The Association of Falling Insulin Requirements With Maternal Biomarkers and Placental Dysfunction: A Prospective Study of Women With Preexisting Diabetes in Pregnancy

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OBJECTIVE

To investigate the association of falling insulin requirements (FIR) among women with preexisting diabetes with adverse obstetric outcomes and maternal biomarkers longitudinally in pregnancy.

RESEARCH DESIGN AND METHODS

A multicenter prospective cohort study of 158 women (41 with type 1 diabetes and 117 with type 2 diabetes) was conducted. Women with FIR of ≥15% from the peak total daily dose after 20 weeks' gestation were considered case subjects (n = 32). The primary outcome was a composite of clinical markers of placental dysfunction (preeclampsia, small for gestational age [≤5th centile], stillbirth, premature delivery [<30 weeks], and placental abruption). Maternal circulating angiogenic markers (placental growth factor [PlGF] and soluble fms-like tyrosine kinase 1 [sFlt-1]), placental hormones (human placental lactogen, progesterone, and tumor necrosis factor-α), HbA_{1c}, and creatinine were studied serially during pregnancy.

RESULTS

FIR ≥15% were associated with an increased risk of the composite primary outcome (odds ratio [OR] 4.38 [95% CI 1.9–10.3]; $P < 0.001$), preeclampsia (OR 6.76 [95% CI 2.7–16.7]; $P < 0.001$), and was more common among women with type 1 diabetes (36.6 vs. 14.5%; $P = 0.002$). Creatinine was modestly elevated among women with FIR ≥15%; however, there was no difference in HbA_{1c}. The ratio of sFlt-1 to PlGF was significantly higher among women with FIR at 25, 30, and 36 weeks, with differences maintained in the subgroup that developed preeclampsia. There was no difference in placental hormones between the groups.

CONCLUSIONS

This is the first prospective study to associate FIR with altered expression of placental antiangiogenic factors and preeclampsia. FIR are an important clinical sign, among women with preexisting diabetes, that should alert the clinician to investigate underlying placental dysfunction.

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Although the majority of women with preexisting diabetes need increasing doses of insulin with advancing gestation (1), a proportion need a reduction in insulin requirements in late pregnancy, the significance of which remains unclear. Because insulin resistance is driven by placental-mediated hormone production, falling insulin requirements (FIR) are considered to be a marker of fetoplacental compromise, prompting admission to the hospital for maternal and fetal monitoring and early delivery in some cases (2,3).

However, despite the potential importance of this clinical sign, only four studies have investigated women with FIR in the literature. We have previously shown, through a retrospective cohort study, that FIR are associated with preeclampsia, small-for-gestational-age (SGA) babies, and increased admission to the neonatal intensive care unit (NICU) (4). In contrast, other studies have not identified an association with adverse obstetric outcomes (5–7). Previous studies are limited by retrospective design, differing definitions of FIR, limited representation of women with type 2 diabetes, and inadequate statistical power for adverse outcomes associated with placental dysfunction.

There is increasing evidence for the role of placental biomarkers in predicting and diagnosing obstetric conditions associated with placental dysfunction; however, few studies have included women with preexisting diabetes (8–10). An imbalance between proangiogenic factors, such as placental growth factor (PlGF), and antiangiogenic factors, such as soluble fms-like tyrosine kinase 1 (sFlt-1), are thought to play a key role in diseases associated with placental dysfunction (11). FIR among women with preexisting diabetes may be considered a clinical manifestation of the glycemic vascular interface between the fetoplacental unit and the mother; however, no previous studies have examined the association between FIR and placental biomarkers.

Considering this, we conducted the Falling Insulin Requirements Study (FIRST), a prospective multicenter cohort study, specifically powered to examine the relationship between FIR and clinical outcomes of placental insufficiency among women with preexisting diabetes. In addition, we investigated the pathophysiology of FIR through serial examination of placental biomarkers including pro- and antiangiogenic markers as well as hormones of insulin resistance.

RESEARCH DESIGN AND METHODS

FIRST in Pregnancy was conducted at three tertiary referral hospitals with dedicated diabetes in pregnancy services in Sydney, Australia, between June 2013 and October 2015. The study was approved by the Western Sydney Local Health District Ethics Committee.

Women with singleton pregnancy and a diagnosis of preexisting type 1, type 2, or a new diagnosis of overt diabetes on a 75-g oral glucose tolerance test during pregnancy (defined as a fasting blood glucose level of $\geq$7.0 mmol/L [126 mg/dL], a 2-h level of $\geq$11.1 mmol/L [200 mg/dL], or an HbA_{1c} of $\geq$6.5% [48 mmol/mol] [12,13]) were prospectively recruited at their first visit to the dedicated diabetes in pregnancy clinic. Only women presenting prior to 20 weeks and progressing beyond 20 weeks' gestation were included in the study.

Information on baseline demographics and medical and obstetric history were obtained via a structured questionnaire at recruitment. Weight in kilograms, blood pressure, insulin dose (total, basal, and prandial), and carbohydrate intake per meal (quantified in exchanges; 1 exchange = 15 g) were recorded at each review. For women treated with an insulin pump, an upload containing data on average basal and prandial insulin dosing and daily carbohydrate intake for the preceding 1 to 2 weeks was recorded. At all centers, insulin doses were titrated to the same blood glucose target of $\leq$5.5 mmol (100 mg/dL) fasting and $\leq$7 mmol (126 mg/dL) 2 h postprandial by the treating team, and intermittent checks of the glucose monitor were performed to ensure correlation between self-recorded glucose values and those in the machine. Participants were reviewed at least every 4 weeks up to 28 weeks, every 2 weeks until 36 weeks, and weekly thereafter.

FIR were calculated as a percentage from the peak total insulin dose (PTID), defined as the highest total insulin dose in pregnancy, and the trough total insulin dose (TTID), defined as the lowest total insulin dose following the peak, using the following formula:

$$\text{FIR} = (\text{PTID-TTID})/\text{PTID}) \times 100\%$$

Care was taken not to include the period within 1 week of antenatal steroid administration when calculating FIR.

Outcomes

As there is currently no universally accepted definition of placental insufficiency, the primary outcome was defined a priori as a composite of adverse clinical outcomes associated with placental dysfunction in the literature (4,14,15). These included preeclampsia (defined as per the International Society for the Study of Hypertension in Pregnancy 2014 criteria [16]), SGA (birth weight $\leq$5th percentile [17,18]), preterm delivery ($<$30 weeks) (19), placental abruption, and stillbirth ($>$20 weeks). Presence of one or more of these outcomes in the pregnancy was the threshold for attribution of an abnormal primary outcome. Additionally, secondary outcomes were evaluated, including maternal and neonatal clinical outcomes, HbA_{1c}, and serum creatinine, as well as biomarkers associated with placental dysfunction, including the antiangiogenic factor sFlt-1 and proangiogenic factor PlGF (11), and hormones of insulin resistance including tumor necrosis factor-α (TNF-α), human placental lactogen (hPL), and progesterone (20,21).

Laboratory Procedures

Blood and urine were collected from participants at four time points: 14 $\pm$ 1 weeks' (or first visit if presenting later), 24 $\pm$ 1 weeks', 30 $\pm$ 1 weeks', and 36 $\pm$ 1 weeks' gestation. Samples from the end of each trimester were tested immediately for HbA_{1c}, creatinine, urine albumin-to-creatinine ratio (ACR), and protein-to-creatinine ratio (PCR), and results were made available to the treating team. Remaining samples were stored at $-$80°C and analyzed within 18 months for sFlt-1, PlGF, TNF-α, hPL, and progesterone. Biomarkers were analyzed by ELISAs using commercially available kits: sFlt-1, PlGF, and TNF-α (R&D Systems), hPL (DRG Diagnostics), and progesterone (Labor Diagnostika Nord). The interassay coefficients of variation were 11.1, 15.5, 14, 22, and 5.2% for PlGF, sFlt-1, TNF-α, hPL, and progesterone, respectively.

Statistical Analysis

Statistical analysis was performed using SPSS version 23 and S-PLUS version 8.2. The primary analysis was conducted using a case-control model by dichotomizing the cohort into case subjects (defined as women with $\geq$15% FIR after 20 weeks' gestation) and control subjects (women with $<$15% FIR after 20 weeks). The cut point of 15% was chosen, as this

was considered clinically significant in previous studies (4,5,7). For the purpose of statistical analysis, women with overt diabetes in pregnancy were reclassified as type 1 diabetes if positive for GAD antibodies or type 2 diabetes if negative. Differences in baseline characteristics were corrected for type of diabetes by logistic regression. Based on results from our previously published retrospective study, a sample size of 156 women was calculated to give a power of 80% at a type 1 error of 0.05 for the primary outcome using this definition of FIR (4).

Continuous variables were analyzed after log transformation using *t* tests and summarized as median and lower to upper quartile (quartile 1 to quartile 3), whereas Pearson χ^2 or Fisher exact tests were used for categorical variables. Biomarker values were analyzed longitudinally during pregnancy by linear mixed-effects models after log transformation. Correction for maternal age, BMI, ethnicity (Caucasian, Asian, or other), nulliparity, and smoking status was performed for the analysis of angiogenic markers, as previous studies have shown these variables can influence levels (8,22).

RESULTS

One hundred fifty-eight women were included in the final analysis, of which 32 (20.3%) fit the case definition of FIR ≥15%. The median reduction in insulin requirements among the case group was 25.2% (20.9–43.7%), substantially greater than the chosen cut point of 15%. The percent fall in insulin requirements was similar whether the dose was calculated in units or corrected for weight (units per kilogram).

A comparison of maternal baseline and pregnancy characteristics between women with and without FIR ≥15% is summarized in Table 1. Forty women (25.3%) had type 1 diabetes, 94 (59.5%) had type 2 diabetes, and 24 (15.2%) were diagnosed with overt diabetes during pregnancy, of whom 1 was reclassified as type 1 diabetes and 23 as type 2 diabetes. A greater proportion of women with type 1 diabetes developed FIR ≥15% compared with type 2 or overt diabetes (37.5 vs. 14.9 vs. 12.5%, respectively; $P = 0.012$). Treatment with aspirin during pregnancy was higher among women with FIR ≥15%, independent of the type of diabetes, although overall, very few women were treated with aspirin in this cohort. Caucasian ethnicity was also more common among women with FIR ≥15%; however, this was no longer significant after correction for type of diabetes by regression analysis.

Glycemic control, measured by HbA_{1c} at the end of each trimester (trimester 1: 13 [12–15] weeks; trimester 2: 24 [24–26] weeks; and trimester 3: 36 [35–36] weeks), improved in both groups with advancing gestation; however, there was no significant difference in HbA_{1c} between women with and without FIR ≥15%. Likewise, there was no difference in gestational weight gain and FIR status (Table 1).

Serum creatinine was modestly higher in women with FIR ≥15% throughout pregnancy and remained independently associated with FIR even after correction for type of diabetes (Table 1). To further assess the relationship between creatinine and FIR, the change in creatinine from the start to the end of the second and third trimesters was calculated (Δ creatinine). Women with FIR ≥15% had a significantly greater increase in creatinine during the third trimester (6.5 [4–12] vs. 4 [−2 to 8] μmol/L; $P = 0.044$) (Supplementary Fig. 1). There was no difference in ACR or PCR between the two groups.

Clinical Outcomes

In total, 33 (20.9%) women were positive for the primary composite outcome. Women with FIR ≥15% had a significantly greater risk of developing the composite outcome compared with those without (odds ratio 4.38 [95% CI 1.86–10.3]; $P < 0.001$) (Table 2). This remained significant after women who had reduced carbohydrate intake were excluded (46.2 vs. 15.9%; $P = 0.001$) and when FIR were calculated using weight-corrected dose (41.7 vs. 14.8%; $P = 0.001$) and weight-corrected basal dose (36.7 vs. 17.2%; $P = 0.018$).

The increased incidence of the composite outcome among women with FIR was largely driven by the increased risk of preeclampsia (odds ratio 6.76 [95% CI 2.74–16.70]; $P < 0.001$) (Table 2). Approximately half of the women with preeclampsia had FIR ≥15%. Of these women, the majority ($n = 9$) had evidence of FIR preceding the clinical diagnosis of preeclampsia by 3 (0–4) weeks (Supplementary Fig. 2). There were no deliveries prior to 30 weeks and no episodes of placental abruption. The incidence of SGA babies or stillbirth was small and did not differ between the groups.

Logistic regression analysis was conducted to analyze the independent predictors of the primary composite outcome and preeclampsia. After correction of factors significantly associated with either FIR or the outcome of interest on univariable analysis, FIR remained an independent predictor of the primary composite outcome but were not independent of third-trimester creatinine for preeclampsia (Supplementary Tables 1 and 2).

Babies of women with FIR ≥15% were more likely to be preterm (<37 weeks) and more likely to require emergency caesarean section and admission to NICU. There was higher antenatal steroid use among women with FIR ≥15% in keeping with earlier deliveries. Results of the neonatal and maternal secondary outcomes are summarized in Table 2.

Placental Biomarkers

Results for sFlt-1 and PlGF are not presented, as the ratio is known to be a more sensitive marker of placental dysfunction (9,23). The sFlt-1/PlGF ratio was significantly higher among women with FIR from 25 weeks onward (Fig. 1*A*). These results remained significant after adjustment for baseline differences in type of diabetes, as well as factors that have previously been shown to lower PlGF levels, including maternal age, BMI, ethnicity, nulliparity, and smoking status (8,22). To account for the effect of preeclampsia on the differences in PlGF and sFlt-1, the analysis was repeated after separating the cohort into those with and without preeclampsia. In total, 27 women developed preeclampsia, of whom 14 also had FIR ≥15%. These women had significantly higher sFlt-1/PlGF ratio at 25 and 36 weeks (Fig. 1*B*). The remaining cohort consisted of 131 women, of whom 18 also had FIR ≥15%. In this subgroup, there were no longer any significant differences in sFlt-1/PlGF ratio between women with and without FIR (Fig. 1*C*).

As the number of women in each subgroup was small and not powered to show differences between groups, we examined if there was a trend of increasing sFlt-1/PlGF ratio across the four subgroups at 36 weeks. We hypothesized that women with no preeclampsia and no FIR (group 1) would have the least placental dysfunction, followed by women

Table 1—Characteristics of women with and without FIR ≥15%

Variables	FIR ≥15% (case subjects, *n* = 32)	FIR <15% (control subjects, *n* = 126)	*P* value	*P* value*
Age (years)	32.0 (28.5–35.0)	32.5 (29.0–36.0)	0.603	—
Prepregnancy BMI (kg/m^2)	27.7 (24.2–33.2)	30.45(26.3–38.1)	0.202	—
Ethnicity				
Caucasian	17 (53.1)	40 (31.7)	0.026	0.180
Asian	12 (37.5)	49 (38.9)		
Other	3 (9.4)	37 (29.4)		
Diabetes			0.012	—
Type 1	15 (46.9)	25 (19.8)		
Type 2	14 (43.8)	80 (63.5)		
Overt	3 (9.4)	21 (16.7)		
Duration of diabetes (years)	5.5 (1.25–15.5)	3 (1–8)	0.134	—
Microvascular complications	7 (21.9)	19 (15.1)	0.35	—
Nulliparity	13 (40.6)	37 (29.4)	0.221	—
Unplanned pregnancy	17 (53.1)	76 (60.3)	0.460	—
Chronic hypertension	6 (18.8)	13 (10.3)	0.223	—
Hypertensive disorder in previous pregnancy†	5 (26.3)	22 (24.7)	0.805	—
Current smoker	4 (12.5)	11 (8.7)	0.507	—
Aspirin use during pregnancy	5 (21.9)	10 (6.3)	0.014	0.010
Preconception HbA_{1c} (%)	7.4 (6.7–9.4)	7.25 (6.1–9.8)	0.628	—
(mmol/mol)	57 (50–79)	56 (43–84)		
Trimester 1 HbA_{1c} (%)	6.9 (6.4–7.7)	6.4 (5.8–7.6)	0.132	
(mmol/mol)	52 (46–61)	46 (40–60)		
Trimester 2 HbA_{1c} (%)	6.2 (5.5–6.8)	5.7 (5.2–6.7)	0.380	
(mmol/mol)	44 (37–51)	39 (33–50)		
Trimester 3 HbA_{1c} (%)	6.2 (5.3–7.1)	6.1 (5.7–7.3)	0.87	
(mmol/mol)	44 (34–54)	43 (39–56)		
Creatinine (μmol/L)				
Trimester 1	46.5 (42–54)	43 (39–48)	0.001	0.015
Trimester 2	46 (40–52)	42 (38–47)	0.043	0.053
Trimester 3	57 (44–63)	46 (40–51)	<0.001	0.001
ACR				—
Trimester 1	0.85 (0–1.7)	0.9 (0–2.1)	0.780	
Trimester 2	1.1 (0–1.8)	0.9 (0–1.7)	0.263	
Trimester 3	1 (0.6–11.1)	1.8 (0.8–3.6)	0.244	
PCR				—
Trimester 1	13 (0–17)	11 (5.9–16)	0.094	
Trimester 2	12.5 (8–21)	16 (9–22.9)	0.717	
Trimester 3	21 (15–60)	23 (16–34.5)	0.145	
Total gestational weight gain (kg)	14.4 (8–18.3)	12.6 (7.5–17.6)	0.860	—

Data represent median (quartile 1 to quartile 3) or *n* (%) as applicable. **P* value correcting for type of diabetes with logistic regression. †Only women with parity ≥1 were included in this analysis (*n* = 108).

with no preeclampsia and FIR (group 2), then women with preeclampsia and no FIR (group 3), and women with preeclampsia and FIR (group 4) would have the greatest placental dysfunction. There was a significant trend for increasing sFlt-1/PlGF ratio from groups 1 to 4, respectively (Fig. 2).

There was no difference in TNF-α, hPL, and progesterone levels between women with and without FIR at any of the four gestational windows examined longitudinally during pregnancy, nor was there a greater reduction in hormone levels over the third trimester among women with FIR ≥15% (Supplementary Table 3).

CONCLUSIONS

FIR are considered a marker of placental insufficiency, although to date, few studies have supported this theory. In this study, consistent with our previously published retrospective analysis (4), we found that FIR of ≥15% increased the risk of having an adverse outcome associated with placental dysfunction by more than fourfold, driven primarily by an increased risk of preeclampsia of more than sixfold.

Furthermore, babies of women with FIR ≥15% were more likely to be delivered early by emergency caesarean section and admitted to the NICU. Although no specific difference in individual neonatal outcomes could be detected, it is likely the higher need for NICU intervention reflects preterm delivery, preeclampsia, and general poorer condition of babies in the FIR group, suggesting poorer placental health overall.

Although the underlying origin of placental dysfunction has not been clearly identified, it is accepted that it causes a

Table 2—Maternal and neonatal outcomes among women with and without FIR ≥15%

Clinical outcome	FIR ≥15% (case subjects, *n* = 32)	FIR <15% (control subjects, *n* = 126)	*P* value
Neonatal outcomes			
Any adverse neonatal outcome†	25 (78.1)	90 (71.4)	0.447
Gestational age of delivery (weeks)	37.4 (35.7–38.1)	38.1 (37.6–38.6)	0.022*
Preterm delivery			
<30 weeks	0 (0)	0 (0)	—
<37 weeks	14 (43.8)	23 (18.3)	0.002*
Stillbirth	0 (0)	1 (0.8)	1.00
Admission to NICU‡	12 (37.5)	26 (20.8)	0.049*
Birth weight	2,992.5 (2,830–3,635)	3,465 (3,085–3,865)	0.043*
Birth weight centile§	47.73 (20.6–94.77)	68.63 (42.13–93.46)	0.308
Large for gestational age (≥90th centile)§	9 (28.1)	37 (29.4)	0.890
Small for gestational age			
≤5th centile§	1 (3.1)	8 (6.3)	0.688
≤10th centile§	4 (12.5)	9 (7.1)	0.301
Jaundice‡	12 (37.5)	38 (30.4)	0.442
Hypoglycemia‡	18 (56.2)	66 (52.8)	0.727
Birth trauma	4 (12.5)	11 (11.2)	0.764
Congenital malformation (major and minor)‡	6 (18.8)	13 (10.4)	0.225
Respiratory distress‡	11 (34.4)	37 (29.6)	0.601
Maternal outcomes			
Primary composite outcome	14 (43.8)	19 (15.1)	<0.001*
Preeclampsia	14 (43.8)‖	13 (10.3)‖	<0.001*
Gestational hypertension	1 (3.1)	7 (5.6)	1.00
Placental abruption	0 (0)	0 (0)	—
Induction of labor	27 (84.4)	98 (77.8)	0.412
Mode of delivery			0.051
Vaginal	6 (18.8)	39 (31)	0.172
Instrumental	1 (3.1)	4 (3.2)	1.00
Elective caesarean	7 (21.9)	45 (35.7)	0.137
Emergency caesarean	18 (56.2)	38 (30.2)	0.006*
Antenatal steroids	9 (28.1)	9 (7.1)	0.003

Data represent median (quartile 1 to quartile 3) or *n* (%) as applicable. **P* value remained significant after adjustment for type of diabetes (1 or 2) by logistic regression. †Composite of stillbirth, admission to NICU, hypoglycemia, jaundice, birth trauma, congenital malformation, respiratory distress, and neonatal death. ‡Stillbirth excluded. §Customized birth weight centile calculated using the gestation network Australian centile calculator (correcting for gestational age, maternal height and weight at booking visit, ethnicity, parity, and fetal sex [40]). ‖Three women in the case group and one woman in the control group were diagnosed with preeclampsia, in the absence of proteinuria, through the criteria of hypertension and organ dysfunction as per the International Society for the Study of Hypertension in Pregnancy (2014) definition (16).

spectrum of obstetric disorders, including placental abruption, preeclampsia, and ultimately intrauterine growth restriction if maternal–fetal exchange is impaired (14,24,25). We hypothesize that FIR are another such clinical expression of this spectrum of disease, manifesting uniquely among women with diabetes as a marker of decreasing insulin resistance in the setting of a failing placenta. The strength of the association demonstrated with preeclampsia supports this hypothesis and suggests a shared pathology contributing to both outcomes.

Our findings are in contrast to three previous studies on FIR, which did not find an association with preeclampsia or other adverse outcomes. A potential reason for the disparate findings were the different definitions used. Achong et al. (7) reported weight-adjusted basal insulin requirements from 30 weeks to delivery in a retrospective study of 54 women with type 1 diabetes, whereas the 5-year retrospective study by McManus and Ryan (5) examined weight-adjusted insulin requirements in 51 pregnancies progressing beyond 36 weeks only. Both studies used a case definition of FIR ≥15% in keeping with our study. The third study examining 236 women with type 1 diabetes by Steel et al. (6) calculated FIR using the PTID and TTID, similar to us, but defined the case group as having FIR ≥30%.

As yet, there is no clear definition of the level and timing at which falling requirements become clinically significant. Further, the possibility of FIR being a manifestation of reduced carbohydrate intake rather than true changes in endogenous needs has been the rationale for using basal- and weight-based changes in insulin dose in previous studies. However, even after excluding women with reduction in carbohydrate intake and applying weight-adjusted basal and total insulin dose, our association with the primary outcome was maintained. It is likely that previous negative findings are because of inadequate power for differences in outcomes, and indeed, this study is the first to apply a prespecified calculated sample size.

Importantly, we found that FIR either preceded or accompanied the onset of preeclampsia in all but three cases, further supporting our hypothesis that FIR may be a milder clinical or early manifestation of placental dysfunction. The temporal association of FIR and outcomes was examined in the study by Steel et al. (6), who also reported FIR preceded the onset of hypertension and preeclampsia in the two case subjects delivered for this indication. Our findings thus open the possibility of using FIR

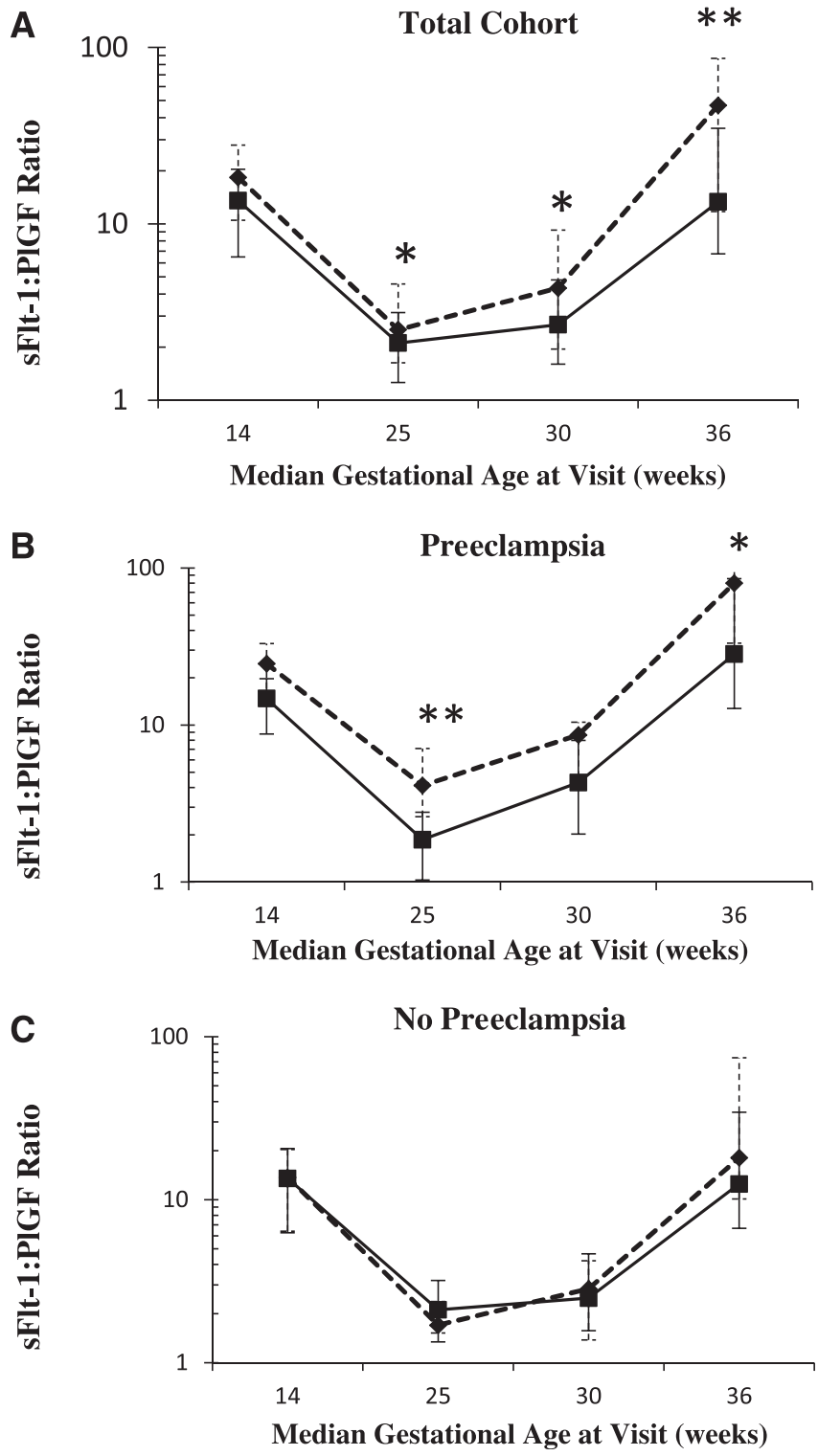

Figure 1—Longitudinal differences in sFlt-1/PlGF ratio between women with FIR ≥15% (case subjects, dashed lines) and FIR <15% (control subjects, solid lines) during pregnancy in the total cohort (*A*), subgroup with preeclampsia (*B*), and subgroup without preeclampsia (*C*). Markers represent median biomarker levels, and error bars represent interquartile range. *y*-axis is on $\log^{10}$ scale. *$P < 0.05$; **$P <$ 0.01 for comparisons at each gestational window conducted on log-transformed data using linear mixed-effects models adjusted for type of diabetes, maternal age, BMI, ethnicity, nulliparity, and smoking status.

as a predictor of preeclampsia or a clinical red flag to alert clinicians to increase monitoring for this condition.

Historically, the mechanism of FIR has been attributed to reduction in hormones mediating insulin resistance; however, there is little evidence for the association between maternal biomarkers and reductions in insulin dose. This is the first prospective study to demonstrate altered levels of placental hormones involved in angiogenesis, with a higher ratio of sFlt-1/PlGF among women with FIR from 25 weeks onwards. PlGF is a vascular endothelial growth factor homolog, which is released in increasing amounts by the placenta during pregnancy and plays a key role in maintaining vascular homeostasis during placental development (26). In contrast, sFlt-1 acts as a negative regulator of angiogenesis and has been found in increased amounts in conditions associated with placental dysfunction including preeclampsia (27,28).

Very few studies have examined pro- and antiangiogenic factors among women with diabetes longitudinally during pregnancy. Yu et al. (29) demonstrated that women with type 1 diabetes and preeclampsia had increased sFlt-1, decreased PlGF, and increased sFlt-1/PlGF ratio compared with non-preeclamptic women. Another study by Cohen et al. (30) including women with both type 1 and type 2 diabetes showed similar results. Although neither of these studies documented changes in insulin requirements, their findings mirror those we observed between women with and without FIR.

As preeclampsia is well known to be associated with derangement of angiogenic factors, a subgroup analysis was conducted to evaluate whether the higher sFlt-1/PlGF ratio among women with FIR was because of the increased incidence of preeclampsia in this group. Despite small numbers, the differences in sFlt-1/PlGF ratio between women with and without FIR were maintained in the subgroup that developed preeclampsia signifying that differences in angiogenic factors cannot be explained by preeclampsia alone. Studies have supported the imbalance of antiangiogenic to proangiogenic factors, released by the placenta as a plausible mechanism for the endothelial dysfunction seen in preeclampsia (31). More recently, extracorporeal removal of sFlt-1 and infusion of PlGF has led to improvement in preeclampsia, further supporting the causal relationship (32,33). The wide clinical spectrum of severity in preeclampsia has been well described and is proportional to maternal levels of pro- and antiangiogenic factors from previous studies

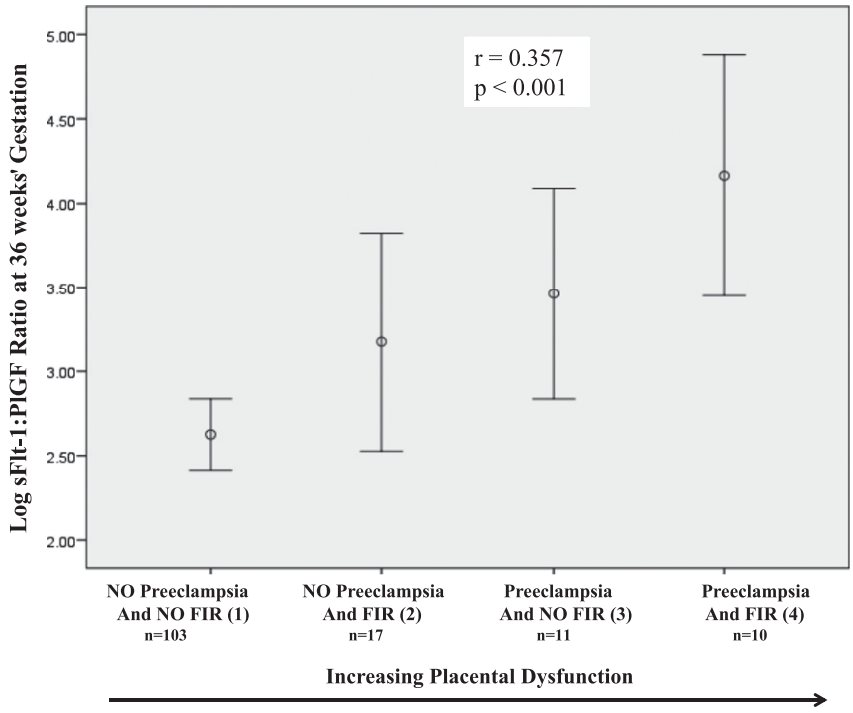

Figure 2—Trend of logarithmically transformed angiogenic biomarkers across four subgroups of increasing placental dysfunction. Markers represent mean, and error bars represent 95% CI for logarithmically transformed sFlt-1/PlGF ratio. FIR ≥15%. *r* is Spearman rank correlation coefficient. *P* value is for Spearman rank correlation across four subgroups with no preeclampsia and no FIR considered the group with least placental dysfunction and preeclampsia and FIR considered the group with greatest placental dysfunction. Only those with blood available at 36 weeks included in the analysis.

(31,34,35). In keeping with this, our findings suggest that women with preeclampsia who manifest FIR may lie on the spectrum of more severe placental dysfunction, as demonstrated by higher sFlt-1/PlGF ratio.

In contrast, we did not find any difference in hPL, progesterone, and TNF-α between the groups. Limited studies have examined changes in placental hormones and insulin requirements and have not demonstrated a correlation between hormone levels and insulin dose (36,37). In keeping with this, we were unable to show any significant differences between women with and without FIR despite examining absolute and relative changes in hormone levels.

In contrast, we detected a significant association between higher serum creatinine level per trimester as well as rising creatinine in third trimester with FIR during pregnancy. Although the absolute difference is small, and the clinical implication of these findings is unclear, the association raises the possibility that there is a renal contribution to the mechanism for FIR, in addition to placental dysfunction. Outside of pregnancy, a declining GFR has been associated with a 30–50% reduction in insulin requirements (38,39); therefore, it is plausible that reductions in the renal clearance of insulin results in FIR in some women during pregnancy, although the degree to which this may have affected our results is unclear. It is possible that vascular dysfunction is a unifying mechanism for FIR manifesting through renal and placental pathways and is consistent with the strong association with preeclampsia and disruption in angiogenic markers in our study. However, the similar rates of albuminuria and proteinuria between groups does not correspond with this hypothesis, and we recognize that using serum creatinine as a surrogate for renal function in pregnancy is far from ideal. Further studies are needed to validate these preliminary findings.

We acknowledge our study has some limitations. Aspirin was not universally commenced in all women, and a small but significantly greater number were treated with aspirin in the FIR ≥15% group. Aspirin is known to reduce the risk of preeclampsia and, as a result, could introduce a negative bias; however, despite this, women with FIR ≥15% were still sixfold more likely to develop preeclampsia, further reinforcing the strength of the association. Further, the titration of insulin requirements was subjective and may have varied by clinician. Ideally, an objective measure of insulin sensitivity would be favored; however, techniques such as insulin clamps and HOMA are less practical. Finally, the analysis of biomarkers at the prespecified gestations may have missed abrupt or sudden changes in hormone levels, and we were unable to measure the entire spectrum of insulin resistance placental hormones. Thus, it is important for our findings to be validated in larger adequately powered studies before discounting the contribution of placental hormones to changes in insulin requirements.

Nevertheless, this is the first study specifically designed to answer this important clinical question using pilot data to ensure adequate power in detecting differences in outcomes. Examination of maternal biomarkers, particularly those associated with placental dysfunction, is a novel method of exploring the mechanism of FIR, which has not been reported before.

The demonstrated changes in pro- and antiangiogenic markers, together with the association with preeclampsia, have important clinical implications, as they suggest FIR are a manifestation of placental dysfunction lying on a similar spectrum to the other diseases. It is likely that FIR are an early indicator of placental dysfunction in women with diabetes, with only a proportion of women subsequently progressing to the more serious clinical manifestations that have typically been associated with disrupted angiogenic markers. Importantly, our data are also reassuring in that over half of the women with FIR did not develop adverse outcomes or have babies requiring NICU admission. Thus, although these findings support the clinical recommendation that all women manifesting FIR (≥15%) should have increased surveillance and investigation for adverse obstetric outcomes, its presence does not necessarily indicate urgent, immediate delivery.

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