction have excess risk for cardiovascular disease and diabetes later in life.	702 women delivered at Magee-Women's Hospital in Pittsburgh, Pennsylvania, and those with preeclampsia or pre-pregnancy diabetes or hypertension were excluded. 1997-2002	The Women and Infant Study of Healthy Hearts is a prospective cohort study. Structured interview and fasting blood sample. Eight years after pregnancy, we estimated the prevalence of metabolic syndrome and its components in a cohort study of women with prior preterm (preterm birth before 37 weeks, n=181) or small for gestational age ([SGA], less than the tenth percentile, n=192) births, compared with women with term	Women with a prior preterm birth had higher blood pressure, triglycerides, and LDL-cholesterol compared with those in a term control group. Women with prior SGA births were leaner and more likely to smoke compared with those with term births. Women with prior preterm birth had elevated risk of metabolic syndrome, adjusted for demographic, smoking and body size factors (23% preterm compared with 17% control group; odds ratio [OR] 1.76 [1.06, 2.80]). In women with a prior preterm birth, low HDL (11% preterm compared with 5% control group; OR 2.6 [1.2, 5.2]), hypertriglyceridemia (22% compared with 14%; OR 1.9 [1.2, 2.9]), and elevated glucose (24% compared with 19%; OR 1.5	Large, observational study. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
			births (37 or more weeks, n=306).	[1.0, 2.3]) accounted for this excess metabolic syndrome. In women with SGA, the only element of metabolic syndrome that was aberrant was glucose metabolism. Eight years after pregnancy, women with prior preterm or SGA births had evidence of metabolic syndrome compared with women with term births. Screening and intervention in these women after pregnancy may delay or prevent disease.	
5. Catov et al. [39]	To investigate whether women who deliver preterm have excess risk for metabolic dysregulation independent of pre-pregnancy factors.	1205 Women with at least one birth between baseline (1985– 1986) and year 25 and no metabolic syndrome or diabetes	Multicenter, longitudinal, observational study as part of the CARDIA study group. Cardiometabolic factors were measured pre-pregnancy and at up to five subsequent	Of 315 cases of metabolic syndrome in 17,717 person-years of follow-up, the incidence rate was higher among women with preterm compared with term births (22.0 compared with 16.4 per 1,000 person-years; relative hazard 2.91 [95% confidence interval (CI) 2.75–3.09]). After adjustment for pre-	Large, observational study. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
		before pregnancy. Recruitment from 4 sites: Birmingham, AL; Chicago, IL; Minneapolis, MN; Oakland, CA.	examinations. We estimated the relative hazards of incident metabolic syndrome in women with one or more preterm births (less than 37 weeks of gestation, n 5295) compared with only term births (37 weeks of gestation or greater, n 5910). Self-reported gestational diabetes mellitus, hypertension during pregnancy, and time-dependent weight gain.	pregnancy cardiometabolic factors and covariates, the relative hazard (95% CI) for metabolic syndrome was 1.52 (1.22–1.88) for women with preterm compared with term births. Gestational diabetes mellitus, hypertension during pregnancy, and weight gain only modestly attenuated this association. Elevated blood pressure (36.3% compared with 26.7%, P=.002) and central adiposity (51.5% compared with 44.0%, P=.02) were the individual metabolic syndrome components that were different in women with preterm compared with term births.	
6. Lei et al. [76]	They investigated the correlation between clustering of metabolic risk factors and adverse pregnancy outcomes.	5535 pregnant women who sought health care during their whole gestation in a women's and	Prospective cohort study. Pre-pregnancy overweight/obesity, as well as pregnancy	The number of metabolic risk factors and adverse pregnancy outcomes were positively correlated (P trend<0.001). Compared with women without a metabolic risk factor,	Large prospective cohort study.

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
		children's hospital. January 2012 and December 2014. Guangzhou, Guangdong Province, China.	high triglycerides, low high-density lipoprotein–cholesterol, hyperglycemia and raised blood pressure were defined as metabolic risk factors. Adverse pregnancy outcomes included preterm delivery, small/large for gestational age, preeclampsia, gestational diabetes mellitus, neonatal asphyxia and fetal demise.	women with one metabolic risk factor had a risk (OR=1.67 95% CI 1.42–1.96) of adverse pregnancy outcomes. Women with a cluster of two metabolic risk factors tended to develop more adverse pregnancy outcomes (OR=3.32 95% CI 2.69–4.10), and the risk was much higher in women with a cluster of three or more metabolic risk factors (OR=10.40 95% CI 7.37–14.69). Pregnant women with a cluster of metabolic risk factors are more likely to have adverse pregnancy outcomes.	Rating: Level II, A
7. Magnussen et al. [42]	The purpose of the study is to examine the effect of cardiovascular risk factors before	3506 women who gave birth after participating in the Nord-Trøndelag health study at	Quantitative Population based prospective study.	Unfavorable pre-pregnancy levels of TRG, TC, HDL and glucose were associated with increased risk of preterm delivery and shorter gestational length.	Large well designed population based study.

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
	pregnancy on risk of preeclampsia. Odds ratio of developing preeclampsia.	baseline; of who 133 (3.8) delivered after preeclamptic pregnancy.	Linkage between a Norwegian populations based study HUNT2 and Norway's medical birth registry. Logistical regression analysis was used for adjusted assoc. of CV risk factors with risk of PTD. Linear regression analysis to estimate crude and adjusted assoc. for CV risk factors with risk for preterm delivery.	TRG above 1.6mm/L were associated with a 60% higher risk of PTD (odds ratio, 1.6, 95% confidence interval, 1.0-2.5) although lower levels of TRG (less than 0.7mm/L) were not associated with preterm delivery.	Rating: Level II, B
8. Mudd et al. [35]	The purpose of the study is to examine associations between maternal lipid levels at mid pregnancy and PTD for both medically indicated at SPTD.	221 sPTD and 100 medically indicated preterm delivery women were recruited from 5 Michigan communities	Quantitative Prospective cohort study. A single blood sample was obtained at study enrollment. Blood lipids, i.e. total cholesterol	Odds of SPTD were increased among women with high TC (aOR 1.51, 95% CI 1.06, 2.15) high LDL (aOR 1.42, 95% CI0.99, 2.04) or high TRG (aOR 1.90, 95% CI 1.21, 2.97, and aOR 1.72, 95% CI 1.06, 2.78 for third and fourth quartiles, respectively). Medically	Multi-site, population based prospective study. Rating: Level II, B

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
		from 52 clinics.	(TC), high-density lipoprotein (HDLc), low-density lipoprotein (LDLc) cholesterol, and triglycerides (TG), were measured on a sub-cohort (n=1309).	indicated PTD which had low TC (adjusted odds aOR ratio 2.04 95% CI 1.12, 3.72) low HDL (aOR 1.89, 95% CI 1.04, 3.42) or low LDL (aOR 1.96, 95% CI 1.09, 3.54). Extremely low TC, HDLc, and LDLc were associated with a modest increase in risk of medically indicated preterm delivery, whereas high TC, LDLc and TG modestly increased the risk of sPTD.	
9. Perng et al. [5]	To investigate whether preterm birth is associated with greater ASCVD risk in a longitudinal cohort.	804 women measured blood 3 years postpartum. Preterm delivery (n=54) vs control (751). Data collection	They examined differences in SBP, DBP, insulin resistance (HOMA_IR), TC, HDL, LDL, TRG, CRP, IL-6 at 3 years postpartum between women who delivered prior to 37 weeks.	After adjusting for age, race, pre-pregnancy BMI, parity, marital status, education and SBP during early pregnancy, women with preterm delivery had 3.99% (95% CI: 0.82, 7.16) mmHg SBP ad 7.01 (1.54, 12.50) mg/dL lower HDL than those who delivered at term.	Prospective, observational study. Rating: Level II, B

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
		1999-2002 at a multispecialty clinic at Harvard Pilgrim Health Center.		Preterm birth is related to greater ASCVD risk by 3 years postpartum, as indicated by higher SBP and lower HDL.	
10. Wang et al. [78]	To evaluate the association between early pregnancy lipid profiles and pregnancy outcomes.	5218 singleton pregnant women. 15 hospitals in Beijing China from June 20-June 30th 2013.	Secondary analysis within a larger retrospective study entitled 'Systemic Random Sampling Survey on the Prevalence of Gestational Diabetes Mellitus in Beijing'. Total cholesterol (TC), triglycerides (TG), high-density lipid cholesterol (HDL-C) and low-density lipid cholesterol (LDL-C) levels were divided into quartiles	Women who subsequently developed adverse pregnancy outcomes had higher levels of TC, TG, LDL and lower levels of HDL during early pregnancy (14 gestational weeks). A trend toward an increasing incidence of adverse pregnancy outcomes was noted with increasing levels of TC, TRG and LDL, and decreasing level of HDL. The more numbers of TC, TRG and LDL above 75th percentile and HDL inferior to 25th percentile women had, the higher their risk of developing adverse pregnancy outcomes. Low TRG level was a protective factor for gestational	Large secondary analysis. Rating: II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
				diabetes mellitus (GDM) (1.44 mmol l− 1, odds ratio (OR) = 0.706, 95% CI, 0.586 to 0.852) and LGA infants (o1.44 mmol l − 1, OR = 0.769, 95% CI, 0.631 to 0.936), and low LDL (1.89 mmol l − 1) level was protective factor for preterm birth. High TRG (41.40 mmol l − 1, OR = 1.327, 95% CI, 1.130 to 1.558), TC (44.29 mmol l − 1, OR = 1.250, 95% CI, 1.062 to 1.471), and LDL (42.62 mmol l − 1 , OR = 1.25, 95% CI, 1.069 to 1.480) levels and a low HDL (1.89 mmol l − 1 , OR = 1.190, 95% CI, 1.007 to 1.405) level were associated with increased risk of GDM. A high TRG (41.40 mmol l − 1 , OR = 1.550, 95% CI, 1.025 to 2.343) level was related to high risk of preeclampsia, while a high LDL (42.62 mmol l − 1 , OR = 1.400, 95% CI, 1.100 to 1.781) level was risk	

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
				factor for macrosomia. After adjusting for confounders, early pregnancy TC was an independent risk factor for GDM (adjusted odds ratio [= 1.184, 95% CI, 1.085 to 1.291), TRG level was independently associated with the prevalence of GDM (aOR = 1.253, 95% CI, 1.141 to 1.375) and PE (aOR = 1.245, 95% CI, 1.023 to 1.516), and LDL-C level was significantly associated with risk of GDM (aOR = 1.162, 95% CI, 1.052 to 1.283) and preterm birth (aOR = 1.264, 95% CI, 1.065, 1.501).	

Table 4. The Association of Inflammatory Biomarkers and Preterm Delivery Studies

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
1. Dulay et al. [58]	They studied CRP and IL-6 to see if these molecules can be used as non-invasive biomarkers of intra-amniotic infection (IAI) sub-clinically.	100 pregnant women who had an amniocentesis to rule out IAI in the setting of preterm labor, PPROM or systemic inflammatory response (SIR: pyelonephritis, appendicitis, pneumonia) to infection. June 2004-June 2010 Yale University	Case Control Study. Time-matched maternal serum, urine and AF from 100 pregnant women who had an amniocentesis to rule out IAI in the setting of preterm labor, PPROM or systemic inflammatory response (SIR: pyelonephritis, appendicitis, pneumonia) to infection. We used sensitive immunoassays to quantify the levels of inflammatory markers in the maternal blood, urine and AF compartment.	Maternal blood IL-6 and CRP levels were elevated in women with subclinical IAI. Compared to clinically manifest chorioamnionitis group, women with SIR have higher maternal blood IL-6 levels rendering some marginal diagnostic benefit for this condition. In women with subclinical IAI, the large overlapping confidence intervals and different cutoffs for the maternal blood levels of IL-6, CRP and PCT likely make interpretation of their absolute values difficult for clinical decision-making.	Case control study. Rating: Level II, B.
2. Kramer et al. [79]	The purpose of this study was to examine blood immune	Women with sPTD (n=207) and 2 term	Case control study nested in a large (n=5337)	High maternal matrix metalloproteinase-9 concentration but none of	Nested case control study.

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
	response prior to labor onset.	controls per case (n=444). Montreal Canada	prospective, multi-center cohort.	the other cytokines or CRP was significantly associated with sPTD.	Rating: Level II, B.
3. Lohsoonthorn et al. [53]	To examine the relation between maternal early pregnancy serum CRP and preterm delivery.	1769 subjects were part of the Omega Study, recruited in Tacoma and Seattle Washington between 1996-2002.	Nested, multi- site prospective cohort study of maternal dietary and other risk factors of adverse pregnancy outcomes including preeclampsia, gestational diabetes mellitus and other adverse outcomes of pregnancy.	Elevations in CRP concentrations were associated with the risk of preterm delivery overall. After adjusting for confounding, the OR for highest quartile (≥7.5 vs. <2.0 mg/l) of CRP was 2.04 (95% CI: 1.13-3.69). Stratified analyses indicated that elevated CRP was associated with an increased risk of sPTD (OR=2.15, 95%CI: 0.85-5.42), medically indicated preterm delivery (OR=3.29, 95%CI: 0.98-11.02), and very preterm delivery (OR= 20.6, 95%CI: 2.53-168.03), but not with preterm premature rupture of membranes (OR=1.48, 95%CI: 0.56-3.86).	Multi-site, nested prospective study. Rating: Level II, B.

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
4. Pitiphat et al. [52]	They investigated the relationship between infection and elevations in highly sensitive CRP.	Subjects were 117 women who delivered preterm (<37 weeks' gestation) and 117 controls (term deliveries) matched on age, race/ ethnicity, and smoking status. 1999-2002 Study conducted in Massachusetts between 1999 and 2002.	Nested case-control study was conducted within Project Viva. High-sensitivity CRP assays were performed on early-pregnancy (5.3–19.3 weeks' gestation) plasma samples.	No significant association was found between quartiles of CRP and preterm delivery. However, CRP levels exceeding the threshold defined in the literature were associated with increased risk of preterm delivery (odds ratio= 2.55, 95% confidence interval: 1.05, 6.02 for CRP >8 mg/liter). The association was stronger among cases who experienced spontaneous delivery (odds ratio = 4.64, 95% confidence interval: 0.94, 22.96) versus indicated delivery (odds ratio = 1.42, 95% confidence interval: 0.44, 4.61). These findings suggest that very high CRP levels in early pregnancy are associated with preterm delivery.	Case matched study. Rating: Level II, B.

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
5. Wei et al.[57]	To estimate the association between inflammatory cytokines and the risk of spontaneous preterm birth in asymptomatic women.	Seventeen primary studies comprising 6,270 participants.	This systematic review included observational studies that reported the association between common inflammatory cytokines and spontaneous preterm birth as an outcome in asymptomatic women.	Spontaneous preterm birth was strongly associated with increased levels of interleukin-6 (IL-6) in mid trimester cervico-vaginal fluid (OR 3.05, 95% CI 2.00–4.67) (number needed to treat7 for identifying an additional preterm delivery) and amniotic fluid (OR 4.52, 95% CI 2.67–7.65) (number needed to treat7), but there was no association in plasma specimen (OR 1.5, 95% CI 0.7–3.0). Spontaneous preterm birth was strongly associated with increased C-reactive protein (CRP) levels in mid trimester amniotic fluid (OR 7.85, 95% CI 3.88–15.87) (number needed to treat3), but the association was weak in plasma specimen (OR 1.53, 95% CI 1.22–1.90). Inflammatory cytokine IL-6 in cervico-vaginal fluid and	Well done systematic review. Rating: Level III, B

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
				IL-6 and CRP in amniotic fluid but not in plasma are strongly associated with spontaneous preterm birth in asymptomatic women, suggesting that inflammation at the maternal–fetal interface, rather than systemic inflammation, may play a major role in the etiology of such spontaneous preterm births.	

Table 5. Assocation of Preterm Delivery and Vascular Function Studies.

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
1. Khalil et al. [73]	The aim of this study was to evaluate aortic systolic blood pressure and arterial stiffness at 11-13 weeks gestation.	Singleton pregnancies at 11-13 weeks' gestation. 7843 singleton pregnancies. 244 sPTD 110 indicated preterm delivery defined as prior to 37 weeks. Women attending their routine first trimester ultrasound The study was conducted in London.	Quantitative, prospective, screening study Patients complete a questionnaire on maternal age, racial origin, cigarette smoking during pregnancy, method of contraception, history, parity, previous PTD. Maternal height and weight. Measured PWV, Aix, Central aortic blood pressure (SBPAo)	Compared with women who had term delivery, women who had iatrogenic PTB had significantly higher AIx (1.08 (interquartile range (IQR), 0.91–1.27) multiples of the median (MoM), vs. 1.00 (IQR, 0.86–1.16) MoM) and SBPAo (1.06 (IQR, 0.98–1.15) MoM vs. 1.00 (IQR, 0.93–1.07) MoM). However, there was no significant difference in AIx, PWV or SBPAo between those who had spontaneous PTB and those who had term delivery. These findings were similar for those who had PTB at <34 and <37 weeks' gestation. Women with a medical indication for PTD had significantly higher Aix with no significance in the other measurements.	Prospective Observational Study. Rating: Level II, B
2. Khalil et al. [72]	The aim of this screening study was to investigate first	210 women total	Quantitative Prospective, screening study	Baseline characteristics evaluated with chi2 test. Fishers exact test when	Screening study small

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
	trimester arterial pulse wave analysis and its potential ability to predict preeclampsia.	14 with preeclampsia and 196 controls with singleton pregnancies. The study was conducted in London, England.	recruitment between 2006-2007. Radial artery pressure waveform was recorded between 11-13 weeks gestation. Women were followed up after delivery. Measured PWV, Aix, Central aortic blood pressure.	appropriate. Univariate logistic regression analysis was performed to determine the relationship of each demographic variable and each of the PWA parameters. 14 (6.7%) women developed preeclampsia and 196 remained normotensive. AIx had a detection rate of 79% for all cases of preeclampsia and 88% for early-onset preeclampsia.	preeclampsia cohort. Rating: Level II, B
3. Valensise et al. [80]	The purpose of this study was to evaluate the maternal hemodynamic profile in women with diagnosis of threatened preterm delivery (TPD) to understand the possible pathophysiologic mechanism leading to an increased lifetime risk for future cardiovascular disease.	68 patients with TPD. Rome, Italy.	Prospective observational study. Cervix length assessment, vaginal and rectal swabs, blood inflammatory indexes, fetal vessel Doppler velocimetry, gestational age at the time of delivery and neonatal outcomes were also considered.	The population was divided into two groups according to total vascular resistance (TVR) in Group A≤1000 dynes.sec.cm-5, and Group B>1000 dynes.sec.cm-5. C-reactive protein (CRP) was higher in Group B vs. Group A, suggesting a systemic inflammation status. Group B delivered earlier (32 weeks+4 days vs 38 weeks+2 days, p <0.01), and neonatal outcome was worse vs. Group A. A significant lower cardiac output, stroke volume, peak	Observational. Rating: Level II, B.

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
				velocity of flow, velocity time integral, minute distance, stroke volume index, cardiac index, stroke work, cardiac power, inotropy index in Group B vs. Group A.	
4. Catov et al.[74]	This study considered women with prior preterm birth would have evidence of subclinical atherosclerosis, endothelial dysfunction, and arterial stiffness.	The Women and Infant Study of Healthy Hearts (WISH) cohort study 702 women either delivered preterm (n=181) or term (n=306). Women who delivered SGA were excluded (n=190). Delivered between 1997-2002 in Pittsburg, PA.	Four to 12 years after pregnancy, blood pressure and fasting lipids were analyzed, and women underwent evaluation, following standardized protocols, of carotid intima-media thickness (IMT), brachial flow mediated dilation (FMD), and pulse wave velocity (PWV). Women with prior preterm (<37 weeks, n=181) or term births (>=37 weeks, n=306) were compared.	Women with a prior preterm vs. term birth had higher blood pressure, on average, and a more atherogenic lipid profile. They also had marginally higher IMT (0.579 standard error [SE] 0.005— vs. 0.567 [0.004] mm, p=0.06), adjusted for body size, demographics, and smoking. IMT differences were greater among those with non-preeclamptic-indicated preterm birth (0.034mm, p=0.05) and PTB<34 weeks (0.024mm, p=0.04) compared to those with term births. These differences appeared to be explained in part by the atherogenic lipid elevations in women with preterm birth. Women with prior preterm birth <34 weeks tended to	Larger prospective observational study. Rating: Level II, A

Citation	Purpose	Sample/ Setting	Methods	Results	Comments & JHNEBP Rating
				have lower FMD, but results were not statistically significant. PWV did not differ according to preterm birth.	

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Chapter 2: Association of Spontaneous Preterm Delivery and Postpartum Vascular Function

Introduction

In women, atherosclerotic cardiovascular disease (ASCVD) remains the leading cause of death worldwide and surpasses all cancer deaths combined. [2] Despite advances in cardiovascular medicine, 1 out of 3 women will die from ASCVD[3]. Although many ASCVD risk factors are well established, less is known about a woman's response to pregnancy, which may be an early marker for later maternal ASCVD risk. [4] Spontaneous preterm delivery (sPTD) has been associated with later development of maternal ASCVD. [1, 5-11] Strikingly, women who experience sPTD, defined as preterm delivery proceeded by spontaneous labor, premature rupture of membranes, or premature rupture of membranes (PROM) prior to 37 weeks, have a three-fold increased risk of ASCVD later in life. [1]

Other studies indicate that the relationship of pregnancy and delivery to future ASCVD risk may be influenced by the severity and type of preterm delivery (i.e., spontaneous vs. medically indicated).[6, 12-15] In contrast to sPTD, medically indicated preterm delivery occurs when a healthcare provider makes the decision to initiate parturition due to a medical condition such as preeclampsia, hypertension or a placental disorder.[16, 17] Compared to controls, medically indicated preterm delivery has been associated with increased arterial stiffness measured in the peripartum and postpartum period and is considered a marker of increased atherosclerotic cardiovascular risk[1, 15, 18-23] and endothelial dysfunction.[24] Traditionally, abnormal vascular function has been characterized by increased arterial stiffness[25], a measurement of rigidity within the arterial wall.[26] Preclinical vascular dysfunction associated with adverse pregnancy outcomes (APOs) may predispose women to subsequent development of future ASCVD.[15, 27-3031] This has

been well characterized by evidence of increased arterial stiffness measured in early pregnancy in medically indicated preterm delivery and preeclampsia literature.[12, 32-34] However, less is known about vascular function during and after delivery in women who experience sPTD. There is only one study that suggests decreased arterial stiffness in early pregnancy amongst women with sPTD, compared to women with medically indicated preterm delivery.[24]

Drastic hemodynamic changes occur in a woman's body during and after pregnancy.[35] Increases in cardiac output, heart rate and systolic blood pressure during pregnancy mimic a normal response to exercise seen on a treadmill stress test.[36, 37] At the time of delivery and immediately postpartum, sudden decreases in cardiac output, heart rate and blood pressure are observed.[35] In turn, these cardiovascular changes during peripartum recovery resemble those expected after a cardiac treadmill stress test.[35, 36] Thus, some researchers have posited that a women's response to pregnancy, as evidenced by whether or not she is able to carry full term, may serve as her first physiological "stress test".[29] By extension, this hypothesis suggests that evaluation of peripartum vascular changes should not be avoided, as has been the traditional approach of researchers in the past. Rather, study of peripartum vascular and hemodynamic variables may reveal latent abnormalities in the vasculature. Because the vast majority of women in globally will bear at least one child,[38] pregnancy may be an underutilized early opportunity to identify future risk of ASCVD. The aim of this study was to assess arterial stiffness in women who experience sPTD $\leq$ 34 weeks of gestation immediately (24-72 hours) postpartum compared to control women who deliver $\geq$39 weeks.

Methods

Defining Preterm Delivery

Preterm delivery is a common adverse pregnancy outcome (APO) that affects 9.6% of women who give birth in the United States.[39] Of those women who deliver prematurely, there are two primary types: spontaneous or medically indicated. Approximately 75% of preterm deliveries are the result of sPTD.[16] Spontaneous preterm delivery includes preterm labor which leads to delivery or PROM.[16] In contrast, medically indicated preterm delivery can result from induction of preterm labor or from preterm cesarean delivery for maternal or fetal indications.[16] Severity of preterm delivery is defined as late preterm (34-37 weeks gestation), early preterm (<34-32 weeks gestation) and very preterm delivery (<32 weeks gestation).[6, 23] We selected to study women who delivered ≤ 34 weeks, because it is an accepted marker for early preterm delivery and has been used by other investigators in the field.[24, 40]

Design and Study population

We performed a matched case-control study, in which women with and without sPTD were matched by age (±5 years), parity, route of delivery and self-reported race/ethnicity. Women with sPTD (≤ 34 weeks) and control women with term delivery (≥ 39 weeks)[41] were considered eligible. Women were recruited from the Labor and Delivery unit at Cedars-Sinai Medical Center, which draws from a diverse metropolitan population, where approximately 6700 deliveries occurred in 2016. The sample for the study included a total of 40 participants, 20 sPTD and 20 normal controls. The Principal Investigator (MM) evaluated potential participants with and without sPTD cohort using the following inclusion criteria: 1) age ≥ 18 years; 2) able to speak English and/or Spanish; 3) competent to give informed consent; 4) singleton birth; 5) no medical indication for preterm delivery, such as hypertensive disorders of pregnancy, gestational diabetes or preeclampsia; and 6) no prior history of ASCVD, including ischemic heart disease (IHD), acute myocardial infarction, acute coronary syndrome, coronary or peripheral

revascularization, stroke, trans-ischemic attack, diabetes (ASCVD equivalent), gestational diabetes or smoking.

Measurements

Pulse Wave Analysis: Pulse Wave Analysis (PWA) is a combination of a forward pressure wave created by ventricular contraction and a reflected wave.[42] It is measured noninvasively by augmentation index (AIx) corrected for heart rate 75 bpm (AIx75) and carotid-femoral pulse wave velocity (PWV). Both measures are strong and independent predictors of CVD risk in diseased and at-risk middle-aged and older subjects, [43-45] although prognostic value in young populations is unclear.

AIx is affected by the speed of wave travel, which is reflected by the amplitude of the waveform.[46] PWA records the brachial pulse waveform through measurements taken by an arm cuff and derives a central pulse pressure (CPP) waveform of the ascending aorta. Measuring AIx75 is noninvasive, accurate and reproducible;[42, 45] it is expressed as a percent and is calculated from the difference between the first and second pulse waves, divided by the CPP. Central blood pressure (CBP) waveform indices for this study were estimated using an oscillometric cuff-based device (Sphygmocor Xcel, AtCor Medical, West Ryde, Australia).[47] Measurement involved the placement of a BP cuff on the upper-arm and automatic recording of standard oscillometric brachial BP, immediately after which the cuff automatically re-inflated to sub-diastolic pressure and is held for a ten second period. The brachial artery waveform was captured during this period. The brachial BP waveforms were calibrated with the brachial systolic pressure and brachial diastolic pressure values as measured during the first inflation. A device specific brachial-aortic generalized transfer function was then applied to derive a central

BP waveform. Integrated software completed the AIx75 calculation, which controls for heart rate.[48, 49]

Pulse Pressure: In addition to measuring AIx75, we calculated central pulse pressure (CPP) with cuff-based oscillometry at the brachial artery which is defined as the difference between systolic and diastolic BP. CPP appears better related to future cardiovascular events than is peripheral PP.[42]

Pulse Wave Velocity: Carotid-femoral PWV is an independent predictor of cardiovascular risk and mortality in diseased and older mid-life to older subjects.[25] It is a direct measurement of the aorto-iliac pathway which assesses for arterial stiffness[42] by measuring the speed of a pressure wave along a length of artery over time. The stiffer the artery, the higher the PWV.[46] Pulse wave velocity is measured as the time difference between the foot of the carotid pulse, measured by a tonometer, and the foot of the pulse from a thigh cuff inflated to a set pressure.[51] Both pulses are measured simultaneously. The foot of the pulse is determined using the intersecting tangent method. To measure carotid femoral PWV, the measured carotid to thigh pulse time is adjusted to a carotid to femoral pulse time based on the femoral to thigh distance. The measured carotid to femoral distance is divided by the adjusted time to produce carotid to femoral PWV.

To control for operator variability and accuracy, all testing was completed by a single investigator (MM), who received training and was proctored in conducting study measurements prior to the initiation of the study.

Procedures

This study was approved by the internal review board at Cedars-Sinai Medical Center (IRB#32089). Patients on the Labor and Delivery unit who might potentially deliver ≤ 34 weeks or >39 weeks were referred to the study team by their treating physician. If the patient indicated interest in learning more about research opportunities, the study team contacted the patient either prior to the delivery, if possible, or within the first 24 hours after delivery. At an initial meeting, the study team introduced the study and provided a copy of the study consent or information sheet. Informed consent was obtained prior to any research procedures.

After enrollment and between 24-72 hours post-delivery, inpatient testing was conducted in the patient's private room at a time when minimal visitors were present and no other health care activities were scheduled. A chart review was conducted to record clinical data (age, parity, prenatal care) and to document all medications given to the participant during delivery. Collection of medication history is important as tocolytics such as magnesium are given to women to help treat sPTD and for fetal neuroprotection and have the potential to impact vascular function. Each participant completed a self-report questionnaire to obtain demographic data. Vascular procedures (AIx75, CPP, PWV) were completed in the patient's room while in bed. Study procedures took no more than one hour.

All women in the sPTD group had their placentas sent to pathology for potential confirmation of chorioamnionitis. This was indicated because chorioamnionitis is a common complication of pregnancy associated with significant maternal, perinatal, and adverse outcomes including postpartum infection and preterm delivery. [52] If chorioamnionitis was confirmed, it was documented in the medical chart and/ or on the pathology report.

Sample Size

Power analysis was calculated for the primary aim (AIx75) using G*Power 3.1.[53] Data from Khalil et al. were used to provide estimates of AIx75 interquartile ranges of 12.18 %, and 11.36% for the sPTD and term control groups, respectively.[24] The formula IQR/2 was used to estimate a standard deviation.[54] Using these estimates together with paired t-tests, we estimated that 20 subjects in each group would yield 80% power to detect at least a 7.6% difference between groups at the 0.05 significance level, assuming a conservative estimate of zero correlation within matched pairs, or a 3.4% difference assuming 0.8 correlation within matched pairs.

Data Analysis

Data were analyzed using SPSS release 21 for Windows (Chicago, IL), SAS version 9.3 (Cary, NC), and R version 3.2. For all analyses, the significance level was set at 0.05. To examine the equality of the two groups, unmatched baseline sociodemographic (educational level and marital status) and unmatched clinical variables and cardiovascular risk markers (pre-pregnancy BMI, family history of ASCVD, use of fertility treatments) were compared using conditional logistic regression models where the outcome was the group (sPTD vs control) and the models were adjusted for the variable in question and stratified by match. Mixed effects linear regression models with a random effect for match were used to compare outcomes of interest (AIx75, CPP,PWV) between the groups (sPTD vs. control) and adjust for potential confounding effects among other variables measured. For post-hoc comparisons of groups in these outcomes, a Tukey-Kramer adjustment was made to the p-values.

Results

Patient Characteristics

A total of 42 participants, 22 with sPTD and 20 comparisons with term delivery enrolled in the study. Two of the sPTD women declined participation after signing consent because of emotional stressors surrounding their preterm delivery. Therefore, 40 women (20 sPTD and 20 matched controls with term delivery) completed AIx75 and CPP measurements 24-72 hours postpartum, and constitute the sample included in this report. Of the 40 women, 34 completed PWV measurements. One woman (5%) in the control group and 5 (25%) women in the sPTD group did not have PWV calculated due to the inability to capture a quality waveform. Table 2 presents demographic and clinical characteristics of the sPTD and control groups. There were no differences demographically between the two groups. Among the sPTD group, 16/20 (80%) experienced PROM.

Group Differences in Measures of Arterial Stiffness

Augmentation Index. Women with sPTD had significantly lower AIx75 compared to controls matched by age, race/ethnicity, parity, and route of delivery (Table 3). To explore our findings regarding group differences in AIx75 and PP, we examined two factors: presence of chorioamnionitis and administration of magnesium sulfate, both of which have potential to influence vascular tone.[55, 56] Women with sPTD and chorioamnionitis both clinically and/ or on pathology report (n=8) had significantly lower AIx75 than matched controls (n=8) (Table 3). Of note, all eight women with a diagnosis of chorioamnionitis experienced PROM. There were no differences between sPTD and matched controls in PWV (5.12 ± 1.62 m/s vs 5.58 ± 1.51m/s, p=0.1219).

Table 4 presents a model of adjusted tests for pair-wise comparisons of arterial stiffness measures across three groups (control [n=20], sPTD with chorioamnionitis [n=8], sPTD with no chorioamnionitis [n=12]), which demonstrated that the sPTD with chorioamnionitis group

significantly differed for AIx75 (p=0.001) compared to controls. In post-hoc Tukey adjusted tests for pair-wise comparisons of the groups, women with sPTD without chorioamnionitis had a trend toward lower AIx75 than controls.

Among sPTD women who received magnesium sulfate (n=10), their infant's gestational age was significantly less than sPTD women who did not receive magnesium sulfate (n=10) (30 weeks ± 15 days vs 32.5 weeks ± 19 days, p=0.002) respectively. Table 5 describes the frequency of tocolytics medication administration in controls and women with sPTD. We did not see associations between AIx75 in women with sPTD who received tocolytics, specifically in regards to magnesium sulfate (n=10), nifedipine (n=5), progesterone (n=3), indomethacin (n=3) or betamethasone (n=16) compared to women with sPTD who did not receive tocolytics. In post-hoc Tukey adjusted tests for pair-wise comparisons of the groups, there were no differences in AIx75 between women with sPTD who received magnesium versus women with sPTD who did not receive magnesium. However, statistical significance in women with sPTD who received magnesium versus their matched control had lower Aix75, and women with sPTD and chorioamnionitis who received magnesium had further lower Aix75 compared to matched controls (Table 5). There was no difference in women with sPTD and chorioamnionitis who did not receive magnesium versus matched controls, and no differences in women with sPTD without chorioamnionitis who did not receive magnesium versus matched controls (Table 5).

Central Pulse Pressure. Women with sPTD had significantly lower CPP compared to normal controls matched by age, race, parity, and route of delivery (Table 6). A mixed model linear regression (n=40which included three groups (control [n=20], sPTD with chorioamnionitis [n=8], sPTD with no chorioamnionitis [n=12]) showed that at least one of the three groups had a

different average CPP value (p=0.003) (Table 6). There was lower CPP in the sPTD group with chorioamnionitis (n=8) compared to controls (Table 6).

Women with sPTD who received magnesium (n=10) versus controls (n = 10) had significantly lower CPP (29.2 ± 5.35 mmHg vs 33.9 ± 4.15 mmHg, p=.04) (Table 6). Statistical significance remained in women with sPTD who did not receive magnesium (n=10) versus controls (n=10) (29 ± 5.66 mmHg vs 35.30 ± 5.40 mmHg, p=0.03). In a within-group comparison of women with sPTD, we found no associations between CPP and receipt of tocolytics (Table 6). Interestingly, women with sPTD and chorioamnionitis who received magnesium (n=5) compared to matched controls (n =5), CPP was not significantly different (28 ±7.35 mmHg vs 34.60 ± 4.98 mmHg, p= 0.11) respectively. However, women with sPTD and chorioamnionitis who did not receive magnesium (n=3) versus controls had lower CPP (24.67 ±4.93 vs 34.60 ±4.74, p= 0.03). Lastly, there were no differences in CPP for women with sPTD without chorioamnionitis who did not receive magnesium (n=7) versus matched controls (n=7) (30.86 ± 5.15 mmHg vs 35.86 ± 5.96 mmHg, p=0.53).

Pulse Wave Velocity. Women with sPTD did not have significant differences compared to normal controls matched by age, race, parity, and route of delivery (5.12 ± 1.62 m/s vs 5.58 ± 1.51m/s, p=0.1219) respectively. Women with sPTD who received magnesium (n=6) versus controls (n = 6) had significantly lower PWV (4.52 ± 0.97 m/s vs 5.54 ±1.49 m/s, p=.02). A mixed model linear regression (n=34) which included three groups (control [n=19], sPTD with chorioamnionitis [n=5], sPTD with no chorioamnionitis [n=10]) showed no difference between groups (p=0.56). There was no significance in women with sPTD who did not receive magnesium (n=9) versus controls (n=9) (5.52 ± 1.88 vs 5.62 ± 1.61 m/s, p=0.98). Women with sPTD and chorioamnionitis who received magnesium (n=3) compared to matched controls (n=3)

were not significantly different (4.27 ±1.42 m/s vs 5.44 ± 1.31 m/s, p= 0.23) respectively. There were no differences in women with sPTD and chorioamnionitis who did not receive magnesium (n=2) versus matched controls (n=2) (5.75 ± 0.49 m/s vs 4.80 ± 0.95 m/s, p=0.97) respectively. Lastly, there were no differences in PWV for women with sPTD without chorioamnionitis who did not receive magnesium (n=7) versus matched controls (n=7) (5.46 ± 2.16 vs 6.03 ± 1.78 m/s, p=0.98).

Discussion

In a matched case-control study of women with sPTD compared to controls, our findings show differences 24-72 hours postpartum in two putative measures of arterial stiffness, AIx75 and CPP. We did not find differences in PWV, another measure of arterial stiffness. Our use of matched controls likely ameliorated the potential effects of age, self-reported race/ethnicity, parity and route of delivery. Our subgroup and modeling analyses suggest the hypothesis that these findings might be related to physiological differences between the two groups secondary to exposure to environmental differences such as prior medication administration (magnesium) and/ or infection.[57]

Exposures to External and Internal Environmental Factors

Chorioamnionitis in Preterm Delivery: A potential explanation for relatively lower arterial stiffness in women with sPTD compared to controls may be related to chorioamnionitis. Infection such as chorioamnionitis accounts for 10% of all preterm births,[16] although the mechanisms behind its implications for preterm birth are not fully understood.[10, 16] Our data suggest that women with sPTD and chorioamnionitis had a lower AIx75 compared to those women who did not have clinical symptoms diagnosed by their physician or placental pathological evidence of chorioamnionitis (Tables 3-4). Preterm labor is often accompanied by

inflammation or infection[10] and may not manifest itself clinically. However, all eight of our PROM mothers were diagnosed with chorioamnionitis, which is a common finding secondary to microbial invasion associated with PROM.[58]

Tocolytic Medication to Treat Impending Preterm Delivery. Medications given to women with threatened preterm delivery may also have an impact on vascular smooth muscle tone affecting mainly the small muscular arteries but not the elastic aorta.[57] Several pharmacological treatment strategies are considered standard of care for preterm delivery and are commonly used prior and up to the time of delivery.[59] These tocolytics may include indomethacin, nifedipine, magnesium sulfate, progesterone and betamethasone. Many of these medications are used to postpone or prevent preterm delivery. However their use has not resulted in decreased rates of sPTD.[16]

The short-term use of magnesium sulfate in obstetric care in preterm delivery for fetal neuroprotection is common in clinical practice;[60] in our study, it was given to ten women with sPTD prior to 32 weeks gestation (Table 5). Magnesium has known vasodilatory properties which affect vascular function.[55, 61] Although the half-life of magnesium is unknown, investigators have evaluated AIx75 in preeclampsia and have documented drops in vascular resistance up to 24 hours after magnesium sulfate infusion.[55] Our findings correlate with Rogers et al.[55] who found that women with preeclampsia who received magnesium sulfate for neuroprotection in the third trimester had significantly lower AIx75 than women who did not receive magnesium sulfate. Their findings suggest that magnesium sulfate effects may last as long at 24 hours postpartum in patients and appears to compound the vasodilatory effects of sPTD.

Women with the lowest AIx75 in our cohort were those women with sPTD who received magnesium and also had a diagnosis of chorioamnionitis. The likely explanation is the

compounding of vasodilatory effects from the administration of magnesium, localized chorioamnionitis infection, and early gestational age.[55, 62, 63] In our sample, magnesium administration alone was not associated with differences in AIx75 in women with sPTD (Table 5).

Differences in Vascular Function between Controls vs sPTD

In prior studies, arterial stiffness in sPTD measured by xxx at time xxx has been compared to adverse pregnancy outcomes such as medically indicated preterm delivery secondary to preeclampsia or hypertension in pregnancy. Investigators have assumed that sPTD would have increased arterial stiffness to a degree similar to that which was described in the preeclampsia literature.[50] Our results concur with Khalil's findings that women with sPTD have lower arterial stiffness than matched controls. However, Khalil et al.[24] studied arterial stiffness in women with all-cause preterm delivery (sPTD plus medically indicated preterm delivery) compared to controls at a single time point (11 to 13 weeks gestation). They found that women with medically indicated preterm delivery had significantly higher arterial stiffness than women with sPTD and term delivery during early pregnancy.[24] Interestingly, they saw a decreased systemic arterial response to sPTD <34 weeks compared to controls.[24]

This is further supported by our finding regarding CPP, since AIx75 is expressed as a percentage of CPP.[64] We saw significantly lower CPP in the sPTD group compared to controls, which was not expected, as diastolic blood pressure and AIx75 are inversely related in young subjects compared to direct relations in older subjects.[65] AIx75 is a measure influenced by smooth muscle tone compared to CPP or PWV, although our data demonstrating lower CPP and AIx may indicate our sPTD are behaving more like older subjects (ref). Pathophysiological conditions and drugs can influence smooth muscle changes that impact these measures.

On the other hand, PWV is dominantly a measure of arterial stiffness and is therefore less influenced by pathophysiologic conditions or drugs that alter smooth muscle tone.[42] This is because PWV directly measures the more elastic, aorto-iliac pathway and has been previously described as being most clinically relevant as the aorta and its branches are in closest proximity to the left ventricle and are mostly responsible for pathophysiological effects of arterial stiffness.[42] Our findings provide evidence that the three measures of arterial stiffness we examined (PWV, AIx75 and CPP) are not interchangeable, similar to prior work (ref), and provide a more detailed picture of vascular function that includes large artery elasticity and smaller artery tone. For example, when clinical researchers interrogate vascular function with pharmacologic probes directed at nitric oxide endothelial function pathways, AIx75 is altered while PWV is not.[57]

Severity of Prematurity

A prior study[22] demonstrated that women who experience early preterm delivery have a higher risk of subsequent IHD than women who experience late preterm or early term deliveries. Hastie et al. linked three Scottish data sources to comprise 750,350 women who delivered live, singleton infants following their first pregnancy. The authors looked for group differences in later risk maternal risk of ASCVD between women with medically indicated preterm delivery and those with sPTD.[22] In this retrospective cohort, the investigators demonstrated independent associations between preterm birth and IHD death (hazard ratio [HR] 2.26, 95% CI 1.88-2.71) and IHD events (HR 1.58, 96% CI 1.47-1.71). Strikingly, there was a stronger association of sPTD with IHD in the very preterm delivery cohort (HR 3.23, 95% CI 2.17-4.8). Further investigation in long-term IHD outcomes in women experiencing preterm deliveries <32 weeks' gestation is warranted.

Strengths and Limitations

Our study has several strengths. Most notably, it is the first of the kind to compare measures arterial stiffness immediately (i.e., 24-72 hours postpartum) in women with sPTD and controls. It was performed in a large tertiary care medical center in a diverse metropolitan city, which supports its external validity. The use of a single operator increases intra-rater reliability in their ability to obtain arterial stiffness measures, in addition quality control software verifies accuracy between 3 measurements.[66] However, this is a pilot study and a core lab was not utilized. Despite the fact that study measures were taken during an often hectic time in the lives of new mothers, our retention rate was strong (95%) which further supports internal validity.

We acknowledge several limitations. One potential limitation is that all our women who delivered less than 32 weeks received magnesium, therefore it is difficult to differentiate if the effects of magnesium played the primary role for decreased arterial stiffness or if it was related to gestational age and very preterm delivery. Although we found group differences in AIx75 and CPP, we are unable to generalize based on this data for the following reasons. We were limited with a one-time, immediate post-partum measure, which may not be a representative of later measures because unique physiological changes occur at that time point. One major issue was the inability to obtain quality PWV waveforms in all women. This was related mostly to positioning and the hectic hospital room environment. Controlling the patient room environment such as noise, temperature and interruptions such as a baby crying during vascular testing may have maternal physiologic implications. Finally, women in the two groups had inherently different postpartum experiences which may have affected their testing. Most notably, women in the sPTD group did not have their infants in their rooms, while most of the women in the normal control group had infants and spouses in their rooms during testing. This potentially allowed for group differences due to different clinical environments between the two groups.

Conclusion

Women with sPTD have significantly lower AIx75 and CPP, but not PWV, compared to their matched normal controls. This could be related to physiological differences between the two groups secondary to exposure to environmental differences such as prior medication administration and/or infection. Alternative time and prospective repeated measures might be considered to both understand these results as well as further better investigate mechanistic links between sPTD, how it differs mechanistically from other APOs and its association with increased maternal ASCVD risk. Further research is required to further understand the cardiovascular changes immediately after pregnancy.

Tables

Table 1. Demographic and Clinical Characteristics of Controls and Spontaneous Preterm Delivery

		Controls N=20		*sPTD N=20*		
		Mean	Std Dev	Mean	Std Dev	p value
Age		32.7	5.59	33.3	5.8	0.28
BMI		26.02	5.1	23.97	3.87	0.10
SBP		105.7	19.94	112.2	12.78	0.72
DBP		66.3	7.7	70.75	11.83	0.25
Hemoglobin		10.61	1.33	10.80	1.46	0.68
Gestational age in weeks		39.6	1.09	30.79	2.87	**<.001**
Parity		1.25	0.44	1.40	0.75	0.42
		Number	Percent	Number	Percent	
Race/Ethnicity	Asian	4	20%	4	20%	0.70
	Black	3	15%	3	15%	
	Latina	4	20%	4	20%	
	White	8	40%	8	40%	
Marital Status	Never Married	2	10%	2	10%	0.12
	Presently Married	14	70%	17	85%	
	Living with other	4	20%	1	5%	
Education	Some school	1	5%	1	5.26%	0.33
	High School	2	10%	1	5.26%	
	Assoc. Degree	2	10%	1	5.26%	
	College Graduated	10	50%	11	57.89%	
	Master's Degree	5	25%	4	21.05%	
	Doctoral	0	0	1	5.26%	
Family Income	<35,000	6	32%	4	21%	0.36
	>35,000	13	68%	15	79.5%	
Clinical Characteristics	Caesarean Delivery	5	25%	7	35%	0.73
	Positive for Chorioamnionitis	0	0	8	40%	
	Received Magnesium Sulfate	0	0	10	50%	

Table 2. The Association Between Chorioamnionitis and Vascular Function in sPTD vs Controls

	Controls N=20		*All sPTD N = 20*		*sPTD + Chorioamnionitis N=8*		*sPTD - Chorioamnionitis N=12*	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
AIx75 (%)	39.85	15.19	21.35**	16.07	13.5**	13.67	26.58	15.89
PP (mmHg)	34.6	4.74	29.10*	5.36	26.75**	6.39	30.67	4.12
PWV (m/s)	5.58	1.5	5.12	1.62	4.86	1.31	5.25	1.8

Post-hoc pair-wise Tukey-Kramer adjusted all variables.

*Compared to +Chorioamnionitis, $p < .01$

** Compared +Chorioamnionitis, $p < .001$

Table 3. Mixed Model for Augmentation Index Adjusted for Control, sPTD with Chorioamnionitis, sPTD without Chorioamnionitis (N = 40)

Group	Vs. Group	Est.	Std. Error	DF	t Value	Pr > |t|	P-value*
sPTD + Chorioamnionitis (n = 8)	sPTD - Chorioamnionitis (n= 12)	-13.11	7.26	37	-1.80	0.08	0.19
sPTD + Chorioamnionitis (n = 8)	Control (n = 8)	-26.36	6.49	28.1	-4.06	0.0004	**0.001**
sPTD – Chorioamnionitis (n= 12)	Control (n = 12)	-13.26	5.62	23.9	-2.36	0.03	0.06

* Using Tukey-Kramer adjustment for pairwise comparisons

.

Table 4. Tocolytic Use (N = 40) and Mixed Model for Augmentation Index Adjusted for Tocolytic Use in sPTD (N = 20)

		Control N=20		sPTD N=20		
		#	%	#	%	p value
Magnesium (n=10)		0	0	10	50	**<.001**
Nifedipine (n=5)		0	0	5	25	**.001**
Indomethacin (n=3)		0	0	3	15	**.041**
Progesterone (n=3)		0	0	3	15	**.009**
Betamethasone (n=16)		0	0	16	80	**<.001**
sPTD Group	**Vs sPTD Group**	**Est.**	**St. Error**	**DF**	**T value**	**Adj. p value**
+ Magnesium (n=10)	- Magnesium (n=10)	5.5	7.40	37	0.75	0.74
+ Nifedipine (n=5)	- Nifedipine (n=15)	-5.73	8.56	37	-0.67	0.78
+ indomethacin (n=3)	-Indomethacin (n=17)	-13.41	10.17	36.90	-1.32	0.40
+ Progesterone (n=3)	-Progesterone (n=17)	-11.67	8.41	36.70	-1.39	0.36
+ Betamethasone (n=16)	- Betamethasone (n=4)	10.18	9.15	37	1.11	0.51x

Table 5. Mixed Models for Pulse Pressure Adjusted for Presence/Absence of Chorioamnionitis (N = 40) and Presence/Absence of Tocolytics (N = 20)

Group	Vs. Group	Est.	Std. Error	DF	t Value	Pr > |t|	Adj P-value*
sPTD + Chorioamnionitis (n = 8)	sPTD - Chorioamnionitis (n= 12)	3.95	2.36	36.70	1.68	0.10	0.23
sPTD + Chorioamnionitis (n = 8)	Control (n = 20)	7.87	2.15	28.40	3.65	0.001	**0.003**
sPTD – Chorioamnionitis (n= 12)	Control (n = 20)	3.92	1.88	24	2.09	0.05	0.11
sPTD Group	**Vs sPTD Group**	**Est.**	**St. Error**	**DF**	**T value**	**Adj. p value**	
sPTD with Magnesium (n=10)	sPTD without Magnesium (n=10)	-0.11	2.21	36.80	-.0.05	0.99	
sPTD with nifedipine (n=5)	sPTD without nifedipine (n=15)	-5.60	2.60	36.40	-.2.16	0.09	
sPTD with indomethacin (n=3)	sPTD without indomethacin (n=17)	0.28	3.37	36.80	0.08	0.10	
sPTD with Progesterone (n=3)	sPTD without Progesterone (n=17)	0.77	2.78	36.80	0.28	0.96	
sPTD with Betamethasone (n=16)	sPTD without Betamethasone (n=4)	-2.51	2.97	36.60	-0.85	0.68	

* Using Tukey-Kramer adjustment for pairwise comparisons

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Chapter 3: Association of Spontaneous Preterm Delivery and Postpartum Cholesterol and Inflammation.

Introduction

Atherosclerotic cardiovascular disease (ASCVD) remains the number one killer of women nationwide.[1] Many ASCVD risk factors, particularly elevated levels of serum lipids, are well established. Less is known about a woman's cardiovascular response to pregnancy, which has been posited to be an early marker of future maternal cardiovascular risk.[2] Spontaneous preterm delivery (sPTD), an adverse pregnancy outcome (APO), has been associated with a three-fold increased risk of maternal cardiovascular disease later in life. Women who experience sPTD are more likely to have maternal dyslipidemia and later development of ASCVD than women who experience normal, term deliveries.[3-7] The association of sPTD with subsequent future maternal ASCVD risk suggests that a women's response to pregnancy as evidenced by whether she can carry to full term serves as her first physiological "stress test".[2] However, mechanistic pathways linking the association between sPTD and future maternal ASCVD risk is unknown.

Current data suggest a clustering of vascular, inflammatory and traditional cardiac risk markers in women with sPTD which may predispose women to additional future ASCVD risk. Therefore, sPTD may provide an early signal for future cardiovascular events. The purpose of this study was to determine if women who experience sPTD $\leq$ 34 weeks of gestation have abnormal cholesterol or inflammatory markers immediately postpartum compared to women who deliver $\geq$ 39 weeks and to establish effect sizes for future investigation.

Methods

Study population

We performed a matched case control study of women with sPTD. Women with sPTD (≤ 34 weeks) were matched by age ±5 years), parity, route of delivery and self-reported race to women with term delivery (controls) (≥ 39 weeks). All participants resided in the metropolitan Los Angeles and surrounding areas, which offer an abundant and diverse population. Women were recruited from the Labor and Delivery unit at Cedars-Sinai Medical Center, which experienced approximately 6700 deliveries in 2016. The sample included a total of 38 participants, 19 sPTD and 19 term delivery controls. Inclusion criteria included: 1) age ≥ 18; 2) ability to speak English and/or Spanish 3) competent to give informed consent; 4) singleton birth; 5) no medical indication for preterm delivery (i.e. hypertensive disorders of pregnancy, preeclampsia; and 6) no prior history of CVD, including acute myocardial infarction, acute coronary syndrome, coronary or peripheral revascularization, stroke, trans-ischemic attack, diabetes (ASCVD equivalent), gestational diabetes or smoking. For the control group, additional criteria included: 1) matched in age ± 5 years, self-reported race, route of delivery and parity to a sPTD participant, and 2) delivery ≥ 39 weeks.

Defining Preterm Delivery

Among women who deliver prematurely, there are two primary types of preterm delivery, spontaneous or medically indicated. Spontaneous preterm delivery can occur either with intact membranes or with premature rupture of fetal membranes (PROM). Medically indicated preterm delivery can result from induction of preterm labor or from preterm cesarean delivery for maternal or fetal indications.[8] Severity of preterm delivery has been defined as late preterm (34-37 weeks gestation), early preterm (<34-32 weeks gestation) and very preterm delivery (<32 weeks gestation). [4, 9] We selected to study women with sPTD ≤ 34 weeks because this timeframe is an accepted marker for early preterm delivery and has been used by other investigators in the field.[4, 10, 11]

Procedures

This study was approved by the internal review board at Cedars-Sinai Medical Center (IRB#32089). Clinicians referred patients who might potentially deliver ≤ 34 weeks to the research team. Informed consent was obtained prior to the research procedures. After enrollment and between 24-72 hours post-delivery, inpatient testing was conducted in the patient's private room at a time when minimal visitors were present and no other health care activities were scheduled. A chart review was conducted to record clinical data (age, parity, prenatal care) and to document all medications given to the participant during and after delivery. Each participant completed a self-report questionnaire to obtain demographic data and provided a peripheral blood sample.

Laboratory Measurements

Cholesterol Panel

During the first 24-72 hours postpartum and prior to hospital discharge, non-fasting peripheral blood draw was performed by a registered research nurse trained in phlebotomy. The cholesterol panel included total cholesterol (TC), high density lipoprotein calculated (HDLc), low density lipoprotein calculated (LDLc) and triglycerides (TRG). Since there is limited data on cholesterol levels immediately postpartum, the time point was selected based on patient access and convenience. We used vacutainer tubes containing EDTA and placed tubes on ice until delivered to the Cedars-Sinai Center for Clinical and Translational Science Institute (CTSI) for processing. All samples were centrifuged at 1000 x g, aliquoted, and stored at –80°C. Assays were performed in batches every two weeks and sent by CTSI via currier service to LabCorp corporation for processing. Processing was completed based on a LabCorp proprietary protocol.[12] Both TC and TRG were processed with enzymatic methodology. LDLc and HDLc were measured by calculation methodology.[12]

Interleukin-6

Samples were centrifuged at 1000 x g, aliquoted, and stored at –80°C. Samples were sent in batches every two weeks by CTSI via currier service to LabCorp corporation for processing. interleukin-6 (IL-6) was measured by sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (R & D Systems, Inc., Minneapolis, MN). Wells of microtiter plates coated with murine anti-human IL-6 monoclonal antibody were filled with 100 µL of a buffered protein base (1% bovine serum albumin (BSA), 5% sucrose in PBS with 0.05% NaN3). Plasma samples and standards (recombinant human IL-6 in a buffered protein base) were added and incubated for 2 hours at room temperature. Then wells were aspirated and washed four times, and 200µL horseradish peroxidase were added and incubated for 2 hours. The wells were aspirated and washed four times again, followed by the addition of 100 µL substrate solution mixture (1:1 mixture of H2O2 and tetramethylbenzidine) for 20 minutes at room temperature. The reaction was stopped by the addition of 50 µL of stop solution (2NH2SO4) and the optical densities were read using a microplate reader at 450 nm with correction wavelength set at 540 nm. IL-6 concentrations were derived from a standard curve. Sensitivity of this test has been reported at 0.055 pg/ml, and coefficients of variation have been reported as < 10%.[13]

HS-CRP

Highly sensitive C-reactive protein (CRP) was measured by high sensitivity sandwich ELISA (Dade Behring Diagnostics, Marburg, Germany). The assay is standardized against the international reference preparation from plasma proteins, CRM 470. Diluted samples were added to wells of a microtiter plate pre-coated with rabbit anti-CRP polyclonal antibodies. The plates were incubated at room temperature for 1 hour and then washed. A peroxidase-labeled rabbit anti-CRP antibody were added to each well and incubated for another hour. After a second wash, tetramethylbenzidine was added to each well and the reaction was stopped after 20 minutes. The

optical density of each well was determined at 450 nm. CRP concentrations were derived from a standard curve. [14] The assay has a functional sensitivity of 0.1 mg/l, and interdilution coefficients of variation are reported at 7%. [14, 15]

Power Analysis

Power analysis was calculated for the primary aim (HDL) using G*Power 3.1.[16, 17] Data from Bartha et al.[5] were used to provide estimates of HDL standard deviations of 18.4, and 18.2 for the sPTD and term control groups, respectively.[5] Using these estimates together with paired t-tests, we estimated that 20 subjects in each group would yield 80% power to detect at least a difference of 23.5 mg/dL between groups at the 0.05 significance level, using a conservative estimate of zero correlation within matched pairs, or a difference of 10.5 with a correlation of 0.8 within matched pairs.

Data Analysis

Data were analyzed using SPSS release 21 for Windows (Chicago, IL), SAS version 9.3 (Cary, NC), and R version 3.2. For all analyses, the significance level was set at 0.05. Log transformations were performed for continuous data that were not normally distributed. To examine the equality of the two groups, unmatched baseline sociodemographic (educational level and marital status) and unmatched clinical variables and cardiovascular risk markers (pre-pregnancy BMI, family history of CVD, route of delivery) were compared using conditional logistic regression models where the outcome was group (sPTD vs controls) and the models were adjusted for the variable in question and stratified by match. Mixed effects linear regression models with a random effect for match were used to compare the main study outcomes between the groups (sPTD vs. control) and adjust for potential confounding effects among unmatched variables. For post-hoc comparisons of groups in these outcomes a Tukey-Kramer adjustment was made to the p-values.

Results

The original sample for the study included 42 participants, 22 with sPTD and 20 controls with term delivery. Three of the sPTD women declined participation after signing consent because of emotional stressors surrounding their preterm delivery. One additional sPTD woman declined the blood draw due to emotional stressors surrounding the preterm delivery. We excluded her matched control due to exclusion criteria. A total of 38 women completed cholesterol and inflammatory marker testing.

Table 1 presents demographic and clinical characteristics of the sPTD and control groups. The mean age at enrollment for sPTD and controls was 33 ± 6 vs. 32 ± 5 years ($p = 0.21$), respectively. There were no differences in demographics between the two groups. Among the sPTD group, 15 out of 19 women experienced PROM; the remaining four women had contractions which resulted in sPTD. Among the women who experienced PROM, seven had evidence of chorioamnionitis either clinically or on pathology report.

Group comparisons of cholesterol and inflammatory markers are presented in Table 2. After adjusting for group (sPTD vs control), BMI, SBP at baseline, educational level and marital status, the HDL difference between groups was still significant ($p=0.0048$). However, none of the other factors were significantly associated with HDL. Compared to controls, women with sPTD had significantly reduced HDL (59.37 ± 12.52 mg/dL vs 67.63 ± 13.1 mg/dL, $p = 0.035$), respectively.

In a sub analysis to evaluate the influence of chorioamnionitis on CRP, we found some trends in CRP levels in a three-group analysis (sPTD with chorioamnionitis, sPTD without chorioamnionitis, and controls). Differences approached significance in two sets of comparisons: between the two groups of sPTD women with and without chorioamnionitis (100.85 ± 68.99

mg/L vs 59.65 ± 105.84 mg/L, p = .056) and between controls and women without chorioamnionitis (71.26 ± 51.16 vs 59.65 ±105.84, p = .055) (Table 3).

Discussion

We found significantly lower HDLc cholesterol levels during the early postpartum period (24-72 hours) in women who experienced sPTD compared to matched controls who delivered at term. Women with sPTD demonstrated HDLc levels below those considered protective against ASCVD risk, while women who experienced a term delivery had HDLc levels similar to those of young adult, non-pregnant women.[18] This relationship remained significant after adjustment of potential confounders, including BMI, SBP, education level, and marital status.

Among traditional ASCVD risk markers, lower HDLc cholesterol is considered a promoter of atherogenic profiles and has an established inverse relationship with ASCVD risk.[19-21] The women in our study (sPTD or controls) did not have prior histories of dyslipidemia, diabetes or ASCVD and would have been considered to be at low cardiovascular risk prior to delivery. Although others have found an association of sPTD with TRG,[22] we did not see differences between sPTD and controls in TRG or in TC or LDL. This is likely attributed to timing of the blood collection. Catov et al.[11] collected pre-pregnancy specimens and saw differences in TC, TRG. This was replicated in early pregnancy prior to 15 weeks' gestation.[22] As pregnancy progresses TRG and LDL increase to supply nutrients to the fetus. Therefore, collecting blood specimens in the early postpartum stage may be less beneficial.[23]

Based on our findings, it is plausible that normal changes in lipid variations during pregnancy and in the early postpartum period could have influenced our findings. During pregnancy, increased levels of estrogen stimulate dynamic changes to lipids resulting in increases of TC, HDLc, LDLc and TRG, which nadir in the second trimester.[23, 24] At the end of pregnancy, elevations in TRG provide maternal energy in the form of fats, sparing glucose and

amino acids for the use of the fetus.[25] Unlike previous studies, the peripheral blood draw in our study occurred 24-72 hours after delivery. This may be insufficient time to allow the cholesterol to normalize after delivery.

Several other factors could have explained our findings. First, the women in our study were non- fasting due to the nature of being early postpartum, which lends itself to variability in breastfeeding or utilizing a breast pump. Second, a non-fasting state could have influenced blood cholesterol levels, but generally has greater impact on TRG and smaller impact on HDLc and LDLc.[26] A final consideration may have been our modest sample size.

Our findings are consistent with other reports regarding the relationship of preterm delivery to HDL levels over time after delivery. Perng et al.[27] found that women with preterm delivery had lower HDL than women with term deliveries three years postpartum, which suggests that women may have related increases in overall ASCVD risk factors. Further, there may be increased cardiometabolic changes in women with sPTD over time resulting in additional ASCVD risk. Catov and colleagues[7, 28] studied women a decade after delivering sPTD, and reported higher SBP and increased atherogenic lipid profiles. This phenotype appears to progress. In a unique second report, Catov et al. reported that women who delivered prior to 34 weeks had increased metabolic syndrome independent of their pre-pregnancy metabolic status, and pregnancy complications remained up to 25 years postpartum.[28]

Together with previous reports, our data highlight the limitations of the current ASCVD risk score. Because age continues to be a major determinant of 10-year ASCVD risk for both men and women,[29] the ASCVD risk score is formulated for individuals aged 40-79 years, and is not able to calculate 10-year risk in individuals less than 40 years of age. [30] However, clinicians can improve risk stratification and potentially provide earlier identification of future ASCVD

risk by identifying women with low HDL, a known cardiac risk factor, and assessing for APO history such as sPTD.

Inflammation in sPTD

Approximately 75% of preterm delivery is the result of sPTD. Preterm labor is often accompanied by inflammation or infection[31] which may not be clinically manifested. Infection accounts for 10% of all preterm births,[32] although the mechanisms behind causes of preterm birth are not fully understood.[8, 31] Understanding the relationship of inflammation to sPTD is complicated because birth, itself, has been described as an inflammatory process.[8] Although we did not find group differences in CRP and IL-6, other investigators have reported elevated CRP and IL-6 levels in women with preterm delivery compared to controls.[5, 6, 33] Increased levels of this inflammatory biomarkers may be due to secondary infectious causes, such as chorioamnionitis.[31] In our study, we completed a three-group (sPTD with chorioamnionitis, sPTD without chorioamnionitis and controls) sub-analysis and found that average CRP levels tended to be lowest in the sPTD group without chorioamnionitis and highest in the sPTD group with chorioamnionitis). One explanation may be that in women with sPTD, clinical chorioamnionitis is difficult to diagnose clinically[8] or potentially the chorioamnionitis infection is isolated to intra-amniotic inflammation[34] and less detectable in the peripheral blood sample.[35]

Strengths and Limitations

Our data supports similar findings by other investigators before, during and after pregnancy.[6, 22, 36] Because our report is a preliminary finding from a study designed for within subject comparison over time,[37] our sample size may not have been sufficient to identify group differences during the immediate postpartum period alone. We present a single time point from a single center, with no baseline or follow up data. We also acknowledge environmental variability, in that most infants of women who experienced sPTD required neonatal intensive

care and were not rooming in with their mothers, while most infants of normal control mothers were rooming in. This situation may have increased variability between groups in other factors, breastfeeding vs utilizing a breast pump and meal patterns in relationship to blood sample collection.

Conclusions

We conducted a matched case control study of 19 women with sPTD and 19 controls who were matched to account for age, self-reported race, parity and route of delivery. We found that women with sPTD have lower HDLc compared to controls 24-72 hours postpartum. HDLc plays an important role in ASCVD prevention and lower levels may predispose younger women with sPTD to increased ASCVD risk later in life. Future strategies for clinical practice may include identification of lower HDLc levels after lactation and assessment of APO history to identify potentially at-risk women and therefore provide earlier, more vigilant ASCVD prevention and monitoring.

Table 1. Demographic and Clinical Characteristics of sPTD and Control Groups.

		sPTD N=19	*Control N=19*	
Variables		**Mean ±SD**	**Mean ±SD**	**p value**
Age		32.95±5.66	32.16 ±5.18	0.21
Pre-Pregnancy BMI km/m^2		23.99±3.97	26.13 ±5.01	0.08
SBP mmHg		104.18± 13.38	105.05 ± 9.83	0.81
DBP mmHg		72.05 ±10.62	68.18 ±7.76	0.20
Hemoglobin g/dL		10.61 ±1.33	10.68±1.33	0.82
Gestational age in weeks		39.9 ±0.96	31±2.59	**<.001**
Parity		1.25 ±0.44	1.40±0.75	0.42
		Number/ Percent	Number/Percent	
Race	Asian	4 (21%)	4 (21%)	0.50
	Black	3 (16%)	3 (16%)	
	Latina	4 (21%)	4 (21%)	
	White	8 (42%)	8 (42%)	
Marital Status	Never Married	2 (10.5%)	2 (10.5%)	0.25
	Presently Married	14 (74%)	16 (84%)	
	Living with other	3 (16%)	1 (5%)	
School	Some school	1 (5%)	1 (5%)	0.33
	High School	2 (10.5%)	1 (5%)	
	Assoc. Degree	2 (10.5%)	1 (5%)	
	College Graduate	9 (47%)	11 (58%)	
	Master's Degree	5 (26%)	4 (21%)	
	Doctoral	0	1 (5%)	
Family Income	<$35,000	4 (21%)	6 (32%)	0.36
	>$35,000	15 (79%)	13 (68%)	

Table 2. Results from Cholesterol and Inflammatory Markers in sPTD vs Control Groups.

		sPTD Group (n = 19)	Control Group (n = 19)	
Cholesterol Parameter	**Reference Ranges**	**Mean ± SD**	**Mean ± SD**	**Paired test p value**
TC (mg/dL)	100-199	213.79 ±29.42	203.68 ± 37.16	0.30
TRG (mg/dL)	0-149	216.21 ±101.80	193.79 ±66.36	0.32
HDL (mg/dL)	>39	59.37 ±12.52	67.63 ±13.10	**0.04**
LDL (mg/dL)	0-99	113.33 ±28.53	97.32 ±34.60	0.15
CRP (mg/L)	0-3	74.8 ±94.07	72.97 ±51.97	0.23
IL-6 (pg/mL)	0-15.5	19.31 ±40.36	10.93 ±8.20	0.52

Table legend: TC- Total Cholesterol, TRG- Triglycerides, HDL- High Density Lipoprotein calculated, LDL-Low Density Lipoprotein Calculated, hsCRP- Highly Sensitive C-Reactive Protein, IL-6- Interleukin-6.

Table 3. Group Differences in CRP and IL-6 with Mixed Model Three-Group Analysis of Chorioamnionitis Effects.

Group	CRP (Mean ± SD)	IL-6 (Mean ± SD)
sPTD + Chorioamnionitis	100.85 ±68.99	12.13 ± 13.35
sPTD - Chorioamnionitis	59.65 ±105.84	23.5 ± 50.16
Control	72.97 ± 51.97	10.93 ± 8.2

Group	Comparison Group	Estimate	Standard Error	t value	Adjusted p-value
Group LS means differences in log CRP					
Control	sPTD + Chorioamnionitis	-0.37	0.51	-.73	0.75
Control	sPTD – Chorioamnionitis	1.01	0.41	2.43	0.06
sPTD + Chorioamnionitis	sPTD – Chorioamnionitis	1.38	0.56	2.44	0.06
Group LS means differences in log IL-6					
Control	sPTD + Chorioamnionitis	0.13	0.43	0.31	0.95
Control	sPTD – Chorioamnionitis	0.23	0.35	0.67	0.79
sPTD + Chorioamnionitis	sPTD – Chorioamnionitis	0.10	0.47	0.22	0.97

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