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RESEARCH ARTICLE

Preconception maternal retinal arteriolar narrowing and fetal growth throughout pregnancy: A prospective cohort study

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Abstract

Objective: To investigate the association between preconception maternal retinal arteriolar calibre and fetal growth.

Design, setting and population: A hospital-based, prospective preconception cohort including 369 women with a singleton live birth.

Methods: We collected detailed information on sociodemographic status, pregnancy history and lifestyle, and performed retinal imaging at the preconception visit.

Main outcome measures: We retrieved medical records documenting fetal growth biometrics (e.g., abdominal circumference [AC], head circumference [HC], femur length [FL]) at 11–13, 18–21, 24–28, and 32–34 weeks throughout pregnancy. We then computed the *z* scores for all fetal growth biometrics from 14 weeks of gestation where data were available, referencing the INTERGROWTH-21st fetal growth chart. We used a linear mixed model to estimate the association between maternal preconception retinal arteriolar calibre and fetal growth biometrics *z* scores throughout pregnancy, with random intercept accounting for repeated measures within individuals. We then performed a multivariable linear regression of maternal preconception retinal arteriolar calibre and *z* score changes for all fetal growth biometrics between 24–28 weeks and 32–34 weeks of gestation, after full adjustment.

Results: Maternal preconception generalised retinal arteriolar narrowing was consistently associated with a reduction in fetal AC *z* scores (−0.34; 95% CI −0.66 to −0.03) throughout pregnancy. In addition, women with preconception generalised retinal arteriolar narrowing tended to have significantly reduced *z* score changes in AC (−0.41; 95% CI −0.90 to −0.001) and fetal FL (−0.55; 95% CI −1.00 to −0.10) between 24–28 weeks and 32–34 weeks of gestation, respectively.

Conclusions: Our findings suggest that women with narrower preconception retinal arterioles had smaller fetuses, evidenced by reductions in AC and FL *z* score throughout pregnancy.

KEYWORDS
arteriolar narrowing, fetal growth, pregnancy, retinal microvasculature, trajectory

1 | INTRODUCTION

Fetal size and fetal growth trajectories are essential indicators of fetal health and may have long-term health implications over the lifespan.[1] Fetal growth is heavily modulated by placental function, with the placenta serving the fetus's critical respiratory, hepatic, and renal functions.[2,3] Multiple factors may affect fetal growth,[4,5] such as intrinsic fetal factors (e.g., chromosomal abnormalities,[6] genetic syndromes,[7] intrauterine infections,[8] multiple pregnancies[9]), maternal factors (e.g., malnutrition,[10] smoking,[11] hypertension,[12] severe anemia[13]), and uteroplacental factors (e.g., inadequate placentation, placental structural abnormalities and changes in placental implantation and attachment).[2,14–16] Among them, reduced uteroplacental perfusion associated with maternal vascular disease is responsible for 25–30% of fetal growth restriction cases,[17] and it is the most common cause of fetal growth restriction in non-anomalous fetuses.[18] However, the evaluation of antenatal uterine blood flow using Doppler ultrasound lacks consistency in reliability and reproducibility,[19] and there is no non-invasive approach to assess maternal microvascular circulation during pregnancy.

Retinal imaging—an advanced technology accessible to most populations—has been applied clinically to study general microcirculation due to its non-invasive and reproducible advantages.[20,21] Abnormal retinal vascular morphology, such as retinal arteriolar narrowing, is associated with cardiometabolic risks (e.g., smoking, depression, stress, obesity),[22,23] systemic diseases (e.g., hypertension, diabetes)[24,25] in general populations, hypertensive disorder during pregnancy[20] and gestational diabetes mellitus.[26] Our previous study even showed that maternal arteriolar narrowing during mid-pregnancy was associated with smaller birth size at delivery.[27]

Based on current evidence, retinal arteriolar narrowing might indicate adverse maternal vascular circulation in general and further shed light on the underlying vascular mechanism of fetal growth restriction and uteroplacental perfusion. However, no previous study has investigated the association between maternal preconception retinal arteriolar calibre and changes in fetal growth throughout pregnancy. Therefore, in this prospective preconception cohort in Singapore among 369 women with a singleton live birth, we explored the novel association between women's pre-gravid retinal arteriolar narrowing and fetal growth throughout pregnancy.

2 | METHODS

2.1 | Study population

Participants in the present study were from the Singapore PREconception Study of long-Term maternal and child Outcomes (SPRESTO, ClinicalTrials.gov, NCT03531658) from February 2015 to October 2017,[28] in a tertiary government hospital in Singapore (KK Women's and Children's Hospital). We recruited Chinese, Malay, or Indian women without pre-existing chronic hypertension, without pre-existing type 1 or type 2 diabetes, aged 18–45 years, and planning to conceive naturally within 12 months. We published the prospective cohort profile earlier and described the study objective and protocol in detail.[28] The study did not involve any participant in the development of the research. The study was conducted according to the guidelines under the Declaration of Helsinki and approved by the SingHealth Centralized Institute Review Board (2014/629/D). All participants provided written informed consent at study entry.

We recruited 1032 eligible women during the preconception phase and 480 women among them successfully conceived within a 12-month follow up.[28,29] After excluding 36 dropouts and 75 with subsequent pregnancy loss, we included 369 women with preconception retinal examination and a singleton live birth in the final analysis (Figure 1).

2.2 | Examination of retinal microvasculature

Trained photographers performed retinal examination using a 45-degree non-mydriatic retinal camera (Canon CR-1, 40D SLR digital retinal camera backing, Canon Inc.) at study entry during the preconception screening. The photographers took two retinal photographs centred on each eye's optic disc and macula without pharmacological pupillary dilation. Subsequently, we randomly assigned retinal images to two graders blinded to the participants' bio-data. According to a standard protocol, the graders assessed all retinal vessels beyond 25 μm in width crossing through 0.5–2.0 disc diameters (zone C) from the optic disc margin.[20,26] The retinal arteriolar calibre (central retinal arteriolar equivalence, CRAE) was evaluated and quantitatively measured using a semi-automated computer-based program (Singapore I Vessel Assessment [SIVA] version 4.0, Singapore Eye Research Institute) (Figure SS1). CRAE (measured in micrometres) is defined as the average width of retinal arterioles.[20,26] Based on a previous publication, we further defined retinal arteriolar abnormal morphology, namely 'generalised retinal arteriolar narrowing' as the lowest 20% of the study population CRAE value.[24] The inter- and intra-grader intraclass correlation coefficient achieved at 0.98 for CRAE in a randomly selected 10% subset of re-graded retinal photos, was published earlier.[29]

2.3 | Fetal growth measurement throughout pregnancy

We reviewed pregnant participants during the following research visits at 6–8 weeks (visit 1), 11–13 weeks (visit 2), 18–21 weeks (visit 3), 26–28 weeks (visit 4) and 32–34 weeks (visit 5) of gestation throughout pregnancy and until delivery. Pregnant women received an ultrasonographic examination at all research visits except for visit 1. Trained sonographers performed ultrasonographic assessment based on standard operating procedures; they used identical equipment (model: Aloka SSD-4000) and assessed a series of fetal growth biometrics from 11–13 weeks to 32–34 weeks of gestation as

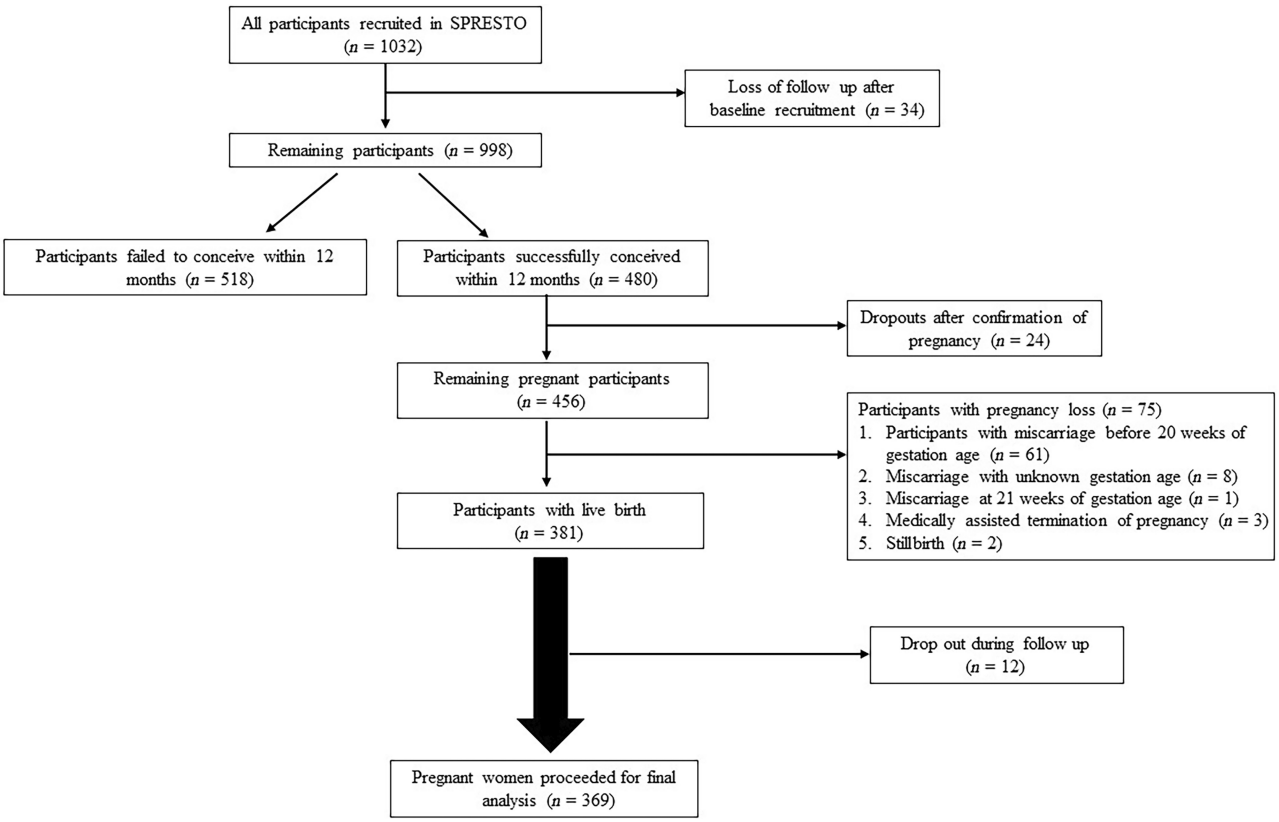

FIGURE 1 Study analysis flow chart.

follows: head circumference (HC, mm), biparietal diameter (BPD, mm), abdominal circumference (AC, mm) and femur length (FL, mm), based on standard views (Fetal Medicine Foundation).[30,31] We then used the INTERGROWTH-21 formula to compute the estimated fetal weight (EFW, g) for 24–28 weeks and 32–34 weeks based on the HC and AC.[32] In addition, we generated *z* scores for all fetal growth biometrics according to gestational age where data were available (AC, HC, FL and BPD: 14 weeks of gestation onwards; EFW: 22 weeks of gestation onwards), with reference to the INTERGROWTH-21st fetal growth standards chart.[33] Emerging evidence has shown that fetal growth in later pregnancy instead of earlier pregnancy might be a stronger tracking coefficient for adverse birth outcomes such as stillbirth, placental insufficiency and intrauterine growth restriction (IUGR).[34,35] Therefore, we further calculated *z* score changes from mid- to late pregnancy as an exploratory outcome by using the following formula: *z* score at visit 5 (32–34 weeks of gestation) – *z* score at visit 4 (24–28 weeks of gestation).

2.4 | Covariates

During recruitment, trained research coordinators conducted in-person interviews and measured the participants' weight, height, and blood pressure at the study clinic. Covariates collected using questionnaires at study entry included sociodemographic factors, health history, menstrual characteristics, and lifestyle behaviours. In detail, they were maternal age, ethnicity (Chinese versus Malay versus Indian versus others), college education (yes versus no), parity (nulliparous versus parous), Asian-specific cutoffs for body mass index (BMI) (underweight [BMI < 18.5 kg/m^2] versus normal weight [18.5 kg/m^2 ≤ BMI ≤ 22.9 kg/m^2] versus overweight [23.0 kg/m^2 ≤ BMI < 27.4 kg/m^2] versus obese [BMI ≥ 27.5 kg/m^2]),[36] systolic blood pressure (SBP), diastolic blood pressure, physical activity in the past 7 days classified based on the International Physical Activity Questionnaire guidelines (inactive versus minimally active versus HEPA [health-enhancing physical activity] active),[37] smoking (never versus past or current), alcohol intake (never versus past or current), micronutrient supplements intake in the past 3 months (yes versus no). In addition, a 2-hour 75 g two-time-point oral glucose tolerance test was performed at the clinic during the preconception visit. Prediabetes was diagnosed based on fasting glucose concentration at 6.1 mmol/L or higher and/or 2-hour glucose concentration at 7.8 mmol/L or higher, according to World Health Organization (WHO) guidelines.[38] Clinical diagnosis of pre-eclampsia was collected from medical records at delivery.

2.5 | Statistical analysis

Distributions of major characteristics of study participants were examined, and mean (standard deviation [SD]) or count (percentage) were tabulated accordingly.

Due to the nature of repeated outcome assessment on fetal growth biometrics for all participants, we applied a generalised linear mixed model (GLMM) to examine the temporal relationship between preconception retinal arteriolar calibre and fetal growth measurements as in raw values and *z* scores throughout pregnancy, respectively. The best performance of GLMM was based on covariance structure, including the smallest value in Akaike Information Criterion and Bayesian Information Criterion. We applied the following two models for the regression on fetal growth biometrics in raw values: Model 1 adjusting for gestational age; Model 2 adjusting for gestational age, maternal age, ethnicity (Chinese versus Malay versus Indian versus others), BMI at study entry (underweight versus normal weight versus overweight versus obese), prediabetes at study entry (yes versus no), maternal preconception SBP, smoking (current or past smoker versus non-smoker) and fetal sex. Since *z* scores were calculated according to gestational age, we applied another model (Model 3) for the regression on fetal growth biometrics in *z* scores. This model adjusted for maternal age, ethnicity (Chinese versus Malay versus Indian versus others), BMI at study entry (underweight versus normal weight versus overweight versus obese), prediabetes at study entry (yes versus no), smoking (current or past smoker versus non-smoker), maternal preconception SBP and fetal sex. Due to the better correlation between fetal growth parameters in the mid- to late pregnancy and actual birth size,[39,40] we further investigated maternal preconception retinal arteriolar calibre and the *z* score changes for all fetal growth biometrics between 24–28 weeks and 32–34 weeks of gestation, using multivariable linear regressions.

To test the robustness of the study findings, we performed sensitivity analyses by including the following variables: maternal college degree, nulliparity, smoking history, SBP, physical activity (inactive versus minimally active versus HEPA active), and supplement intake at study entry. We investigated potential effect modification by antenatal gestational age for each research visit, age (≥35 years versus <35 years), and BMI (underweight/normal weight versus overweight/obese) with retinal microvascular features in relation to the fetal growth trajectory. Moreover, we performed multiple imputations to address the issue of missingness in our cohort (<10% missing data on covariates). We created ten imputed data sets to reduce sampling variability from the imputation process. We included all covariates from the analysis model (maternal age, ethnicity, college education, nulliparity, SBP, smoking, physical activity, micronutrient dietary supplements intake, BMI, fasting and 2-hour glucose concentration at study entry, and fetal growth parameters at each research visit) in the imputation model. The results were pooled over ten imputed data sets using Rubin's rules.[41,42] In order to support our hypothesis that pre-pregnancy maternal suboptimal systemic microcirculation reflected by retinal arteriolar narrowing might lead to subsequent placental circulatory underdevelopment, we compared the categorical variables of maternal preconception generalised arteriolar narrowing and pre-eclampsia in the ensuing pregnancy.

Statistical analyses were conducted using PASW software version 27.0 (SPSS; IBM, Armonk, NY, USA) and R Software (version 3.1.2, R Development Core Team).[43] We provided regression coefficient (β or mean difference) with 95% CI for all estimates and set a significant *p* value at 0.05.

3 | RESULTS

Maternal characteristics, including age, ethnicity, socioeconomic status, reproductive health, body composition, blood pressure and lifestyle at study entry before conception, are shown in Table 1, along with fetal sex among women recruited at baseline and women with ($n = 381$) and without ($n = 651$) a singleton live birth in this preconception cohort.

There were 12 cases of loss of follow up, so we included a final number of 369 pregnant women in our final analysis. After adjusting for major confounders – including maternal age, BMI at study entry, prediabetic status, and maternal smoking – maternal preconception generalised retinal arteriolar narrowing was associated with reductions in AC raw values (β −3.19 mm; 95% CI −6.14 to −0.23) and AC *z* scores (−0.34; 95% CI −0.66 to −0.03) throughout pregnancy (Tables 2 and 3). Similarly, per SD narrowing in maternal preconception retinal arteriolar calibre (8.62 μm) was associated with a reduction in AC *z* score (−0.13; 95% CI −0.22 to −0.001) throughout gestation (Table 3).

In addition, we examined the association between maternal preconception generalised retinal arteriolar narrowing and changes in fetal size during mid- to late pregnancy, which was suggested as a better proxy for actual birth size at delivery.[39,40] After adjusting for maternal age, ethnicity, BMI, prediabetic status, fetal sex, and maternal smoking, mothers with preconception generalised retinal arteriolar narrowing were susceptible to significant reductions of *z* score changes in both fetal AC (−0.41; 95% CI −0.85 to −0.001) and fetal FL (−0.55; 95% CI −1.00 to −0.10) between 24–28 weeks and 32–34 weeks of gestation, respectively, compared with mothers without preconception generalised retinal arteriolar narrowing (Table 4). Figure 2 shows the case of a mother with preconception generalised retinal arteriolar narrowing, whose fetus subsequently had reduced *z* scores in AC and FL between 24–28 weeks and 32–34 weeks of gestation, compared with a mother without such a condition.

Interestingly, we did not find any significant association between preconception maternal CRAE and other fetal growth parameters such as HC, BPD and EFW. All the significant associations reported above remained significant after multiple imputations (Table S1), and sensitivity analysis by additionally adjusting for physical activity, supplementary intake and nulliparity at the preconception phase (Table S2). In terms of potential effect modifiers, we

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TABLE 1 Maternal characteristics at preconception phase in SPRESTO women ($n = 1032$).

Characteristics	Women in SPRESTO ($n = 1032$) Mean ± SD or *N* (%)	Women with a live birth in SPRESTO ($n = 381$) Mean ± SD or *N* (%)	Women without a live birth ($n = 651$) Mean ± SD or *N* (%)	*p* value*
Baseline visit at preconception phase				
Age, year-old	30.8 ± 3.8	29.9 ± 3.2	31.3 ± 4.0	<0.001
Ethnicity				
Chinese	743 (72)	281 (76.2)	462 (69.7)	0.02
Malay	159 (15.4)	51 (13.8)	108 (16.3)	
Indian	95 (9.2)	22 (6.0)	73 (11.0)	
Others	35 (3.4)	15 (4.1)	20 (3.0)	
Maternal College degree, Yes	635 (62.3)	266 (72.1)	369 (56.8)	<0.001
Nulliparous, Yes	653 (63.5)	230 (62.3)	423 (64.0)	0.63
BMI at study entry				
Underweight (<18.5 kg/m^2)	84 (8.4)	23 (6.4)	61 (9.6)	<0.001
Normal weight (18.5–22.9 kg/m^2)	456 (45.7)	203 (56.2)	253 (39.8)	
Overweight (23.0–27.4 kg/m^2)	266 (26.7)	81 (22.4)	185 (29.1)	
Obese (≥27.5 kg/m^2)	191 (19.2)	54 (15)	137 (27.5)	
SBP, mmHg	105.1 ± 9.7	103.9 ± 8.9	105.8 ± 10.1	0.003
Fasting plasma glucose, mmol/L	4.8 ± 0.8	4.7 ± 0.4	4.9 ± 0.9	<0.001
2-hour plasma glucose, mmol/L	6.0 ± 2.1	5.7 ± 1.3	6.2 ± 2.4	<0.001
Prediabetes at preconception, yes	113 (11.4)	26 (7.0)	87 (13.1)	0.003
Physical activity				
Inactive	164 (16.3)	61 (16.7)	103 (16.1)	0.21
Minimally active	505 (50.2)	171 (46.7)	334 (52.2)	
HEPA active	337 (33.5)	134 (36.6)	203 (31.7)	
Smoking exposure				
Never smokes	904 (89.4)	339 (92.1)	565 (87.9)	0.10
Smoker	45 (4.5)	11 (3.0)	34 (5.3)	
Ex-smoker	62 (6.1)	18 (4.9)	44 (6.8)	
Alcohol intake, Past or current				
Never drinks	695 (67.3)	252 (68.3)	443 (68.0)	0.63
Past or current drinker	337 (32.7)	117 (31.7)	208 (32.0)	
Anxiety (STAI score ≥75th centile), Yes	224 (26.2)	74 (24.3)	150 (27.2)	0.35
Depression (EPDS score ≥14), Yes	80 (9.2)	23 (7.5)	57 (10.2)	0.19
Supplement intake, Yes	674 (66.7)	264 (71.7)	410 (63.9)	**0.01**
Preconception CRAE, μm	119.05 ± 8.83	119.82 ± 8.62	118.61 ± 8.91	0.05
Fetal sex, male	–	200 (54.2)	–	

Note: Significant findings were shown in bold.

Abbreviations: BMI, body mass index; CRAE, central retinal arteriolar equivalent; HEPA, health-enhancing physical activity; SBP, systolic blood pressure; SD, standard deviation; SPRESTO, Singapore PREconception Study of long-Term maternal and child Outcomes; STAI, Stait–Trait Anxiety Inventory.

*Student's *t* test or chi-square test.

did not find any significant interaction between maternal preconception generalised retinal arteriolar narrowing and gestational age, maternal age (<35 versus ≥35 years), or Asian-specific BMI categories (underweight/normal weight versus overweight/obese) in relation to fetal growth biometrics like AC (Table S3). Finally, among nine women who were clinically diagnosed with pre-eclampsia form their medical records, eight had preconception generalised arteriolar narrowing (Fisher's *t* test <0.05) (Table S4).

4 | DISCUSSION

In our preconception and birth cohort, we analysed 369 singleton pregnancies that resulted in a live birth. We found that pregnant women with preconception generalised retinal arteriolar narrowing consistently had smaller fetuses throughout pregnancy, as evidenced by reduced *z* scores in both AC and FL between mid- and late pregnancy. Such findings remained significant after multiple imputations and sensitivity analyses.

TABLE 2 Association of preconception maternal retinal arteriolar calibre and fetal growth biometrics in raw values throughout pregnancy using multivariable generalised linear mixed model ($n = 369$).

Preconception maternal retinal vascular calibre	Fetal growth biometric in raw values across pregnancy (11–13, 18–21, 24–28 and 32–34 weeks of gestation)							
	AC, mm		HC, mm		FL, mm		BPD, mm	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
CRAE, per SD ↓ (SD, 8.62 μm)	−1.02 (−2.16 to 0.13)	−0.74 (−1.93 to 0.46)	−0.54 (−1.58 to 0.50)	−0.06 (−1.12 to 1.00)	−0.18 (−0.43 to 0.06)	−0.16 (−0.41 to 0.10)	−0.02 (−0.34 to 0.29)	0.12 (−0.20 to 0.44)
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Generalised retinal arteriolar narrowing, yes versus no (ref)	**−3.60 (−6.45 to −0.73)**	**−3.19 (−6.14 to −0.23)**	−1.28 (−3.85 to 1.29)	−0.46 (−3.07 to 2.14)	−0.07 (−0.67 to 0.54)	−0.02 (−0.65 to 0.62)	−0.37 (−1.16 to 0.41)	−0.18 (−0.98 to 0.61)

Note: Generalised arteriolar narrowing, the lowest 20th centile in the study population CRAE value. Model 1, adjusting for gestational age; Model 2, adjusting for gestational age, maternal age, race/ethnicity, pre-pregnancy BMI (underweight versus normal weight versus overweight versus obese), prediabetes at preconception (yes versus no), maternal smoking (non versus past/current), maternal preconception systolic blood pressure and fetal sex. Significant findings were shown in bold.

Abbreviations: AC, abdominal circumference; BMI, body mass index; BPD, biparietal diameter; CI, confidence interval; CRAE, central retinal arteriolar equivalent; FL, femur length; HC, head circumference; SD, standard deviation.

TABLE 3 Association of preconception retinal arteriolar calibre and fetal growth biometrics *z* scores throughout pregnancy using multivariable generalised linear mixed model ($n = 369$).

Preconception maternal retinal vascular calibre	Fetal growth biometric *z* scores from 14 weeks of gestation onwards							
	AC *z* score		HC *z* score		FL *z* score		BPD *z* score	
	Crude model	Model 3	Crude model	Model 3	Crude model	Model 3	Crude model	Model 3
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
CRAE, per SD ↓ (SD, 8.62 μm)	**−0.13 (−0.25 to −0.01)**	**−0.13 (−0.22 to −0.001)**	−0.07 (−0.18 to 0.05)	−0.01 (−0.13 to 0.11)	−0.08 (−0.19 to 0.02)	−0.08 (−0.19 to 0.03)	0.001 (−0.14 to 0.14)	0.06 (−0.09 to 0.20)
	Crude Model	Model 3	Crude Model	Model 3	Crude Model	Model 3	Crude Model	Model 3
	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Generalised retinal arteriolar narrowing, yes vs. no (ref)	**−0.39 (−0.70 to −0.07)**	**−0.34 (−0.66 to −0.03)**	−0.22 (−0.51 to 0.08)	−0.14 (−0.43 to 0.16)	−0.10 (−0.37 to 0.17)	−0.10 (−0.38 to 0.19)	−0.12 (−0.48 to 0.23)	−0.05 (−0.41 to 0.30)

Note: Generalised arteriolar narrowing, the lowest 20th centile of the study population CRAE value. Model 3, adjusting for maternal age, race/ethnicity, pre-pregnancy BMI (underweight versus normal weight versus overweight versus obese), prediabetes at preconception (yes versus no), maternal smoking (non versus past/current), maternal preconception systolic blood pressure and fetal sex. Significant findings were shown in bold.

Abbreviations: AC, abdominal circumference; BPD, biparietal diameter; CI, confidence interval; CRAE, central retinal arteriolar equivalent; FL, femur length; HC, head circumference; SD, standard deviation.

Fetal size and growth trajectories are essential indicators of fetal health, which are heavily modulated by placental function.[1,2,44] Traditional studies suggested that multiple adverse fetal and maternal factors such as genetic syndromes, intrauterine infections, multiple pregnancies, malnutrition and smoking are related to growth restriction in utero.[4,5] Emerging evidence has shown that uteroplacental factors (e.g., inadequate placentation, structural abnormalities, changes in placental implantation and attachment)[2,14–16] are responsible for at least a quarter of fetal growth restriction cases.[17] However, as mentioned above, the evaluation of uterine blood flow during pregnancy via Doppler ultrasound is compromised by relatively low reliability and reproducibility, as well as

TABLE 4 Multivariable linear regressions of preconception maternal retinal arteriolar calibre and changes of *z* scores of fetal growth biometrics between 24–28 weeks and 32–34 weeks of gestation ($n = 369$).

	Fetal growth biometric *z* score changes between 24–28 weeks and 32–34 weeks of gestation									
	Changes of AC *z* score		Changes of HC *z* score		Changes of FL *z* score		Changes of BPD *z* score		Changes of EFW *z* score	
Preconception maternal retinal arteriolar calibre	Crude model β (95% CI)	Model 3 β (95% CI)	Crude β (95% CI)	Model 3 β (95% CI)	Crude β (95% CI)	Model 3 β (95% CI)	Crude model β (95% CI)	Model 3 β (95% CI)	Crude β (95% CI)	Model 3 β (95% CI)
CRAE, per SD ↓ (SD, 8.62 μm)	−0.11 (−0.28 to 0.07)	−0.12 (−0.30 to 0.07)	−0.01 (−0.18 to 0.16)	0.03 (−0.15 to 0.21)	−0.10 (−0.29 to 0.08)	−0.08 (−0.26 to 0.12)	0.04 (−0.12 to 0.20)	0.10 (−0.07 to 0.27)	−0.02 (−0.19 to 0.15)	−0.01 (−0.18 to 0.17)
	Crude Model Mean difference (95% CI)	Model 3 Mean difference (95% CI)	Crude Model Mean difference (95% CI)	Model 3 Mean difference (95% CI)	Crude Model Mean difference (95% CI)	Model 3 Mean difference (95% CI)	Crude Model Mean difference (95% CI)	Model 3 Mean difference (95% CI)	Crude Model Mean difference (95% CI)	Model 3 Mean difference (95% CI)
Generalised retinal arteriolar narrowing, yes vs. no (ref)	**−0.39 (−0.83 to −0.001)**	**−0.41 (−0.85 to −0.001)**	−0.09 (−0.51 to 0.33)	−0.04 (−0.47 to 0.40)	**−0.58 (−1.10 to −0.13)**	**−0.55 (−1.00 to −0.10)**	−0.14 (−0.56 to 0.27)	−0.11 (−0.53 to 0.31)	−0.14 (−0.55 to 0.27)	−0.14 (−0.55 to 0.28)

Note: Generalised arteriolar narrowing, the lowest 20th percentile in the study population CRAE value. Model 3, adjusting for, maternal age, race/ethnicity, pre-pregnancy BMI (underweight versus normal weight versus overweight versus obese), prediabetes at preconception (yes versus no), maternal smoking (non versus past/current), maternal preconception systolic blood pressure and fetal sex. Significant findings were shown in bold.

Abbreviations: BMI, body mass index; CRAE, central retinal arteriolar equivalent; CI, confidence interval; AC, abdominal circumference; HC, head circumference; FL, femur length; BPD, biparietal diameter; EFW, estimated fetal weight.

the lack of a non-invasive approach to assess maternal vascular circulation during pregnancy.

The examination of the retina has become routine to determine the presence and severity of retinal vascular damage among patients with hypertension or diabetes.[21,24] As a commonly assessed clinical feature, the retinal vascular calibre has correlated well with general circulation, inflammatory status, oxidated stress levels, and endothelial dysfunction in vivo.[22,23] Therefore, such a state-of-the-art, non-invasive and reproducible technique has been widely adopted by epidemiological and clinical research to understand the aetiology of systemic disease (i.e., hypertension and diabetes),[45] underlined by retinal microvasculature.

Our previous work in Growing Up Towards Healthy Outcomes (GUSTO) has applied this retinal imaging technique to pregnancy health outcomes in the past decade. We observed a series of exciting associations between suboptimal retinal microvascular features (e.g., narrowing arterioles, widening venules, sparse fractal, enlarged vascular branching angle, higher vascular curvature tortuosity) and common determinants of fetal growth such as maternal obesity,[46] gestational diabetes mellitus,[26] hypertensive disorder during pregnancy risk,[20] psychological stress[47] and nutrition intake in pregnant women.[48] Novel work in this SPRESTO preconception cohort also identified biologically plausible relationships of suboptimal retinal vascular geometry (e.g., sparser arteriolar fractal, higher arteriolar and venular curvature tortuosity) with prolonged time-to-pregnancy,[29] and incident spontaneous abortion.[49] Hence, retinal microcirculation might indicate overall female cardiometabolic and reproductive health and potentially reflect the general uteroplacental and ovarian–uterine circulation.

A prospective study involving a cohort of 129 women recruited from Sydney, Australia, reported that maternal CRAE was significantly reduced throughout pregnancy in the pre-eclampsia group.[50] Similarly, 63 women identified in the Generation R study follow up ($n = 3391$) with an episode of pre-eclampsia had prolonged retinal arteriolar narrowing even 6 years after pregnancy.[51] In other words, retinal arteriolar narrowing indicated that peripheral vascular resistance during pregnancy might be considered a transient response to increased blood pressure and a result of cumulative exposure to endothelial damage and microvascular impairment in vivo.[52,53] Our study found that pregnant women with generalised retinal arteriolar narrowing bore fetuses with smaller AC throughout pregnancy, specifically a drop in *z* scores of both AC and FL from mid- to late pregnancy. Interestingly, most women in this condition also developed pre-eclampsia at delivery. Such findings indirectly implied that a compromised maternal vascular environment might adversely impact placental perfusion and fetal development in utero, which could be crucially important during later pregnancy when stillbirth,[54] placental insufficiency[55] and IUGR may occur.[34,35] These features could help provide insights into the pathogenesis of suboptimal fetal growth in utero and provide a potentially novel angle to indicate subsequent fetal growth as early as preconception. Our ongoing study will continue to investigate the associations between

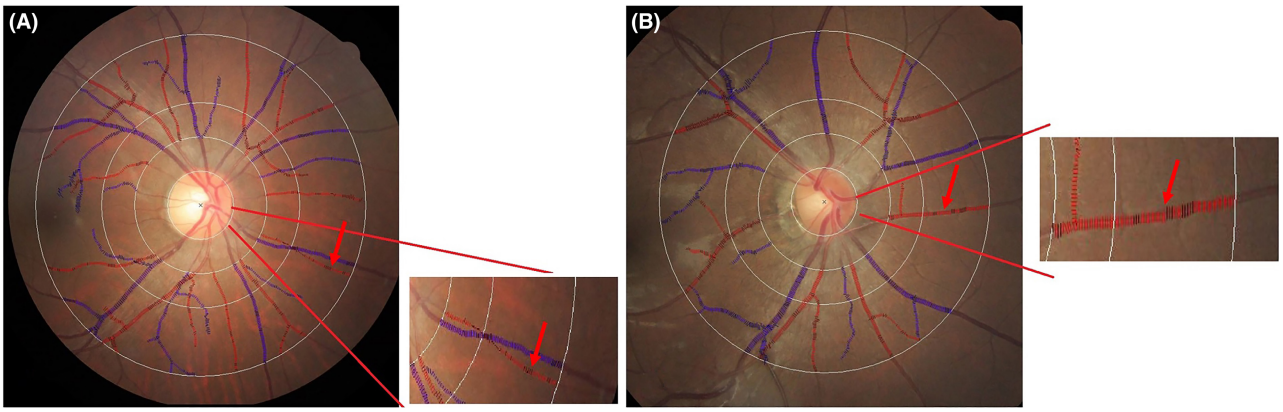

FIGURE 2 Examples of central retinal arteriolar equivalent (CRAE) values in pregnant women with and without suboptimal fetal growth. (A) A woman with preconception generalised retinal arteriolar narrowing (CRAE = 109.56 μm) and a suboptimal fetal growth with a 1.75 *z* score reduction in abdominal circumference (AC) and a 1.38 *z* score reduction in femur length (FL) between 24–28 weeks and 32–34 weeks of gestation, respectively. (B) shows a pregnant woman without preconception generalised retinal arteriolar narrowing (CRAE = 152.86 μm) and a normal fetal growth with a 0.72 *z* score increment in AC and a 0.29 *z* score increment in FL, respectively.

preconception maternal retinal microvasculature and utero-feto-placental perfusion.

To the best of our knowledge, this is the first study exploring the temporal relationship between retinal microvascular features at the preconception phase and fetal growth trajectories throughout pregnancy. The strengths of our study include standardised protocols, validated assessment of retinal microvascular characteristics, and detailed information on a comprehensive database of confounders at the preconception phase relating to reproductive health, together with multiple fetal growth scans. Nevertheless, our study is not without limitations. First, as the result of missing data on spousal characteristics, such as husband's weight and height, residual confounding bias might exist, which could be associated with fetal size.[56] Second, potential selection bias might exist because our analyses were performed in a subset of Asian women with higher education levels and a lower prevalence of being overweight and obese from the original preconception cohort. Third, we reported a relatively lower conception rate than other cohorts investigating natural conception rates, which might indicate a pre-existing subfertility issue among some of our participants that might affect the general circulation in both the retina and placenta. Therefore, our results were not generalisable to other populations.

5 | CONCLUSION

Our prospective preconception cohort showed an association between maternal generalised retinal arteriolar narrowing and a smaller fetal size throughout pregnancy, specifically reductions of *z* score changes in AC and FL *z* scores from mid- to late pregnancy. Maternal preconception retinal arteriolar narrowing might indirectly indicate decreased placental perfusion, leading to suboptimal fetal growth in utero.

AUTHOR CONTRIBUTIONS

L-JL designed and conducted the study, collected data, performed statistical analysis and drafted the manuscript. RC conducted the statistical analysis. JKYC supervised the statistical analysis and critically revised the manuscript; KHT and YSC designed and conducted the study; JGE acquired the data and conducted the research; LLS and ZW critically revised the manuscript; CZ supervised the statistical analysis and revised the manuscript.

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Data will be made available to the editors of the journal for review or query upon request. Individual participant data (including data dictionaries) underlying the results reported in this article after deidentification will be available to investigators whose proposed use of the data for research purposes has been approved by an independent review committee from the SPRESTO cohort. The data are available between 9 and 36 months following article publication.

ETHICS APPROVAL

The study was conducted according to the guidelines under the Declaration of Helsinki and approved by the SingHealth Centralized Institute Review Board (2014/629/D).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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