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Placental Growth Factor as an Indicator of Maternal Cardiovascular Risk After Pregnancy

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BACKGROUND: Angiogenic placental growth factor (PlGF) concentrations rise during pregnancy, peaking at the end of midpregnancy. Low PlGF concentrations during pregnancy are associated with pregnancy complications with recognized later-life cardiovascular risk. We hypothesized that low PlGF concentrations, especially in midpregnancy, identify not only a subset of women at risk for pregnancy complications but also women with greater cardiovascular risk factor burden after pregnancy regardless of pregnancy outcome.

METHODS: In a population-based prospective cohort study of 5475 women, we computed gestational age–adjusted multiples of the medians of early pregnancy and midpregnancy PlGF concentrations. Information on pregnancy complications (preeclampsia, small for gestational age, and spontaneous preterm birth) was obtained from hospital registries. Six years after pregnancy, we measured maternal systolic and diastolic blood pressures, cardiac structure (aortic root diameter, left atrial diameter, left ventricular mass, and fractional shortening), carotid-femoral pulse wave velocity, and central retinal arteriolar and venular calibers. Blood pressure was also measured 9 years after pregnancy.

RESULTS: Women were on average 29.8 (SD, 5.2) years of age in pregnancy, were mostly European (55.2%), and 14.8% developed a pregnancy complication. Quartile analysis showed that especially women with midpregnancy PlGF in the lowest quartile (the low-PlGF subset) had a larger aortic root diameter (0.40 mm [95% CI, 0.08–0.73]), left atrial diameter (0.34 mm [95% CI, –0.09 to 0.78]), left ventricular mass (4.6 g [95% CI, 1.1–8.1]), and systolic blood pressure (2.3 mm Hg [95% CI, 0.93–3.6]) 6 years after pregnancy than women with the highest PlGF. Linear regression analysis showed that higher midpregnancy PlGF concentrations were associated with a smaller aortic root diameter (–0.24 mm [95% CI, –0.39 to –0.10]), smaller left atrial diameter (–0.75 mm [95% CI, –0.95 to –0.56]), lower left ventricular mass (–3.9 g [95% CI, –5.5 to –2.3]), and lower systolic blood pressure (–1.1 mm Hg [95% CI, –1.7 to –0.46]). These differences persisted after the exclusion of women with complicated pregnancies.

CONCLUSIONS: Women with low PlGF in midpregnancy have a greater aortic root diameter, left atrial diameter, and left ventricular mass and higher systolic blood pressure 6 and 9 years after pregnancy compared to women with higher PlGF, including women with uncomplicated pregnancies. The pathophysiological implications of lower PlGF concentrations in midpregnancy might provide insight into the identification of pathways contributing to greater cardiovascular risk factor burden.

Key Words: cardiovascular diseases ■ placenta growth factor ■ pre-eclampsia ■ pregnancy outcome ■ risk factors

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Clinical Perspective

What Is New?

- This study identifies low maternal placental growth factor levels in midpregnancy to be associated with a greater aortic root diameter, left atrial diameter, and left ventricular mass and higher systolic blood pressure 6 and 9 years after pregnancy compared with women with high midpregnancy placental growth factor levels.
- These associations were observed in women with and without a complicated pregnancy (ie, affected by preeclampsia, small for gestational age, and spontaneous preterm birth).

What Are the Clinical Implications?

- These results suggest that a woman's response to the cardiovascular challenges of pregnancy, measured by midpregnancy placental growth factor, could provide insight into the pathophysiological mechanisms leading to future cardiovascular disease in parous women.

Pregnancy is accompanied by extensive maternal hemodynamic changes that allow proper placental implantation, growth, perfusion, and fetal development. This process requires a tight balance between proangiogenic (eg, placental growth factor [PlGF]) and antiangiogenic (eg, soluble fms-like tyrosine kinase-1 [sFlt-1]) factors. Dysregulation of PlGF and sFlt-1 is proposed to reflect stress to the syncytiotrophoblast of the placenta, which produces these factors.[1] In response to stress, the syncytiotrophoblast will decrease the production of PlGF and release more sFlt-1 into the maternal circulation. Consequently, sFlt-1 will bind to circulating PlGF and further decrease PlGF availability. Women with reduced PlGF and increased sFlt-1 are more at risk of a complicated pregnancy (preeclampsia, spontaneous preterm birth [sPTB], and children born small for gestational age [SGA]).[2] Furthermore, findings from an animal study demonstrate that low PlGF is associated with abnormal cardiovascular remodeling during pregnancy (eg, excessive increase in left ventricular [LV] mass, and systolic blood pressure [SBP]).[3] The pregnancy complications associated with reduced circulating PlGF are also associated with an increased risk of cardiovascular disease (CVD) in later life.[4] The largest difference in PlGF concentration between women with a complicated pregnancy and those with an uncomplicated pregnancy can be observed during midpregnancy. Longitudinal studies examining PlGF across pregnancy showed that low midpregnancy PlGF was associated with earlier onset of preeclampsia, which is the form of preeclampsia with the highest risk for later-life CVD.[4,5] sFlt-1 levels differ less profoundly throughout pregnancy between both groups.[1] Low PlGF and high sFlt-1 can lead to vascular inflammation and endothelial dysfunction.[6] Endothelial dysfunction can persist for at least 15 years after pregnancy and plays an important role in the development of CVD.[7,8]

We propose that lower PlGF concentrations, most evident in midpregnancy when PlGF rapidly increases, might identify women at increased risk for developing not only complicated pregnancies but also greater cardiovascular risk factor burden years before the onset of CVD.[9] The aim of this study was to determine whether lower PlGF concentrations during midpregnancy were associated with a disadvantageous cardiovascular risk factor profile 6 years after pregnancy as evaluated by measurement of pulse wave velocity (PWV), fractional shortening (FS), aortic root diameter (AOD), left atrial diameter (LAD), LV mass, blood pressure, and central retinal arteriolar and venular calibers and 9 years after pregnancy as assessed by blood pressure. We also examined whether these associations were valid not only for women with complicated pregnancies but also for women with uncomplicated pregnancies.

METHODS

The data, analytical methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Design and Study Population

This study was embedded in the Generation R Study, a population-based prospective cohort from early pregnancy on.[10,11] The Medical Ethics Committee of the Erasmus Medical Center Rotterdam, the Netherlands, approved the study. Written informed consent was obtained from all participants. We included women with a live-born singleton, available data on midpregnancy PlGF, and at least 1 available cardiovascular measurement of pregnancy or follow-up. We excluded women with chronic hypertension before pregnancy, women with preexisting disease (eg, CVD), and women who were pregnant (n=190 and 51, respectively) or were receiving antihypertensive treatment (n=46 and 67, respectively) during the 6- or 9-year follow-up measurements. To attempt to exclude the effect of increased blood pressure, women with only gestational hypertension during the index pregnancy (gestational hypertension without sPTB or SGA) were excluded. Women with self-reported gestational hypertension or preeclampsia after an uncomplicated index pregnancy were included in the total analyses but excluded from the analyses in the uncomplicated pregnancy group. The final population for analysis comprised 5475 women (Figure), of whom 4664 had an uncomplicated index pregnancy and 811 had a complicated index pregnancy.

In addition to cardiovascular risk factors after pregnancy, we studied cardiovascular adaptation during pregnancy to support our hypothesis. Cardiovascular adaptation to pregnancy has been examined previously in the Generation R Study.[2] Coolman et al[2] examined the association of placental biomarkers in early and midpregnancy (PlGF and sFlt-1)

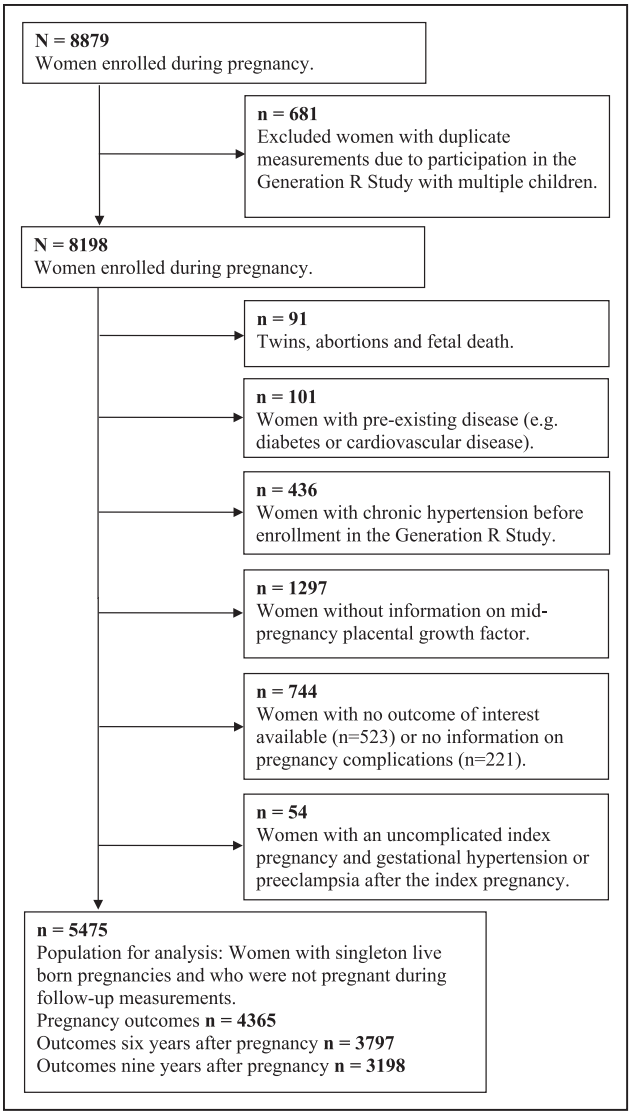

Figure. **Flowchart.**

with placental function (uterine artery [UtA] resistance index), placental weight, birth weight, and pregnancy complications (sPTB, fetal growth restriction, and preeclampsia).

Complicated Pregnancy

Complicated pregnancies studied included pregnancies with preeclampsia, SGA, or sPTB. We obtained information on clinically diagnosed preeclampsia from medical records that were cross-checked with the original hospital charts.[12] Preeclampsia was defined with the International Society for the Study of Hypertension in Pregnancy criteria that were in effect at the time of the study as new-onset SBP ≥140 mm Hg or diastolic blood pressure (DBP) ≥90 mm Hg after 20 weeks of gestation and the presence of proteinuria with no evidence of urinary tract infection in a random urine sample.[13] Midwife and hospital registries provided information on the child's gestational age at birth, birth weight, and sex. We defined sPTB as the spontaneous onset of labor before 37 weeks of gestation. SGA was used as a surrogate for fetal growth restriction and was defined as a birth weight below the 10th percentile adjusted for gestational age and sex of the child. Information on gravidity, parity, and the occurrence of gestational hypertension and preeclampsia in pregnancies other than the index pregnancy was obtained in an interview 9 years after pregnancy.

Placental Growth Factor

Our primary analysis was PlGF measured at midpregnancy (mean, 20.6 [SD, 1.1] weeks of gestation) We also measured PlGF in early pregnancy (mean, 13.5 [SD, 2.0] weeks of gestation). Analyses were performed in nonfasting venous blood samples. Details of processing procedures have been described previously.[2,11] The Department of Clinical Chemistry of the Erasmus Medical Center analyzed PlGF concentrations using an immunoelectrochemoluminence assay on the Architect System (Abbott Diagnostics BV, Hoofddorp, the Netherlands). The between-run coefficients of variation were 4.7% at 24 pg/mL and 3.8% at 113 pg/mL for PlGF.[2] PlGF varies with gestational age and is not normally distributed. Therefore, we constructed PlGF gestational age–adjusted standardized multiple of the median scores, which we used in all regression models.[14] Table 1 presents absolute PlGF concentrations.

Hemodynamic, Demographic, and Anthropometric Measurements During Pregnancy

We obtained information on maternal age, ethnicity, educational level, gravidity, parity, prepregnancy weight, smoking, chronic disease during pregnancy, chronic hypertension before pregnancy, and medication use through questionnaires given repeatedly during the index pregnancy.

In the second (mean, 20.6 [SD, 1.1] weeks of gestation) and third (mean, 30.4 [SD, 1.0] weeks of gestation) trimesters of pregnancy, we performed Doppler velocimetry of the uterine arteries. The UtA pulsatility index (peak systolic velocity–end-diastolic velocity)/time averaged) of the left and right UtAs was measured near the crossover with the external iliac artery. We recorded 3 consecutive uniform waveforms by pulsed Doppler ultrasound for each measurement. For further analyses, we used the mean of these 3 measurements and the mean of the left and right UtAs.[15] In addition to the UtA pulsatility index measurement in the third trimester, we assessed the presence of notching (either left- or right-sided) in flow velocity waveforms. The sonographer was blinded to previous measurements and pregnancy outcomes. Doppler measurements showed a high intraclass correlation coefficient value (>0.80) with corresponding low coefficient of variation value (<10%), which indicates adequate reproducibility.[16]

Trained research assistants wearing usual clothing (ie, no white coats) measured SBP and DBP in early, mid, and late pregnancy in the right upper arm with the validated Omron 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe BV, Hoofddorp, the Netherlands). Before the measurement, women sat in an upright position with back support and relaxed for 5 minutes. The mean value of 2 blood pressure readings over a 60-second interval was documented.

At study enrollment (during pregnancy) and in midpregnancy, we measured maternal height (centimeters) and weight

Table 1. Baseline and Follow-Up Characteristics (n=5475)

Pregnancy	
Age at enrollment, mean (SD), y	29.8 (5.2)
Non-European ethnicity, %	44.8
No education/primary school, %	13.0
Prepregnancy BMI, median (90% range), kg/m^2	22.6 (18.6–31.6)
Lean and normal, %	72.8
Overweight, %	19.9
Obese and morbidly obese, %	7.3
SBP midpregnancy, mean (SD), mm Hg	116.1 (11.6)
DBP midpregnancy, mean (SD), mm Hg	66.5 (8.9)
PlGF early pregnancy, median (90% range), pg/mL	47.5 (17.8–151.3)
PlGF midpregnancy, median (90% range), pg/mL	146.8 (91.4–524.4)
Smoking, %	28.2
Nulliparous, %	59.5
Pregnancy outcomes, %	
sPTB	3.8
SGA (<10th percentile)	10.3
Preeclampsia	1.5
Gestational hypertension	0.3
Male	50.4
Follow-up 6 y after pregnancy (n=3797)	
Follow-up interval, median (90% range), y	6.0 (5.7–7.3)
BMI, median (90% range), kg/m^2	24.5 (19.7–35.1)
SBP, mean (SD), mm Hg	118.2 (11.7)
DBP, mean (SD), mm Hg	70.2 (9.2)
Smoking, %	21.2
Pregnant more than once, %	91.1
Gravidity, median (90% range), n	3.0 (1.0–9.0)
Cardiovascular medication, %	0.2
Follow-up 9 y after pregnancy (n=3198)	
Maternal age, mean (SD), y	41.0 (4.8)
BMI, median (90% range), kg/m^2	24.6 (20.0–35.0)
SBP, mean (SD), mm Hg	113.4 (11.6)
DBP, mean (SD), mm Hg	67.9 (7.7)
CVD, %	31 (1.0)
Pregnant more than once, %	92.0
Parity, median (90% range), n	3.0 (1.0–6.0)
Preeclampsia after the index pregnancy, %	0.7
Ever preeclampsia, %	2.5
Gestational hypertension after the index pregnancy, %	1.0
Ever gestational hypertension, %	3.5

Values are percentages for categorical variables, means (SDs) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution. Imputed values are shown for confounders.

BMI indicates body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; PlGF, placental growth factor; SBP, systolic blood pressure; SGA, small for gestational age; and sPTB, spontaneous preterm birth.

(kilograms) without shoes. Thereafter, body mass index (BMI) was calculated and categorized as lean and normal BMI (<25.0 kg/m^2), overweight BMI (≥25.0 and <30.0 kg/m^2), and obese and morbidly obese BMI (≥30.0 kg/m^2). We obtained information on prepregnancy weight with a questionnaire at study enrollment. Prepregnancy weight was highly correlated with measured early pregnancy weight (Pearson correlation coefficient [r]=0.95, P<0.001).

Maternal Hemodynamic and Anthropometric Measurements 6 Years After Pregnancy

Six years after pregnancy (90% range, 5.7–7.3 years), women returned for assessment. Information was obtained by questionnaires on the number of pregnancies after the index pregnancy and medication use. We measured maternal height and weight without shoes and calculated BMI. Blood pressure was measured twice in the seated position with the validated automatic sphygmomanometer Datascope Accutorr Plus (Paramus, NJ). The average of both blood pressure measurements was used for further analyses.

Carotid-femoral PWV was measured with an automatic noninvasive, validated device (Complior, Artech Medical, Pantin, France) to assess arterial wall stiffness. The device measures the distance between the recording sites at the carotid (proximal) and femoral (distal) arteries. We performed 2-dimensional M-mode echocardiographic measurements using the ATL-Philips Model HDI 5000 (Seattle, WA) or the Logiq E9 (GE Medical Systems, Wauwatosa, WI) devices. FS, AOD, and LAD were measured. LV mass was calculated from the equation derived by Devereux et al.[17] Intraobserver and interobserver intraclass correlation coefficients were described previously and demonstrated good repeatability and reproducibility.[18]

We took unilateral digital retinal photographs of maternal retinal vascular calibers of the left eye using a Topcon digital retinal camera (model TRC, NW300) with image resolution set to 4096 and 3072 pixels. A semiautomatic computer imaging program measured the 6 largest retinal arteriolar and venular calibers of these photographs, located one-half to 1 disc diameter from the optic disc margin. The average of the 6 largest retinal arteriolar and retinal venular calibers was used as central retinal arteriolar and central retinal venular equivalents.[19] Two graders who were blinded to participants' characteristics operated the semiautomatic computer imaging program. Grader-specific SD scores are used for both central retinal arteriolar and central retinal venular equivalents. Interclass correlation coefficients between graders are excellent for both retinal arteriolar calibers (0.77) and retinal venular calibers (0.87). This suggests adequate reproducibility.

Blood Pressure 9 Years After Pregnancy

Blood pressure was measured 9.7 years after pregnancy (90% range, 9.4–10.4 years) with the validated automatic sphygmomanometer Datascope Accutorr Plus. Women were supine while blood pressure was measured 4 times in the right upper arm. Unlike 6 years after pregnancy when we used the average of 2 blood pressure measurements while the subject was

sitting, the average of the last 3 blood pressure measurements with the subject supine was used for our analyses at 9 years.

Statistical Analyses

We examined baseline and follow-up characteristics within the total population (Table 1). To reduce potential bias caused by missing data, we imputed missing values in covariates used as confounders in the regression analyses through multiple imputation procedures. Data were imputed according to the Markov chain Monte Carlo method, assuming no monotone missing pattern. Data were analyzed in each set separately, and pooled estimates from the 5 imputed data sets were used to report the effect estimates and their 95% CIs.[1] For the multiple imputation procedure, we performed 10 iterations.[20] We used Statistical Package of Social Sciences version 24.0 for Windows (IBM Corp, Armonk, NY). In the total population for analysis, 4.1% had missing information on ethnicity, 7.9% on educational level, 0.8% on gravidity during pregnancy, 0.4% on DBP at study enrollment, 18.2% on prepregnancy BMI, 11.1% on smoking during pregnancy, 28.1% on gravidity 6 years after pregnancy, 2.4% on SBP 6 years after pregnancy, 4.2% on BMI 6 years after pregnancy, and 9.0% on parity 9 years after pregnancy. We performed linear and logistic regression analyses to relate midpregnancy and early pregnancy PlGF to cardiovascular adaptation to pregnancy and cardiovascular risk factors 6 and 9 years after pregnancy (Tables 2 and 3 and Tables I and II in the online-only Data Supplement, respectively). The quartile analysis (specifically the lowest quartile) was determined and analyzed to allow comparisons from previous studies using similar strategies and to address the subset of subjects with low PlGF (Table 3 and Table II in the online-only Data Supplement).[5,21,22] The association between PlGF and cardiovascular adaptation to pregnancy has been studied previously in the Generation R Study through the UtA resistance index.[2] In this study, we examine different measurements of cardiovascular adaptation to pregnancy, namely UtA pulsatility index and the presence of notching, to characterize pregnancy in the subset of women with low PlGF (Tables III and IV in the online-only Data Supplement). However, we realize these measurements do not describe a novel finding; therefore, we included them in the online-only Data Supplement to support our main findings. The regression models comparing PlGF values with later-life cardiovascular markers include covariates selected on the basis of their associations with the outcome of interest from previous studies or a change in effect estimate of >10%. The confounder models included the following variables, depending on the outcome of interest: maternal age at enrollment, gravidity during pregnancy, gestational age at blood sampling in pregnancy, ethnicity, educational level, smoking during pregnancy, change in SBP between early pregnancy and midpregnancy, SBP at the time of PlGF measurement, time interval between pregnancy and follow-up, pulse at the time of PWV measurement, and central retinal vascular caliber 6 years after pregnancy. The BMI models included change in BMI between prepregnancy and midpregnancy or prepregnancy BMI, in addition to the confounder model. We repeated all analyses after stratification into the presence or absence of complicated pregnancies during the index pregnancy (preeclampsia, SGA, or sPTB). We applied a Benjamini-Hochberg procedure controlling for false discovery rate at the 0.05 level.[23] We examined a possible trend over the quartiles presented in Table 3 through a linear contrast analysis. We also tested whether there was interaction between PlGF and gravidity or parity for any of our outcomes; this was not the case. Finally, a nonresponse analysis was carried out to test for differences in baseline characteristics between women included in this study and those with a midpregnancy PlGF measurement but no outcome of interest and those without a midpregnancy PlGF measurement or any outcome of interest (Table V in the online-only Data Supplement).

RESULTS

Subject Characteristics

Table 1 shows baseline characteristics during pregnancy and 6 and 9 years after pregnancy. The majority of women were European, highly educated, with lean or normal prepregnancy BMI, nonsmokers, and nulliparous during the index pregnancy. Six years after pregnancy, BMI was higher compared with before pregnancy, and 91% of women had been pregnant more than once. Nine years after pregnancy, BMI remained similar to BMI 6 years after pregnancy.

Results 6 Years After Pregnancy

Table 2 shows that the lower the midpregnancy PlGF concentrations were, the higher AOD, LAD, LV mass, and SBP were. In addition, when PlGF was divided into quartiles, women with the lowest midpregnancy PlGF concentrations had a higher AOD, LAD, LV mass, and SBP 6 years after pregnancy compared with women with the highest PlGF concentrations (Table 3). Women with medium-high PlGF 6 years after pregnancy also had a higher SBP and DBP than women with high PlGF. These differences persisted after the exclusion of women with complicated index pregnancies from the analyses. We observed no association between midpregnancy PlGF concentrations and FS and central retinal arteriolar and venular calibers 6 years after pregnancy for women in the total population or women with uncomplicated pregnancies (Tables 2 and 3).

Last, lower early pregnancy PlGF concentrations were associated with a higher SBP and AOD after pregnancy (Tables I and II in the online-only Data Supplement).

Blood Pressure 9 Years After Pregnancy

Lower midpregnancy PlGF levels were associated with a higher SBP 9 years after pregnancy (Tables 2 and 3). Quartile analysis showed that women with low PlGF or medium-high PlGF in midpregnancy had a higher SBP and DBP 9 years after pregnancy than women with high PlGF (Table 3). Early-pregnancy PlGF was not associated

Table 2. Association of Midpregnancy PlGF With Cardiovascular Risk Factors 6 and 9 Years After Pregnancy

	Confounder Model β (95% CI)	*P* Value	BMI Model β (95% CI)	*P* Value	Adjusted *P* Value
Total population					
At 6 y after pregnancy (n=3797)					
AOD, mm*	−0.24 (−0.39 to−0.10)	0.001	−0.24 (−0.39 to −0.10)	0.001	0.003
LAD, mm*	−0.75 (−0.95 to −0.56)	<0.001	−0.75 (−0.95 to −0.56)	<0.001	<0.001
LV mass, g*	−3.9 (−5.5 to −2.3)	<0.001	−3.9 (−5.5 to −2.3)	<0.001	<0.001
PWV, m/s†	0.05 (−0.01 to 0.11)	0.13	0.05 (−0.01 to 0.12)	0.12	0.19
FS*	−0.18 (−0.43 to 0.08)	0.18	−0.18 (−0.44 to 0.08)	0.17	0.21
Central retinal arteriolar caliber (SDS)‡	−0.03 (−0.08 to 0.03)	0.30	−0.03 (−0.08 to 0.03)	0.30	0.33
Central retinal venular caliber (SDS)‡	−0.01 (−0.06 to 0.05)	0.77	−0.01 (−0.06 to 0.05)	0.77	0.77
SBP, mm Hg*	−0.88 (−1.5 to −0.28)	0.004	−0.88 (−1.5 to −0.28)	0.004	0.009
DBP, mm Hg*	−0.33 (−0.80 to 0.14)	0.17	−0.33 (−0.81 to 0.14)	0.17	0.21
At 9 y after pregnancy (n=3198)					
SBP, mm Hg*	−1.1 (−1.7 to −0.45)	0.001	−1.1 (−1.7 to −0.46)	0.001	0.004
DBP, mm Hg*	−0.48 (−0.91 to −0.05)	0.03	−0.47 (−0.90 to −0.05)	0.03	0.06
Uncomplicated pregnancies					
At 6 y after pregnancy (n=3270)					
AOD, mm*	−0.28 (−0.44 to −0.12)	0.001	−0.28 (−0.44 to −0.12)	0.001	0.004
LAD, mm*	−0.81 (−1.0 to −0.60)	<0.001	−0.81 (−1.0 to −0.60)	<0.001	<0.001
LV mass, g*	−4.4 (−6.2 to −2.7)	<0.001	−4.4 (−6.2 to −2.7)	<0.001	<0.001
PWV, m/s†	0.06 (−0.01 to 0.13)	0.08	0.06 (−0.01 to 0.13)	0.08	0.15
FS*	−0.20 (−0.47 to 0.09)	0.17	−0.20 (−0.47 to 0.08)	0.17	0.23
Central retinal arteriolar caliber (SDS)‡	−0.03 (−0.08 to 0.03)	0.37	−0.03 (−0.08 to 0.03)	0.37	0.41
Central retinal venular caliber (SDS)‡	−0.01 (−0.07 to 0.05)	0.74	−0.01 (−0.07 to 0.05)	0.74	0.74
SBP, mm Hg*	−0.95 (−1.6 to −0.30)	0.004	−0.95 (−1.6 to −0.30)	0.004	0.009
DBP, mm Hg*	−0.29 (−0.80 to 0.22)	0.27	−0.29 (−0.80 to 0.22)	0.27	0.33
At 9 y after pregnancy (n=2748)					
SBP, mm Hg*	−1.1 (−1.8 to −0.36)	0.003	−1.1 (−1.8 to −0.36)	0.003	0.008
DBP, mm Hg*	−0.35 (−0.81 to 0.11)	0.14	−0.34 (−0.80 to 0.12)	0.15	0.23

Values are regression coefficients with β and 95% CI based on linear regression models. Estimates represent the unit increase in the outcome per 1 multiple of the median increase in PlGF. The adjusted *P* value adjusts for multiple comparisons via the Benjamini-Hochberg method.

AOD indicates aortic root diameter; BMI, body mass index; DBP, diastolic blood pressure; FS, fractional shortening; LAD, left atrial diameter; LV, left ventricular; PlGF, placental growth factor; PWV, pulse wave velocity; SBP, systolic blood pressure; and SDS, SD score.

*Adjusted for maternal age at enrollment, gravidity during pregnancy, time interval between pregnancy and follow-up, ethnicity, educational level, smoking during pregnancy, and change in SBP between early pregnancy and midpregnancy (and change in BMI between prepregnancy and midpregnancy in the BMI model).

†Model adjusted as above plus pulse at the time of PWV assessment.

‡Model adjusted as both above plus other retinal vessel.

with blood pressure 9 years after pregnancy (Tables I and II in the online-only Data Supplement).

Nine years after pregnancy, mean blood pressure values were lower compared with 6 years after pregnancy (Table 1), although we would expect blood pressure to increase over time. Results are most likely explained by differences in measurement technique: Blood pressure was measured twice in seated position 6 years after pregnancy, whereas it was measured 4 times in the supine position 9 years after pregnancy.[24] We nonetheless tested whether these results could be explained by the selection of a relatively healthy group 9 years after pregnancy. They could not. Restricting the analyses to women who participated at both moments or women who never used antihypertensive medication or never had a hypertension diagnosis did not change the ratio of our results; neither did stratifying for parity, gravidity, smoking, BMI, ethnicity, education, or having had preeclampsia or gestational hypertension more than once.

Results During Index Pregnancy

The association between PlGF and cardiovascular adaptation to pregnancy has been studied previously in the Generation R Study through the UtA resistance index.[2] Our present study showed that higher midpregnancy

Table 3. Association of Midpregnancy PlGF in Quartiles With Cardiovascular Risk Factors 6 and 9 Years After Pregnancy

Outcome	Low PlGF (MoM ≤0.72; 116.4 pg/mL [62.6–171.4 pg/mL]), β (95% CI)	Medium-Low PlGF (MoM, 0.73–1.0; 176.7 pg/mL [127.6–247.9 pg/mL]), β (95% CI)	Medium-High PlGF (MoM, 1.01–1.40; 243.9 pg/mL [176.5–342.5 pg/mL]), β (95% CI)	High PlGF (MoM ≥1.41; 422.2 pg/mL [260.2–714.8])	*P* for Trend	Adjusted *P* Value
Total population (n=3797)						
At 6 y after pregnancy						
AOD, mm*	0.40 (0.08 to 0.73)	0.22 (–0.10 to 0.54)	0.20 (–0.13 to 0.52)	Referent	<0.001	<0.001
LAD, mm*	0.34 (–0.09 to 0.78)	0.26 (–0.17 to 0.69)	0.40 (–0.03 to 0.83)	Referent	<0.001	<0.001
LV mass, g*	4.6 (1.1 to 8.1)	1.2 (–2.3 to 4.6)	0.74 (–2.8 to 4.2)	Referent	<0.001	<0.001
PWV, m/s†	0.01 (–0.13 to 0.15)	–0.09 (–0.23 to 0.05)	0.02 (–0.12 to 0.15)	Referent	0.33	0.61
FS*	–0.01 (–0.58 to 0.55)	–0.05 (–0.60 to 0.50)	0.10 (–0.46 to 0.66)	Referent	0.42	0.62
Central retinal arteriolar caliber (SDS)‡	–0.09 (–0.20 to 0.03)	–0.10 (–0.22 to 0.01)	–0.04 (–0.16 to 0.08)	Referent	0.62	0.68
Central retinal venular caliber (SDS)‡	0.04 (–0.08 to 0.16)	0.04 (–0.07 to 0.16)	0.02 (–0.10 to 0.14)	Referent	0.86	0.86
SBP, mm Hg*	2.3 (0.93 to 3.6)	1.0 (–0.31 to 2.3)	1.3 (0.04 to 2.7)	Referent	0.005	0.02
DBP, mm Hg*	0.89 (–0.15 to 1.9)	0.63 (–0.40 to 1.7)	1.2 (0.17 to 2.2)	Referent	0.51	0.62
At 9 y after pregnancy (n=3198)				Referent		
SBP, mm Hg*	2.6 (1.2 to 4.0)	1.2 (–0.21 to 2.6)	2.2 (0.82 to 3.6)	Referent	0.04	0.09
DBP, mm Hg*	1.8 (0.81 to 2.7)	0.88 (–0.05 to 1.8)	1.4 (0.48 to 2.3)	Referent	0.46	0.62
Uncomplicated pregnancies (n=3270)					Referent	
At 6 y after pregnancy				Referent		
AOD, mm*	0.45 (0.09 to 0.80)	0.33 (–0.02 to 0.67)	0.30 (–0.05 to 0.64)	Referent	<0.001	<0.001
LAD, mm*	0.28 (–0.20 to 0.75)	0.16 (–0.30 to 0.62)	0.30 (–0.16 to 0.76)	Referent	<0.001	<0.001
LV mass, g*	5.1 (1.3 to 8.9)	2.2 (–1.5 to 5.9)	1.2 (–2.5 to 4.8)	Referent	<0.001	<0.001
PWV, m/s†	0.04 (–0.11 to 0.19)	–0.09 (–0.24 to 0.06)	0.01 (–0.13 to 0.16)	Referent	0.27	0.50
FS*	–0.12 (–0.72 to 0.48)	–0.15 (–0.73 to 0.44)	0.21 (–0.38 to 0.79)	Referent	0.41	0.56
Central retinal arteriolar caliber (SDS)‡	–0.08 (–0.21 to 0.04)	–0.09 (–0.21 to 0.03)	–0.04 (–0.16 to 0.08)	Referent	0.57	0.68
Central retinal venular caliber (SDS)‡	0.07 (–0.06 to 0.20)	0.06 (–0.07 to 0.19)	0.02 (–0.11 to 0.15)	Referent	0.70	0.70
SBP, mm Hg*	1.8 (0.35 to 3.2)	0.67 (–0.71 to 2.1)	0.78 (–0.59 to 2.2)	Referent	0.003	0.008
DBP, mm Hg*	0.82 (–0.29 to 1.9)	0.39 (–0.70 to 1.5)	0.83 (–0.25 to 1.9)	Referent	0.40	0.56
At 9 y after pregnancy (n=2748)				Referent		
SBP, mm Hg*	2.3 (0.84 to 3.8)	0.78 (–0.70 to 2.3)	1.9 (0.40 to 3.3)	Referent	0.047	0.10
DBP, mm Hg*	1.6 (0.58 to 2.6)	0.70 (–0.29 to 1.7)	1.1 (0.17 to 2.1)	Referent	0.62	0.68

Values are regression coefficients with β and 95% CI based on linear regression models and are compared with women with high PlGF. Estimates represent the mean unit increase in the outcome compared with the reference category. The *P* for trend is the result of univariate ANOVA through linear contrast analysis. The adjusted *P* value adjusts for multiple comparisons via the Benjamini-Hochberg method.

AOD indicates aortic root diameter; BMI, body mass index; DBP, diastolic blood pressure; FS, fractional shortening; LAD, left atrial diameter; LV, left ventricular; MoM, multiple of the median; PlGF, placental growth factor; PWV, pulse wave velocity; SBP, systolic blood pressure; and SDS, SD score.

*Adjusted for maternal age at enrollment, gravidity during pregnancy, time interval between pregnancy and follow-up, ethnicity, educational level, smoking during pregnancy, and change in SBP between early pregnancy and midpregnancy (and change in BMI between prepregnancy and midpregnancy in the BMI model).

†Model adjusted as above plus pulse at the time of PWV assessment.

‡Model adjusted as model * above plus other retinal vessel.

PlGF concentrations were associated with a lower risk of notching in late pregnancy (Table III in the online-only Data Supplement). In addition, SBP in early, mid, and late pregnancy and DBP in late pregnancy were lower with increasing concentrations of PlGF. After the exclusion of women with complicated pregnancies from the analyses, results remained similar.

Higher early-pregnancy PlGF was associated with lower UtA pulsatility index in mid and late pregnancy, a lower risk of UtA notching in late pregnancy, a lower

SBP in early pregnancy, and a lower DBP throughout pregnancy (Table IV in the online-only Data Supplement).

Nonresponders

We compared baseline characteristics of women included and not included in the analyses (Table V in the online-only Data Supplement). Women with information available on midpregnancy PlGF and at least 1 outcome of interest (group 1) were compared with women with a midpregnancy PlGF measurement and no outcome of interest (group 2) and women without a midpregnancy PlGF measurement or any outcome of interest (group 3). Women not included in the analyses (groups 2 and 3) were on average 1 to 2 years younger, more often of non-European descent, less educated, more often multiparous, and more often affected by sPTB, SGA, and preeclampsia (Table V in the online-only Data Supplement).

DISCUSSION

This large prospective study demonstrates that PlGF concentrations in midpregnancy were inversely associated with subsequent AOD, LAD, LV mass, and SBP. Moreover, women with low midpregnancy PlGF had a greater AOD, LAD, and LV mass and higher SBP at follow-up than women with high PlGF. These findings were not driven by pregnancy complications.

The effect estimates in our study are modest and of minimal clinical impact at the time of measurement. However, these measures are in young women, and it is likely that in the long term, the magnitude of these findings may increase, making these women more susceptible to CVD. The positive association between AOD, LAD, LV mass, and SBP and the risk of CVD has been shown in multiple studies. In a population-based study of middle-aged individuals, a 1-unit increase in AOD (indexed by height) was associated with a 2.62-fold increased risk of fatal or nonfatal CVD.[25] Another study found that a 1-cm increase in LAD was associated with a 1.25-fold increased risk of ischemic stroke in women.[26] In the Framingham Heart Study, there was a 1.57-fold increased risk of CVD for every 50-g increase in LV mass in 40-year-old women.[27] Higher SBP was associated with increased risk of heart failure.[28] The risk of heart failure increased 1.75-fold for each 20–mm Hg increase in SBP in 30- to 59-year-old men and women.[28]

We posit that low midpregnancy PlGF concentrations or an unknown factor closely associated with low PlGF concentrations reflects a suboptimal cardiovascular status in pregnancy and a higher risk of cardiovascular impairment after pregnancy. We propose 2 pathophysiological mechanisms to explain these associations. First, stress to the syncytiotrophoblast can reduce the production of PlGF and lead to an increase in sFlt-1 levels, which in turn can bind to circulating PlGF levels and further decrease PlGF availability, thereby leading to endothelial cell dysfunction.[21,29] The association between low PlGF levels in pregnancy and endothelial dysfunction has been well established. Endothelial dysfunction can persist for many years after pregnancy and can lead to cardiovascular impairment.[7,8] Second, low PlGF levels in pregnancy could result in suboptimal cardiovascular adaptation and remodeling, as demonstrated in cardiac studies of PlGF-null pregnant mice.[3] PlGF-null mice manifest cardiac findings similar to those of hearts exposed to pressure overload (eg, increased LV mass).[3,30] This abnormal remodeling initiated during pregnancy might persist after pregnancy. The cardiac findings in our study also resemble those found in hearts with pressure overload, which can lead to CVD (eg, heart failure).[31]

PWV, a measurement of the velocity at which the arterial pulse propagates through the arteries, is a measure of arterial stiffness. Six years after pregnancy, midpregnancy PlGF concentrations were positively, although not significantly, associated with PWV, although we expected a negative association. Women with high PlGF smoked more often compared with women with low PlGF (30.3% versus 16.1%, respectively). Smoking has previously been reported to be associated with increased PlGF measured at an average of 17 weeks' gestation.[32] Smoking might explain the tendency to a larger PWV in women with high PlGF. Previous studies showed that at <40 years of age, a rise in PWV precedes a rise in blood pressure, which occurs later in life.[33] Therefore, we expect that higher PlGF will be associated with higher blood pressure in smokers at a later age.

We observed no association between midpregnancy PlGF concentrations and FS. FS measures the degree of shortening of the LV diameter between end diastole and end systole. Low FS is therefore a measure of impaired LV performance.[34] A substantial degree of long-axis systolic dysfunction is necessary to reduce FS, which is impaired below 25%.[35] The women included in our study were young and relatively healthy at the time FS was measured (age, 36.7 [SD, 5.0] years). We hypothesize that the onset of FS in women with low midpregnancy PlGF will start at a later age when systolic function is more impaired.

The microvasculature measurements (central retinal arteriolar and venular calibers) 6 years after pregnancy were not associated with midpregnancy PlGF in pregnancy. However, low maternal midpregnancy PlGF concentrations have been associated with narrower central retinal arteriolar calibers in their offspring, as was shown in a previous report from the Generation R Study.[36] These findings may be the result

of interference of normal vascular growth by low PlGF concentrations or endothelial dysfunction induced by hypoxia in the placental environment owing to low PlGF concentrations. Perhaps this is not pertinent to adult vessels.

Women with low or medium-high midpregnancy PlGF concentrations had a higher blood pressure 6 and 9 years after pregnancy compared with women with high midpregnancy PlGF. Conditions that are associated with low PlGF concentrations in pregnancy (eg, endothelial dysfunction) can also induce an isolated rise in SBP.[37] Women with low PlGF in pregnancy possibly have more endothelial dysfunction after pregnancy than women with high PlGF concentrations. This might explain why SBP especially and DBP to a lesser extent were associated with low PlGF. The positive association between low midpregnancy PlGF and SBP, but not DBP, is consistent with the positive association between low midpregnancy PlGF and LV mass. Previous studies showed that SBP was more strongly associated with LV mass than DBP was.[38] This could be the result of LV wall stress, which has been associated with a higher SBP and LV hypertrophy.

During normal pregnancy, the cardiovascular system adapts to expanded plasma volume with increased preload and decreased afterload.[39] Cardiac remodeling is initiated that consists of LV dilatation, geometric changes, increased myocardial contractility, and therefore increased stroke volume, heart rate, and cardiac output. In normal physiological pregnancy, all geometric and hemodynamic changes usually return to baseline after ≈1 year.[39] However, in women with previous early-onset preeclampsia, asymptomatic LV impairment and diastolic dysfunction can persist at least 4 years after pregnancy.[40,41] Increased wall stress caused by hypertension during preeclampsia might result in persistent cardiac remodeling in these women. In our study, the association of midpregnancy PlGF concentrations with cardiac remodeling as indicated by a larger LAD and LV mass was independent of preeclampsia, SGA, and sPTB.

Similar cardiac changes observed in women with previous preeclampsia are described in women with previous fetal growth restriction and in some women with previous apparently uncomplicated pregnancies.[41,42] In a small proportion of women with uncomplicated pregnancies, these changes can be identified 1 year postpartum.[41,43] It is tempting to speculate that these are women with low PlGF concentrations during pregnancy who do not manifest preeclampsia. This is in line with our hypothesis that midpregnancy PlGF concentration is a distinctive factor associated with cardiovascular function after pregnancy, independent of pregnancy complications.

Our findings on midpregnancy PlGF concentrations and pregnancy outcomes were consistent with previous studies examining PlGF and the uterine vascular bed with Doppler velocimetry.[44] These studies showed that vascular resistance of the uterine arteries was substantially increased in pregnancies affected by preeclampsia or SGA, which is proposed to be evidence for a failed vascular adaptation. We suggest that a high resistance in the uterine vascular bed may be a response to low PlGF because vascular maladaptation during pregnancy with low PlGF was also present in uncomplicated pregnancies. Alternatively, in midpregnancy and later pregnancy, the hemodynamic demands of the syncytiotrophoblast grow beyond the capacity of the uteroplacental circulation, leading to syncytiotrophoblast stress. This stress will result in a decreased production of PlGF and increased production of sFlt-1 by the syncytiotrophoblast. A dysregulation between PlGF and sFlt-1 can lead to endothelial dysfunction.

During pregnancy, the placenta is the main source of a dramatic increase in circulating PlGF of ≈50 times the nonpregnant concentration. Outside of pregnancy, PlGF is expressed mainly by endothelial cells.[45] Contrary to our findings of lower PlGF concentration in pregnancy being associated with later markers of future CVD, a doubling of circulating PlGF outside of pregnancy is associated with several pathological conditions, including tumor growth, plaque formation, and CVD.[46,47] These processes are regulated partly by the effect of PlGF to initiate angiogenesis, vasculogenesis, and inflammation. However, the exact mechanistic details are unclear.[48] PlGF also has a cardioprotective function. During cardiac stress such as a myocardial infarction, cardiomyocytes upregulate PlGF, which is proposed to initiate cardiac remodeling to maintain cardiac function.[49] The explanation for the contrasting association of PlGF during and after pregnancy with CVD is not clear. It may reflect the differing impact of the strikingly dissimilar concentrations of PlGF in the 2 settings or the mechanism responsible for the alteration of PlGF. Outside of pregnancy, high levels of PlGF most likely result from endothelial inflammation, as seen in individuals with cardiac stress.[50] During pregnancy, low circulating PlGF results from a stressed syncytiotrophoblast and placental pathophysiology.[1]

Our results indicate that midpregnancy PlGF concentrations are associated with cardiovascular risk factors 6 and 9 years after pregnancy. Midpregnancy PlGF concentrations might identify women at risk of cardiovascular impairment after pregnancy. Although our effect estimates are small, they indicate clear differences between women with lower and those with high PlGF in midpregnancy. We have to take into account that these women are still young and overall healthy. In the long term, the magnitude of these findings will most likely increase and make these women more susceptible to CVD.

Strengths and Limitations

This study has some limitations. First, placental hemodynamic measurements were carried out in only 2 of 3 research centers because of a lack of equipment. Second, retinal vascular imaging was not obtained from 33.6% of women 6 years after pregnancy because this measurement was introduced into the Generation R Study after recruitment for follow-up had already started. Both the first and second limitations were independent of subject characteristics, and selection bias therefore seems unlikely. Nevertheless, they might have resulted in loss of power and perhaps an underestimation of our results. Third, a relatively healthy population was selected, which might have affected the generalizability of our results. Last, because prepregnancy cardiovascular risk measurements are not available, the observational nature of this study does not allow the inference of causality. Future studies should examine prepregnancy cardiovascular risk measurements in relation to PlGF levels. In addition, future research should examine the consistency of PlGF levels from 1 pregnancy to another to identify whether the association between midpregnancy PlGF levels and cardiovascular measurements differs between a first pregnancy and subsequent pregnancies.

Our study has several strengths. First, the sample size was large, and prospective data were collected from early pregnancy on. Second, all outcomes were obtained following standardized protocols. Third, this is a multiethnic study, which we believe will give a good representation of the general population.

CONCLUSIONS

Lower PlGF concentrations in midpregnancy are associated with greater AOD, LAD, and LV mass and higher SBP 6 and 9 years after pregnancy, regardless of whether pregnancy was uncomplicated or complicated. PlGF concentrations in midpregnancy might provide insight into the pathways contributing to a worse cardiovascular risk profile after pregnancy.

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Disclosures

None.

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