## Associations of maternal angiogenic factors during pregnancy with alterations in cardiac development in childhood at 10 years of age


**Published in:**
American Heart Journal

**Publication status and date:**
Published: 01/05/2022

**DOI (link to publisher):**
10.1016/j.ahj.2022.01.016

**Document Version**
Publisher's PDF, also known as Version of record

# Associations of maternal angiogenic factors during pregnancy with alterations in cardiac development in childhood at 10 years of age

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**Aim** To examine whether maternal angiogenic factors in the first half of pregnancy are associated with offspring left and right cardiac development.

**Methods** In a population-based prospective cohort among 2,415 women and their offspring, maternal first and second trimester plasma PlGF and sFlt-1 concentrations were measured. Cardiac MRI was performed in their offspring at 10 years.

**Results** Maternal angiogenic factors were not associated with childhood cardiac outcomes in the total population. In children born small-for-their-gestational-age, higher maternal first trimester PlGF concentrations were associated with a lower childhood left ventricular mass (-0.24 SDS [95%CI -0.42, -0.05 per SDS increase in maternal PlGF]), whereas higher sFlt-1 concentrations were associated with higher childhood left ventricular mass (0.22 SDS [95%CI 0.09, 0.34 per SDS increase in maternal sFlt-1]). Higher second trimester maternal sFlt-1 concentrations were also associated with higher childhood left ventricular mass (*P*-value <.05). In preterm born children, higher maternal first and second trimester sFlt-1/PlGF ratio were associated with higher childhood left ventricular mass (0.30 SDS [95%CI 0.01, 0.60], 0.22 SDS [95%CI -0.03, 0.40]) per SDS increase in maternal sFlt-1/PlGF ratio in first and second trimester respectively). No effects on other childhood cardiac outcomes were present within these higher-risk children.

**Conclusions** In a low-risk population, maternal angiogenic factors are not associated with childhood cardiac ventricular structure, and function within the normal range. In children born small for their gestational age or preterm, an imbalance in maternal angiogenic factors in the first half of pregnancy was associated with higher childhood left ventricular mass only. (Am Heart J 2022;247:100–111.)

*Keywords:* Maternal PlGF; Maternal sFlt-1; Pregnancy; Childhood cardiac development

Fetal life is a critical period for later cardiovascular development. An accumulating body of evidence suggests that placental and fetal vascular development directly affect fetal cardiac development, and may lead to long-term alterations in cardiac structure and function.[1–3] Placental and fetal vascular development are complex processes in which a tightly regulated balance of pro-angiogenic and anti–angiogenic factors is needed to induce adequate angiogenesis.[4] Placental growth factor (PlGF) is a glycoprotein of the vascular endothelial growth factors (VEGF) family, which is secreted by cytotrophoblasts, and plays a key role in normal neoangiogenesis. Soluble fms-like tyrosine kinase (sFlt-1) is an anti–angiogenic factor which binds to VEGF and PlGF. An imbalance in PlGF and sFlt-1 from early-pregnancy onwards is associated with impaired remodeling of the spiral arteries and suboptimal vascular development of the feto-placental villous tree, which may subsequently lead to alterations in vascular resistance, blood flow, and endothelial function in the feto-placental circulation.[5–7] These hemodynamic alterations in the feto-placental circulation may directly affect fetal cardiac development. In addition, mechanistic studies suggest that PlGF may also directly influence embryonic and fetal cardiac development through influences on cardiac endothelial cells and fibroblasts.[8,9] Already, small studies among animal,

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*Submitted August 26, 2021; accepted January 29, 2022*

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*https://doi.org/10.1016/j.ahj.2022.01.016*

and human populations have shown that lower maternal PlGF levels during pregnancy are associated with cardiac anomalies in the offspring.[10–12] These studies mostly focused on the development of the fetal heart. Thus far, it remains unknown whether an imbalance in maternal angiogenic factors from early pregnancy onwards also has consequences for offspring cardiac development within in the normal range in later life, as important changes occur in the cardiovascular system during the transition from the prenatal period to the postnatal period. Further insight into the longer-term consequences of an imbalance in maternal angiogenic factors during early pregnancy for offspring cardiac development is important from an etiologic perspective and for the development of screening and prevention strategies focused on improving offspring cardiovascular health.[13]

We hypothesized that lower maternal PlGF and higher sFlt-1 concentrations in early-pregnancy may be associated with persistent cardiac structural and functional adaptations in the offspring in response to alterations in the feto-placental circulation and direct effects of PlFG on fetal cardiac development. We specifically expected the right ventricle to be affected in structure and function, as this is the dominant ventricle in the fetal circulation.[14] Cardiac MRI provides high resolution image quality and can produce 3D images, which allows for the most accurate and reproducible assessment of the left and right ventricle without geometric assumptions. We examined in a population based prospective cohort study among 2,415 mothers and their children, the associations of maternal first and second trimester PlGF and sFlt-1 concentrations with childhood left and right cardiac structures and function measured by cardiac Magnetic Resonance Imaging (MRI) at 10 years.

## Methods

### Study design and subjects

This study was embedded in the Generation R Study, a prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands. Approval for the study was obtained from the local Medical Ethical Committee. Written consent was obtained from all participants. In total, 8,879 pregnant women were enrolled between 2001, and 2005. We excluded mothers without plasma PlGF and sFlt-1 concentrations available in the first half of pregnancy ($n$ = 780) and pregnancies not leading to singleton live births ($n$ = 89). We invited a random subgroup of 2,878 children to participate in cardiac MRI measurements at 10 years, of which 2,397 children had good quality cardiac MRI measurements. Because we focused on cardiac changes within the normal range as our main outcome, we excluded children with cardiac abnormalities in their medical history ($n$ = 18). Our final population for analyses consisted of 2,415 mothers and their children (Figure 1).

### Maternal PlGF and sFlt-1 concentrations in the first and second trimester

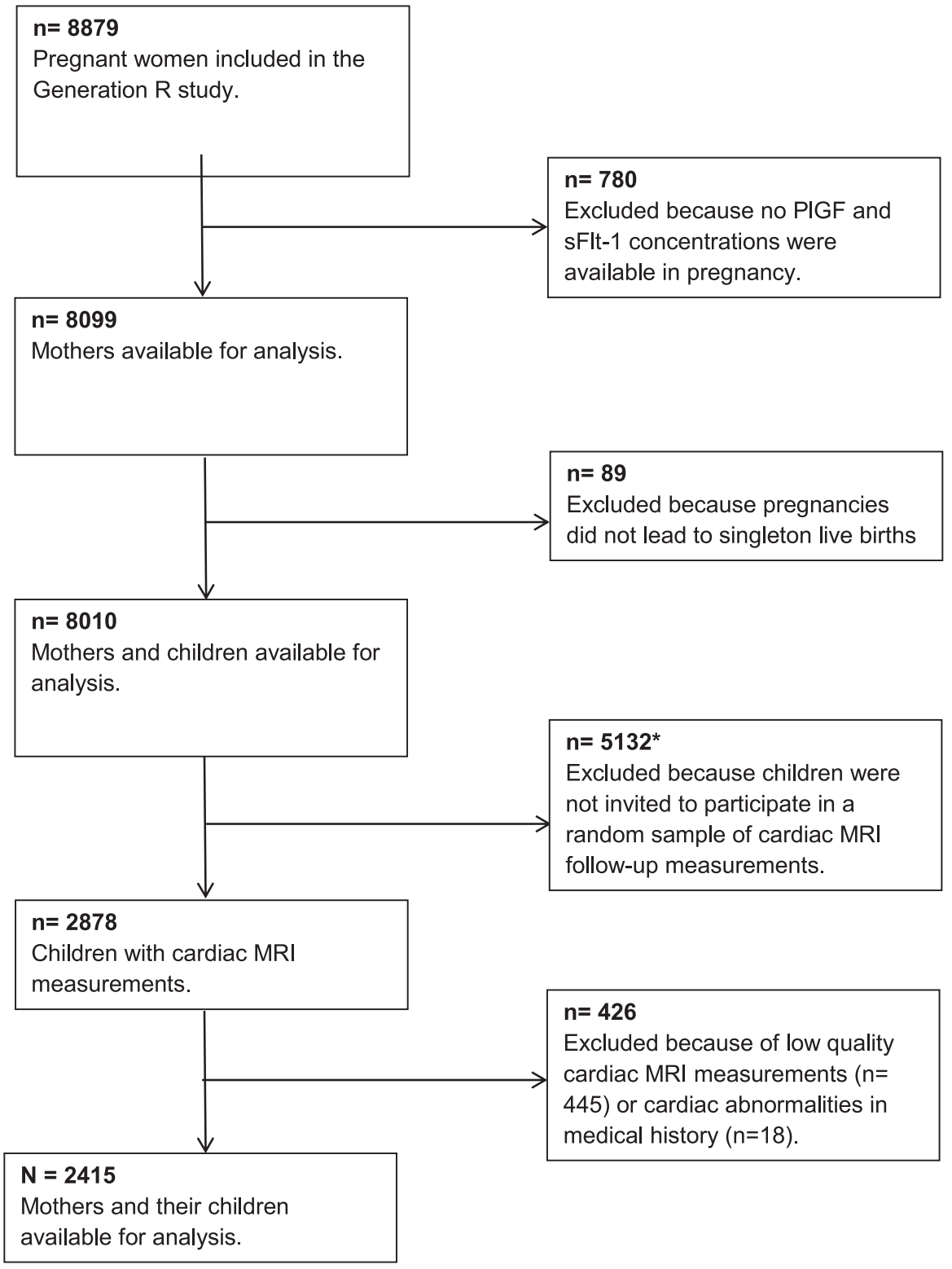
In the first trimester (median 13.2 weeks, 95% range 9.6-7.6 weeks) and second trimester (median 20.4 weeks, 95% range 18.5-23.5 weeks) maternal non–fasting venous blood plasma samples were drawn. Blood samples were centrifuged and thereafter stored at −80 °C. As described previously, maternal PlGF, and sFlt-1 concentrations were analyzed using a prototype of a microparticle-enhanced immunoassay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands).[15] To calculate the reproducibility of our measurements, samples ran twice on multiple assay plates, and we calculated between-run (inter-assay) coefficients of variation (CV). The CV was calculated by dividing the standard deviation of the measurements by the mean of the set. The between-run coefficients of variation were 4.7% at 24 pg/mL, and 3.8% at 113 pg/mL for plasma PlGF and 2.8% at 5.5 ng/mL and 2.3% at 34.0 ng/mL for plasma sFlt-1. The highest detected level was 1,500 pg/mL for PlGF and 150 ng/mL for sFlt-1. The sFlt-1/PlGF ratio was calculated by dividing the sFlt-1 concentration by the PlGF concentration.

Because these measurements were not normally distributed, we log-transformed these measurements for further analyses. We categorized maternal PlGF and sFlt-1 concentrations into quintiles to assess whether associations were restricted to women with relatively high or low PlGF and sFlt-1 concentrations and to explore linearity of the associations. We also constructed standard deviation scores (SDS) of each of the exposures to examine the associations across the full range of maternal PlGF and sFlt-1 concentrations.

### Childhood structural and functional cardiac measurements

At the age of 10 years, we performed cardiac Magnetic Resonance Imaging (MRI) using a wide-bore GE Discovery MR 750 3T scanner (General Electric, Milwaukee, MI), as described in detail previously.[2] We acquired localizer images, followed by ECG gated breath-held scans lasting less than 10 seconds per breath-hold. A short-axis SSFP cine stack was then obtained with basal slice alignment and covering the ventricles and part of the atria with contiguous 8-mm thick slices over several end expiration breath-holds. Image analyses were outsourced to a commercial party (Precision Image Analysis, Kirkland, WA), using Medis QMASS software (Medis, Leiden, the Netherlands), and were performed according to the guidelines of the Society for Cardiovascular Magnetic Resonance (SCMR).[16] We measured childhood left ventricular mass, left and right ventricular end-diastolic

**Figure 1**




Flow chart of the study participants. * For the cardiac MRI measurements, a random subgroup of our participants was invited.

volume and left and right ventricular ejection fraction. For all childhood cardiac outcome measures, we constructed body surface area (BSA) adjusted standard deviation scores (SDS).[2] At the age of 10 years, we calculated child BMI and BSA using height and weight measured without shoes and heavy clothing at our research center. BSA was computed using the Haycock formula (BSA ($m^2$) = 0.024265 × weight (kg)0.5378 × height (cm) 0.3964.[8] Due to the design of our study and the maximum time available per research visit, anthropometric measurements and the cardiac MRI were conducted at 2 separate visits. The majority of the children visited the research center for the cardiac MRI exam within 2 months after anthropometrics measurements were performed. As described in detail previously, we constructed BSA-adjusted standard deviation scores (SDS) for the cardiac outcomes using Generalized Additive Models for Location, Size and Shape using R, version 3.2.0 (R Core Team, Vienna, Austria).[18–20] These models enable flexible modeling, taking into account the distribution of the response variable.[21]

### Covariates

Information on maternal educational level, ethnicity, parity, folic acid supplement use, and weight just before pregnancy was obtained at enrolment through questionnaires.[22] Maternal height was measured at intake without shoes and body mass index (BMI) was calculated. Maternal smoking status and alcohol consumption during pregnancy was assessed through questionnaires in each trimester. Information on child's sex, gestational age, and weight at birth was obtained from medical records.[23] At the age of 10 years, we calculated child BMI using height and weight measured without shoes and heavy clothing. Systolic and diastolic blood pressures (mm Hg) were measured at the right brachial artery, 4 times with 1-minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus TM (Paramus, NJ). Mean systolic and diastolic blood pressure values were calculated, using the last 3 blood pressure measurements.

### Statistical analyses

First, we performed 2 non–response analyses to compare population characteristics of mothers with and without angiogenic factors available and mothers and children participating in the cardiac MRI follow-up to those without. Second, we assessed the associations of maternal first and second trimester PlGF and sFlt-1 concentrations and the sFlt-1/PlGF ratio in quintiles and per SDS change with childhood cardiac outcomes using linear regression models. We constructed multiple models based on previous literature and a Directed Acyclic Graph (DAG) analysis to identify which factors may act as confounders or potential mediators (*Supplementary Figure S1*): (1) a basic model, adjusted for gestational age at blood sampling, child's age, and sex. This model was also adjusted for the time difference between the measurement of the body surface area (BSA) and the MRI, since these measurements were obtained in 2 separate visits; (2) a confounder model, which was considered as the main model. The confounder model was additionally adjusted for maternal educational level, ethnicity, parity, prepregnancy BMI, blood pressure, smoking, alcohol consumption, and folic acid supplement use.[24–26] Variables were selected based on their association with exposures and outcomes or change in effect estimates of >10% in our study sample. If significant associations were found, we further adjusted our analyses for gestational-age-adjusted birth weight, *breastfeeding,* childhood BMI, and systolic blood pressure to examine whether these offspring characteristics act as potential mediators. To examine whether the associations of maternal plasma PlGF and sFlt-1 concentrations with childhood cardiac outcomes were different for fetal sex, gestational age at birth, gestational-age-adjusted birth weight and mothers with gestational hypertensive disorders, we tested for statistical interaction terms.[27,28] As significant interactions of maternal angiogenic factors with gestational age at birth and gestational-age-adjusted birthweight were found for childhood left ventricular mass, we performed further stratified analyses. Gestational-age-adjusted birth weight was separated in 3 subgroups: the lowest decile (small for gestational age), 10th to 90th percentile (appropriate for gestational age), and the highest decile (large for gestational age) of our cohort. Gestational age was divided in preterm birth (<37 weeks of gestation) and term birth (≥37 weeks of gestation). To test the robustness of our findings, we performed a sensitivity analysis, in which we repeated the analyses of our significant associations among women who did not develop any gestational hypertensive disorders. We used multiple imputations for missing values for covariates.[29] The analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (SPSS Inc, Chicago, IL).

## Results

### Subject characteristics and non–response analyses

Table I shows the population characteristics. Median maternal PlGF concentrations were 39.75 pg/mL (95% range 14.35, 188.84) in first trimester, and 194.15 pg/mL (95% range 72.85, 567.98 pg/mL) in second trimester. Median maternal sFlt-1 concentrations were 5.09 ng/mL (95% range 1.91, 14.18 ng/mL) in first trimester and 4.98 ng/mL (95% range 1.54, 16.83 ng/mL) in second trimester. Non–response analysis showed that women with plasma angiogenic factors available were more often European and were higher educated, compared to women without angiogenic factors available (*Supplementary Table S2*). No differences in angio-

**Table I.** Characteristics of mothers and their children in the generation R study ($n = 2,415$)

| | |
|---|---|
| Maternal Characteristics | |
| Age at enrolment, mean (SD), y | 31.0 (4.7) |
| Gestational age at intake, median (95%), wk | 13.8 (10.2-22.2) |
| Prepregnancy BMI, median (95%), kg/m$^2$ | 22.5 (18.0-34.7) |
| Parity, No. nulliparous (%) | 1,429 (59.2) |
| Ethnicity, no. Dutch or European (%) | 1,374 (57.6) |
| Education level, *n* high (%) | 1,211 (52.5) |
| Smoking during pregnancy, *n* yes (%) | 316 (14.6) |
| Folic acid supplement use, *n* yes (%) | 927 (49.4) |
| Gestational hypertensive disorders, *n* yes (%) | 85 (3.6) |
| First trimester PlGF, median (95%), pg/mL | 39.75 (14.35-188.84) |
| First quintile, pg/mL | 7.70-25.50 |
| Second quintile, pg/mL | 25.50-34.30 |
| Third quintile, pg/mL | 34.30-46.30 |
| Fourth quintile, pg/mL | 46.30-72.80 |
| Fifth quintile, pg/mL | 72.80-638.20 |
| Second trimester PlGF, median (95%), pg/mL | 194.15 (72.85-567.98) |
| First quintile, pg/mL | 11.00-128.28 |
| Second quintile, pg/mL | 128.28-169.60 |
| Third quintile, pg/mL | 169.60-221.98 |
| Fourth quintile, pg/mL | 221.98-301.28 |
| Fifth quintile, pg/mL | 301.28-1408.70 |
| First trimester sFlt-1, median (95%), ng/mL | 5.09 (1.91-14.18) |
| First quintile, ng/mL | 0.13-3.49 |
| Second quintile, ng/mL | 3.49-4.53 |
| Third quintile, ng/mL | 4.53-5.71 |
| Fourth quintile, ng/mL | 5.71-7.76 |
| Fifth quintile, ng/mL | 7.76-25.27 |
| Second trimester sFlt-1, median (95%), ng/mL | 4.98 (1.54-16.83) |
| First quintile, ng/mL | 0.04-3.03 |
| Second quintile, ng/mL | 3.03-4.36 |
| Third quintile, ng/mL | 4.36-5.90 |
| Fourth quintile, ng/mL | 5.90-8.23 |
| Fifth quintile, ng/mL | 8.22-62.66 |
| Child Characteristics | |
| Age, mean (SD), y | 10.2 (0.6) |
| Gender, *n* female (%) | 1,258 (51.9) |
| Birth weight, mean (SD), grams | 3,451.7 (550.1) |
| Gestational age at birth, median (95%), wk | 40.3 (36.0-42.3) |
| BMI, median (95%), kg/m$^2$ | 17.0 (14.0-24.3) |
| Body surface area, median (95%), m$^2$ | 1.15 (1.0-1.5) |
| Systolic blood pressure, mean (SD), mm Hg | 103.2 (7.9) |
| Diastolic blood pressure, mean (SD), mm Hg | 58.6 (6.4) |
| Cardiac MRI measures at 10 y of age | |
| Left ventricular mass, median (95%), gram | 47.7 (32.9-73.3) |
| Left ventricular end-diastolic volume, median (95%), mL | 98.8 (69.8-139.1) |
| Left ventricular ejection fraction, median (95%), % | 58.4 (50.2-67.6) |
| Right ventricular end-diastolic volume, median (95%), mL | 98.5 (65.8-144.3) |
| Right ventricular ejection fraction, median (95%),% | 58.2 (49.4-68.1) |

Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %).

genic factors of mothers with children who participated in MRI follow-up measurements as compared to those who did not participate in follow-up measurements were present. (*Supplementary Table S3*).

## Associations of maternal angiogenic factors with childhood cardiac outcomes

In the basic model, a higher maternal first trimester PlGF concentration was associated with a lower childhood left ventricular mass ($P$-value $<.05$), but this association was fully explained by other maternal sociodemographic, and lifestyle characteristics in the confounder model (Table II). No other associations were present of maternal first and second trimester PlGF and sFlt-1 concentrations across the full range with childhood structural and functional cardiac measures. Similarly, no associations of maternal sFlt-1/PlGF ratio with childhood cardiac outcomes were present. *Supplemental Figures S2-S4* show that also no consistent associations of maternal first and second trimester PlGF or sFlt-1 concentrations

**Table II.** Associations of maternal first and second PlGF and sFlt-1 concentrations with childhood cardiac outcomes at 10 years

| Maternal angiogenesis factors | Difference in left ventricular mass (SDS) (95%CI) | Difference in left ventricular end-diastolic volume (SDS) (95%CI) | Difference in left ventricular ejection fraction (SDS) (95%CI) | Difference in right ventricular end-diastolic volume (SDS) (95%CI) | Difference in right ventricular ejection fraction (SDS) (95%CI) |
|---|---|---|---|---|---|
| First trimester | | | | | |
| Maternal PlGF concentrations | | | | | |
| Basic model* | -0.08 (-0.13, -0.02)* | -0.04 (-0.10, 0.02) | 0.04 (-0.03, 0.10) | -0.05 (-0.10, 0.00) | 0.04 (-0.03, 0.10) |
| Confounder model† | -0.06 (-0.11, 0.00) | 0.01 (-0.05, 0.07) | 0.05 (-0.02, 0.11) | -0.00 (-0.06, 0.05) | 0.05 (-0.02, 0.12) |
| Maternal sFlt-1 concentrations | | | | | |
| Basic model* | -0.01 (-0.05, 0.03) | 0.00 (-0.04, 0.04) | -0.03 (-0.7, 0.02) | -0.01 (-0.05, 0.03) | -0.03 (-0.07, 0.01) |
| Confounder model† | -0.01 (-0.05, 0.03) | 0.00 (-0.04, 0.04) | -0.02 (-0.07, 0.02) | -0.02 (-0.06, 0.02) | -0.02 (-0.06, 0.03) |
| Maternal sFlt-1/PlGF ratio | | | | | |
| Basic model* | -0.04 (-0.08, 0.01) | -0.02 (-0.07, 0.03) | 0.05 (0.00, 0.11) | -0.01 (-0.06, 0.03) | 0.06 (0.00, 0.11) |
| Confounder model† | -0.02 (-0.07, 0.03) | 0.01 (-0.04, 0.06) | 0.05 (-0.01, 0.11) | 0.02 (-0.03, 0.07) | 0.05 (-0.01, 0.11) |
| Second trimester | | | | | |
| Maternal PlGF concentrations | | | | | |
| Basic model* | -0.03 (-0.07, 0.01) | -0.02 (-0.05, 0.02) | 0.01 (-0.03, 0.06) | -0.02 (-0.06, 0.02) | 0.01 (-0.04, 0.05) |
| Confounder model† | -0.02 (-0.06, 0.02) | 0.01 (-0.03, 0.05) | 0.01 (-0.03, 0.06) | 0.01 (-0.03, 0.05) | 0.01 (-0.03, 0.06) |
| Maternal sFlt-1 concentrations | | | | | |
| Basic model* | 0.01 (-0.03, 0.04) | 0.00 (-0.03, 0.04) | -0.02 (-0.06, 0.02) | 0.00 (-0.04, 0.03) | -0.03 (-0.07, 0.01) |
| Confounder model† | 0.01 (-0.03, 0.04) | 0.01 (-0.03, 0.04) | -0.02 (-0.06, 0.03) | 0.00 (-0.04, 0.04) | -0.02 (-0.07, 0.02) |
| Maternal sFlt-1/PlGF ratio | | | | | |
| Basic model* | -0.03 (-0.06, 0.01) | -0.01 (-0.05, 0.02) | 0.02 (-0.02-0.07) | -0.01 (-0.05, 0.03) | 0.03 (-0.01, 0.07) |
| Confounder model† | -0.02 (-0.06, 0.02) | 0.00 (-0.04, 0.04) | 0.02 (-0.02, 0.06) | 0.00 (-0.04, 0.04) | 0.03 (-0.02, 0.07) |

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood cardiac outcomes in SDS per 1 SDS increase in maternal PlGF and sFlt-1 concentrations. SDS, standard deviation score.

* Basic model, adjusted for gestational age at intake, gestational age at blood sampling, child's age and sex and time difference between BSA and MRI measurement.

† Confounder model, basic model additionally adjusted for maternal educational level, ethnicity, parity, prepregnancy BMI, blood pressure, smoking, alcohol consumption, and folic acid supplement use.

quintiles and PlGF/sFlt-1 ratio quintiles with childhood cardiac outcomes were present.

## Subgroup analysis

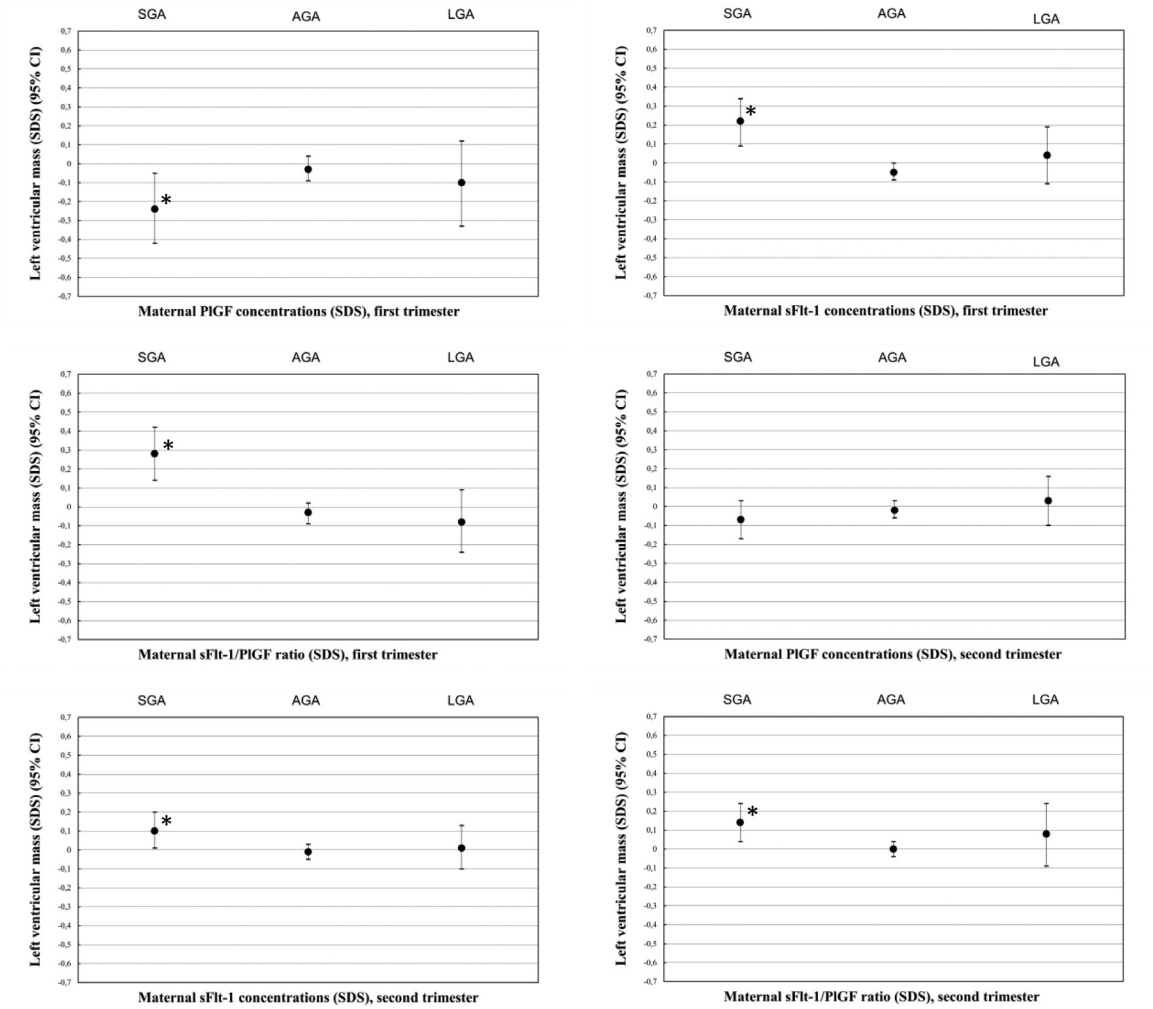
Significant interactions of maternal angiogenic factors with gestational age adjusted birth weight and gestational age at birth were present for childhood left ventricular mass only (*P*-values for the interactions are shown in *Supplementary Table S1).*

In children born small for their gestational age, a higher maternal first trimester PlGF concentration was associated with a lower childhood left ventricular mass (difference: -0.24 SDS (95%CI -0.42, -0.05) per SDS increase in maternal first trimester PlGF concentration), whereas a higher maternal first trimester sFlt-1 concentration was associated with a higher childhood left ventricular mass (difference: 0.22 SDS [95%CI 0.09, 0.34]) per SDS increase in maternal first trimester sFlt-1 concentration) (Figure 2). A higher maternal first trimester sFlt-1/PlGF ratio was also associated with a higher childhood left ventricular mass (*P*-value <.05). In the second trimester, a higher maternal sFlt-1 concentration and higher maternal sFlt-1/PlGF ratio, but not PlGF, were associated with higher childhood left ventricular mass (differences: 0.10 SDS (95% CI 0.01, 0.20) and 0.14 SDS (95% CI 0.04, 0.24) per SDS increase in maternal second trimester sFlt-1 concentration, and sFlt-1/PlGF ratio respectively). Additional adjustment for breastfeeding, childhood BMI, and systolic blood pressure did not explain these associations *(Supplemental Table S4).* No associations were present in children born appropriate for their gestational age or large for their gestational age.

Figure 3 shows that in preterm born children, a higher maternal first trimester and second trimester sFlt-1/PlGF ratio, but not PlGF and sFlt-1 concentrations separately, were associated with a higher childhood left ventricular mass (differences: 0.30 SDS (95% CI 0.01, 0.60) and 0.22 SDS (95% CI 0.03, -0.40) per SDS increase in maternal sFlt-1/PlGF ratio in first and second trimester respectively). Additional adjustment for gestational-age-adjusted birth weight, breastfeeding, childhood BMI, and systolic blood pressure did not explain these associations *(Supplemental Table S5).* No associations among term born infants were present.

We repeated these analyses among children born SGA and preterm and excluded women who did not develop any gestational hypertensive disorders. The effect estimates were similar, but became borderline signifi-

**Figure 2**




Associations of maternal first and second trimester PlGF and sFlt-1 concentrations with childhood left ventricular mass among children born SGA, AGA, and LGA. Values represent regression coefficients (95% confidence interval) from linear regression, that reflect differences in childhood left ventricular mass in SDS per 1 SDS increase in maternal PlGF and sFlt-1 concentrations or PlGF/sFlt-1 ratio analyzed for children born SGA, AGA, and LGA separately. Models were adjusted for gestational age at intake, gestational age at blood sampling, educational level, ethnicity, parity, prepregnancy BMI, blood pressure, smoking, alcohol consumption, folic acid supplement use and child's age and sex and time difference between BSA, and MRI measurement. SGA: small for gestational age, AGA, appropriate for gestational age; LGA, large for gestational age. $*P < .05$.

cant due to smaller sample sizes (*Supplementary Table S6-7).*"

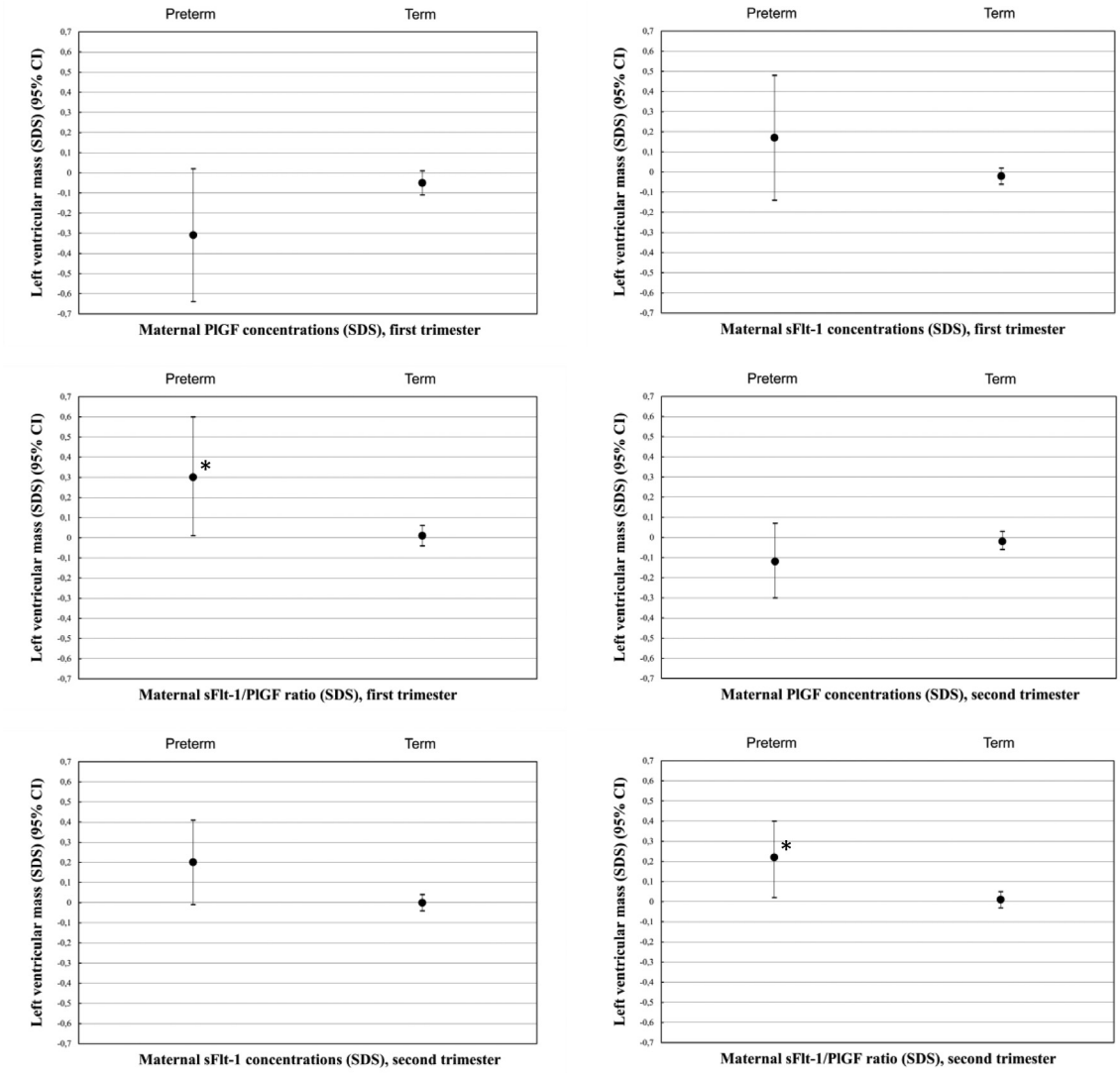
## Discussion

In a low-risk population, no associations of maternal first or second trimester angiogenic factors across the full range with childhood cardiac structural and functional outcomes were present in the total population. Among children born small for their gestational age and children born preterm, an imbalance in maternal angiogenic factors, reflected by lower maternal first and second trimester PlGF concentrations and higher sFlt-1 concentrations, was associated with a higher childhood left ventricular mass only. These associations were not explained by maternal socio-demographic and lifestyle factors, maternal blood pressure or gestational hypertensive disorders, and concurrent childhood BMI or blood pressure.

### Interpretation main findings

Accumulating evidence suggest that feto-placental vascular development is important for fetal cardiac devel-

**Figure 3**




Associations of maternal first and second trimester PlGF and sFlt-1 concentrations with childhood left ventricular mass among children born term and preterm. Values represent regression coefficients (95% confidence interval) from linear regression, that reflect differences in childhood left ventricular mass in SDS per 1 SDS increase in maternal PlGF and sFlt-1 concentrations or sFlt-1/PlGF ratio analyzed for children born preterm (born <37 weeks of pregnancy) and term (born >37 weeks of pregnancy) separately. Models were adjusted for gestational age at intake, gestational age at blood sampling, educational level, ethnicity, parity, prepregnancy BMI, blood pressure, smoking, alcohol consumption, folic acid supplement use and child's age and sex and time difference between BSA and MRI measurement. *$P < .05$.

opment and cardiac structures and function in later life.[1,2,30] However, the underlying mechanisms of these associations remain unclear and it remains challenging to identify children at higher risk of cardiovascular diseases in later life. Previously, we have already shown that a suboptimal fetal and placental vascular function may have a persistent influence on cardiac outcomes in childhood.[2] An imbalance in maternal angiogenic factors during pregnancy may be marker of a poorer placental vascular function among higher risks groups. Measuring maternal PlGF and sFlt-1 concentrations during pregnancy among higher risk groups could aid in the early detection of women and their offspring that may benefit from early intervention possibilities. An adequate balance of maternal pro-angiogenic and anti–angiogenic factors from early-pregnancy onwards is needed for a normal placental, fetal, and postnatal vascular development.[4,31] On the maternal side, maternal PlGF in early-

pregnancy regulates the remodeling of the maternal spiral arteries to establish a desirable low resistance vascular network that enables optimal blood flow to the placenta for oxygen and nutrient supply to the developing fetus.[4] In the fetal compartment, both angiogenesis, and vasculogenesis are needed for the development of the feto-placental villous tree. An impaired feto-placental development and vascularization can lead to hemodynamic alterations, relative hypoxemia and oxidative stress, which can negatively affect fetal cardiac development through increases in ventricular wall stress and remodeling of the length and density of the capillaries per cardiomyocyte.[32,33] Next to the effects on angiogenesis and vasculogenesis, PlGF also seems to directly affect fetal cardiac development. Multiple animal studies have shown that PlGF influences cardiac endothelial cells and fibroblasts and that its levels must be tightly regulated for normal fetal cardiac formation.[8,9,32,34,35] Although maternal PlGF and sFlt-1 cannot pass the placental barrier, maternal angiogenic levels in first half of pregnancy are also a proxy measurement of fetal angiogenic levels.[10] Among human populations, several studies have examined the associations of maternal angiogenic factors with fetal congenital heart diseases. Two case-control studies showed that maternal first, second, and third trimester PlGF concentrations were significantly lower in mothers of children with congenital heart diseases in comparison with the healthy controls.[10,11]

No studies among human populations have yet examined the associations of maternal angiogenic factors across the full range during the first half of pregnancy with childhood cardiac structure and function within in the normal range. We hypothesized that an imbalance in maternal angiogenic factors, reflected by lower maternal first and second trimester PlGF and higher maternal first and second trimester sFlt-1 concentrations, may affect structural and functional childhood cardiac outcomes across the normal range via direct effects on cardiac development, hemodynamic alterations, and suboptimal oxygen supply. Already, multiple studies have shown that lower maternal first and second trimester PlGF and higher sFlt-1 concentrations across the full range are associated with increased risks of adverse birth outcomes in low and high-risk populations.[36,37] Also, a higher sFlt-1/PlGF ratio is associated with fetal growth restriction, preterm delivery and stillbirth.[37-41] In this current study, we showed that in children born small for their gestational age or preterm, an imbalance in maternal first and second trimester PlGF and sFlt-1 concentrations was associated with a higher childhood left ventricular mass. No associations were present with other childhood cardiac outcomes. Also, no association were found between maternal first and second trimester angiogenic factors and childhood cardiac outcomes in term born children or children born appropriate and large for their gestational age. Thus, our findings suggest that in children born preterm or small for their gestational age, an imbalance in maternal angiogenic factors is associated with higher childhood left ventricular mass, but not with other childhood cardiac structural or functional measures.

Our findings suggest that associations of an imbalance in maternal angiogenic factors with fetal cardiac development may be stronger among children exposed to impaired intra-uterine environment. A study in 134 term-born infants has shown that children born from mothers with hypertensive disorders during pregnancy have a smaller right ventricular end-diastolic volume index compared to controls at 3 months of age.[42] Other studies among human populations have shown that children born preterm have a higher left and right ventricular mass later in life, as compared to term born children. Also, among children born SGA it has been shown that they have shorter, and more global ventricles with a slightly larger diameter later in life.[43,44] These effects were even stronger among offspring who also have a higher blood pressure later in life.[45,46] Animal studies suggest a combined effect of reduced placental vascularization and impaired fetal growth on the development of a higher left ventricular mass in later life. These studies have also suggested that a higher left ventricular mass may be induced by a subsequent higher blood pressure and not by increased placental vascular resistance per se.[30] However, in our analyses, additional adjustment for childhood systolic blood pressure did not explain our observed associations. It is well established that intrauterine fetal growth restriction may be characterized by an increased resistance in the umbilical artery, which in severe cases can lead to absent or reversed umbilical artery flow patterns. This leads to an increased workload for the developing fetal cardiovascular system.[47] In our study population, none of our participants in the SGA subgroup had a reversed umbilical artery flow pattern. Furthermore, in a previous study from our cohort, increased umbilical artery pulsatility index was not associated with childhood cardiovascular outcomes among children born SGA.[3] Thus, it seems unlikely that adverse umbilical flow patterns can fully explain the association of maternal angiogenic factors with childhood cardiac outcomes.

Our associations may be explained by effects of the imbalance of maternal angiogenic factors on offspring left ventricular mass via hemodynamic alterations and suboptimal oxygen supply. However, an imbalance of maternal angiogenic factors may also be a marker of a more severe phenotype of intra-uterine fetal growth restriction, leading to preterm birth or SGA at birth. Exclusion of women who developed any gestational hypertensive disorder did not change our findings, which suggests that not only the severity of the adverse intra-uterine environment as reflected by the development of a gestational hypertensive disorder explains our

observed association.[48,49] Further mechanistic and population studies are needed to explore the underlying mechanisms and the combined effects of fetal growth restriction and angiogenic factors on postnatal cardiac development.

The effect estimates for the associations of lower maternal PlGF and higher sFlt-1 concentrations with higher childhood left ventricular mass in children born preterm or small for their gestational age were small and are mainly of interest from an etiologic perspective. For comparison, our observed associations within these higher risk groups are comparable in magnitude to previous reported associations of a low and high birth weight with childhood left ventricular mass.[50] Our results should be considered hypothesis generating as they provide novel insight into the potential role of early-pregnancy angiogenesis on later cardiac development. Previous studies have shown that left ventricular mass tracks from childhood into adulthood.[13] A study in 3,220 adults has shown that a higher left ventricular mass was associated with a higher incidence of cardiovascular disease, mortality from cardiovascular disease, and mortality from all causes, even after correction for age, blood pressure, treatment for hypertension, cigarette smoking, diabetes, obesity, cholesterol concentrations, and electrocardiographic evidence of left ventricular hypertrophy.[51] These findings suggest that even small subclinical differences in childhood left ventricular mass may be related to the development of cardiovascular diseases in later life.

We hypothesized that especially the right ventricle might be affected by suboptimal angiogenesis, as this is the dominant ventricle in fetal circulation.[52] We only observed associations with childhood left ventricular mass and not with right ventricular outcomes. In line with left ventricular outcomes, the associations of maternal angiogenic factors might be stronger with offspring right ventricular mass than with right ventricular end-diastolic volume or ejection fraction. At 10 years, right ventricular mass cannot be measured accurately with MRI because the right ventricular wall is too thin, and is prone to measurement error.[53] Further studies are needed to replicate our findings and to examine whether an imbalance in maternal early-pregnancy angiogenic factors influences offspring left and right ventricular mass development and the risk of cardiovascular diseases later in life.

### Strengths and limitations

The main strengths of this study were the prospective design with data collection already from early-pregnancy onwards and the use of cardiac MRI scans to measure childhood cardiac development in detail. Although only a subgroup of children was invited to participate in cardiac MRI scans, we do not expect that this affected our effect estimates, as maternal angiogenic concentrations did not differ between those participating, and not-participating in cardiac MRI follow-up. The generalizability of our results may be affected by a selection toward a relatively healthy, high-educated study population. We examined the associations of maternal PlGF and sFlt-1 concentrations with multiple cardiac outcomes. As these cardiac outcomes are strongly correlated, we did not correct our analyses for multiple testing. However, chance findings cannot be excluded and our findings need to be replicated in further studies among low and high-risk populations. Finally, the analyses were adjusted for a significant number of confounders. However, as in any observational study, residual confounding may still be a concern.

### Perspectives

In a low-risk population, no associations of maternal first or second trimester angiogenic factors across the full range with childhood cardiac structural, and functional outcomes were present in the total population. Among children born small for their gestational age and children born preterm, an imbalance in maternal angiogenic factors, reflected by lower maternal first and second trimester PlGF concentrations and higher sFlt-1 concentrations, was associated with a higher childhood left ventricular mass only. Our findings should be considered hypothesis generating and are important from an etiologic perspective, as they provide novel insight into the potential role of early-pregnancy angiogenesis on later cardiac development. Further studies are needed to replicate our findings and to identify potential underlying mechanisms.

## Sources of funding

The Generation R Study is financially supported by the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam and the Netherlands Organization for Health Research and Development. Romy Gaillard received funding from the Dutch Heart Foundation (grant number 2017T013), the Dutch Diabetes Foundation (grant number 2017.81.002), the Netherlands Organization for Health Research and Development (NWO, ZonMW, grant number 543003109) and from the European Union's Horizon 2020 research and innovation programme under the ERA-NET Cofund action (no 727565), EndObesity, ZonMW the Netherlands (no. 529051026). Vincent Jaddoe received a grant from the Netherlands Organization for Health Research and Development (NWO, ZonMw-VIDI 016.136.361) and a European Research Council Consolidator Grant (ERC-2014-CoG-648916).

## Authors' contributions

All authors made contributions to qualify as an author according to the criteria stated in the Publication Ethics, and all authors are responsible for the reported research. All authors have read and approved the submission of the manuscript; the manuscript has not been published

and is not being considered for publication elsewhere, in whole or in part, in any language.

## Acknowledgments

The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, Rotterdam, the Rotterdam Homecare Foundation, Rotterdam and the Stichting Trombosedienst and Artsenlaboratorium Rijnmond (STAR), Rotterdam. We gratefully acknowledge the contribution of participating mothers, general practitioners, hospitals, midwives, and pharmacies in Rotterdam.

## Conflicts of interest

We state that we have no financial commitments regarding this publication, or any other conflict of interests.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ahj.2022.01.016.

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