Changes in circulating kisspeptin levels during each trimester in women with antenatal complications

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Abstract

Context: Antenatal complications such as hypertensive disorders of pregnancy (HDP), fetal growth restriction (FGR), gestational diabetes (GDM), and preterm birth (PTB) are associated with placental dysfunction. Kisspeptin has emerged as a putative marker of placental function, but limited data exist describing circulating kisspeptin levels across all three trimesters in women with antenatal complications.

Objective: To assess whether kisspeptin levels are altered in women with antenatal complications.

Design: Women with antenatal complications (n=105) and those with uncomplicated pregnancies (n=265) underwent serial ultrasound scans and blood-sampling at least once during each trimester (March 2014 to March 2017).

Setting: Early Pregnancy Assessment Unit at Hammersmith Hospital, UK.

Participants: Women with antenatal complications: HDP (n=32), FGR (n=17), GDM (n=35) and PTB (n=11), and 10 women with multiple complications, provided 373 blood samples, and a further 265 controls provided 930 samples.

Main outcome: Differences in circulating kisspeptin levels.

Results: Third trimester kisspeptin levels were higher than controls in HDP but lower in FGR. The odds of HDP adjusted for gestational age, maternal age, ethnicity, BMI, smoking and parity were increased by 30% (95%CI 16.47%; p<0.0001), and of FGR were reduced by 28% (95%CI 4.46%; p=0.025), for every 1 nmol/L increase in plasma kisspeptin. Multiple of gestation-specific median values of kisspeptin were higher in pregnancies affected by PTB (p=0.014), and lower in those affected by GDM (p=0.020), but not significantly on multivariable analysis.

Conclusion: We delineate changes in circulating kisspeptin levels at different trimesters and evaluate the potential of kisspeptin as a biomarker for antenatal complications.

Key Terms: fetal growth restriction (FGR), intra-uterine growth restriction (IUGR), hypertensive diseases of pregnancy (HDP), gestational diabetes (GDM), preterm birth (PTB), kisspeptin
Précis

Circulating kisspeptin levels were higher in pregnancies affected by hypertensive disease of pregnancy (HDP), but lower in those affected by fetal growth restriction (FGR). In contrast to the existing literature, we find that HDP is associated with increased circulating kisspeptin levels, in keeping with previously reported increased placental kisspeptin expression.
Introduction

Antenatal complications are common: hypertensive disorders of pregnancy (HDP) affect \( \sim 5\% \) (1), fetal growth restriction (FGR) \( \sim 4\% \) (2), gestational diabetes (GDM) \( \sim 15\% \) (3), and preterm birth (PTB) \( \sim 11\% \) (4) of pregnancies. Such conditions present serious risks to maternal and fetal wellbeing (5). Despite intensive study, the precise mechanisms underlying many antenatal complications remain subject to conjecture (6). However, a shared abnormality in the temporo-spatial regulation of trophoblast invasion during the first trimester, resulting in defective spiral artery transformation has been proposed to underlie HDP, FGR, GDM and PTB (7,8).

The peptide kisspeptin, better known for its stimulatory role in hypothalamic GnRH release (7,8), has emerged as a putative regulator of trophoblast invasion (11,12). Both kisspeptin and its receptor (KISS1R) are highly expressed in placental tissues; kisspeptin is expressed by syncytiotrophoblasts and its receptor by both cytotrophoblasts and syncytiotrophoblasts (13). Kisspeptin constrains trophoblast migration \textit{in vitro}, and decreases expression of matrix metalloproteinases (MMPs) II and IX, which are essential for breakdown of the extracellular matrix during the migratory process (13). Thus, kisspeptin appears to be a key paracrine inhibitor of excessive trophoblast migration, and as such, safeguards physiological placentation.

Syncytiotrophoblasts secrete kisspeptin, along with other placental peptides such as \( \beta \)-human chorionic gonadotropin (\( \beta hCG \)), into the circulation throughout pregnancy (14). However, whereas \( \beta hCG \) levels peak during the first trimester before subsequently declining (15), kisspeptin levels continue to rise with increasing gestation (16). Hitherto, kisspeptin has garnered interest as a biomarker for placental function; low circulating kisspeptin levels have been reported in patients with miscarriage, HDP (17–22), FGR (22–24), and GDM (17–25). Such abnormalities in kisspeptin levels in complicated pregnancies may be contingent on gestation, thus quantification of circulating kisspeptin levels in all three trimesters could provide insight into placental dysfunction in these conditions at different stages of pregnancy.
To date, there are limited data reporting alterations in kisspeptin levels during all three trimesters of complicated pregnancies. Therefore, we aimed to assess whether circulating kisspeptin levels differ in pregnancies complicated by HDP, FGR, GDM and PTB as compared to healthy control pregnancies, and to identify the specific trimesters at which such perturbations become apparent.

**Methods**

**Study Approval**

This study was approved by the National Research Ethics Service (NRES) Riverside Committee London (14/LO/0199) and the North East, Newcastle and North Tyneside Two Research Ethics Committee (17/NE/0121). All participants provided written informed consent in accordance with the declaration of Helsinki.

**Setting and design**

This was a nested case-control study of women with antenatal complications and healthy singleton control pregnancies recruited between March 2014 to March 2017. Women affected by antenatal complications (n=105) and those with uncomplicated pregnancies (n=265) underwent serial ultrasound scans and blood-sampling at least once during each trimester. Women completed a detailed questionnaire regarding demographic details, past medical, gynaecological, and obstetric history. Following recruitment, participants were invited to attend every two weeks during the first trimester for an ultrasound scan and blood test (plasma kisspeptin level). Participants were also assessed at the time of their anomaly scan (18 to 22 weeks of gestation) during the second trimester and once during the third trimester (31 to 36 weeks of gestation). Blood samples for kisspeptin were stored at -80°C until day of kisspeptin assay. Assays for plasma kisspeptin levels were conducted at the end of the study after completion of sample collection had taken place.
Study Participants

Participants were recruited via open advertisements (using posters) in local GP surgeries and hospitals. Pregnant women aged between 18-49 years old with an intrauterine pregnancy on ultrasound in the first trimester (<14 weeks) were invited to participate. Women with miscarriage, pregnancy of unknown location, or ectopic pregnancy were excluded.

A total of 1242 pregnant women were screened and 1045 were recruited to participate in the study. The commonest reasons for withdrawal/exclusion were booking antenatal care at a different hospital, personal choice, and being unable to attend future visits. Of the 1045 recruited patients, 122 were excluded from analysis due to pregnancy termination (n=11), loss to follow-up (n=11), withdrawal (n=5), or miscarriage (n=95). Of the remaining 923 women, 105 women had complicated pregnancies as detailed below and all samples from these women were included in the study. It is important to measure kisspeptin levels in all samples using a radioimmunoassay conducted on the same day to ensure consistent assay characteristics. Due to the large number of samples, it was not practically possible to assay samples from all 818 control women who were not affected by pregnancy complications, and therefore we assayed samples from 265 women, who had spontaneous singleton conception, did not suffer any pregnancy complications, nor any symptoms of possible pregnancy loss in early pregnancy (eg vaginal bleeding), to act as the control group (26).

Diagnostic criteria for pregnancy complications:

Hypertensive disorders of pregnancy (HDP): PIH was defined as raised blood pressure ≥140/90 mmHg on two occasions four hours apart at >20 weeks of gestation in a woman with previously normal blood pressure without proteinuria, growth restriction, or abnormal blood tests (27). Pre-eclampsia (PET) was defined as PIH with proteinuria (urine protein creatinine ratio >0.3 mg/dL or 24 hour urine collection >3 g/24hrs) (27,28). In addition, a diagnosis of PET was applied if PIH occurred...
with either fetal growth restriction or deranged blood tests (thrombocytopenia <100x10^9/L, serum creatinine concentration >1.1 mg/dL, or a doubling of this in the absence of renal disease, or elevated liver transaminases to twice normal concentration), or if they developed eclampsia (27,30). Severe PET was diagnosed in the presence of one of BP ≥160/110 mmHg, visual disturbance, chest pain, shortness of breath, flash pulmonary oedema, seizures or neonatal distress (27,30). Analysis of subgroups of HDP in Figure 2 are presented only as exploratory analyses and should be interpreted with caution as they may be subject to type 2 error.

**Gestational diabetes (GDM):** GDM was diagnosed by an oral glucose tolerance test (OGTT) performed after 20 weeks of gestation. A fasting plasma glucose ≥5.6 mmol/L or a glucose level ≥7.8 mmol/L (2 hours following 75g of glucose) confirmed GDM (29).

**Preterm Birth:** Preterm birth (PTB) was diagnosed in women with delivery after 24 weeks and before 37 completed weeks of gestation (as dated by a routine dating scan), which included both iatrogenic and spontaneous preterm birth (31).

**Fetal Growth Restriction:** Fetal growth restriction (FGR) was used to describe either intrauterine growth restriction (IUGR) or small for gestation age (SGA). IUGR was an ultrasound-based antenatal diagnosis where the estimated fetal weight was <10th centile for gestational age with abnormal umbilical artery doppler results (pulsatility index >95th percentile with or without reverse or absent end diastolic flow) (32,33). SGA was defined in accordance with the World Health Organization (WHO) criteria and WHO centiles, as delivery weight less than the 10th percentile for gestational age, where the final gestational age was estimated using the dating scan (performed at 11-14 weeks gestation) as a reference (34).
Assays

Plasma kisspeptin levels were measured using an in-house radioimmunoassay at Imperial College London (35). The assay has a 10.2% inter-assay and 8.2% intra-assay coefficient of variation. The antibody exhibits <0.01% cross-reactivity with similarly structured RF-amide proteins, such as RF-amide-related peptides (RFRP): RFRP-1, RFRP-2 and RFRP-3. The assay measures all kisspeptin splicing variants, although kisspeptin-54 has been reported to be the dominant circulating form in human pregnancy (16).

Gestational age correction

The gestational age at the time of sample collection was determined to correct for changes in plasma kisspeptin levels with gestation. During the first trimester, pregnancies were dated using crown-rump length (CRL) on ultrasound scan (36). Multiple of median (MoM) values were derived from raw kisspeptin levels in order to correct for gestational age at the time of measurement; first, median hormone levels in healthy controls were determined at each week of gestation, and then each raw hormone level was expressed as a proportion of the median control value for the corresponding gestation. Thus, a MoM of 0.5 denotes a value that is half that of the corresponding median value in healthy controls for that gestation.

Statistical Analysis

Analysis was performed using Prism v8.4.3 (GraphPad) and Stata v13.0 (StataCorp) software packages. Normality was determined using the D’Agostino Pearson test. Parametrically distributed data are summarised as mean ± standard deviation (±SD) and non-parametrically distributed data are summarised as median (interquartile range; IQR). Comparisons between two groups were made using unpaired t-tests for parametrically distributed data or by Mann-Whitney U test for non-parametrically distributed data. Comparisons between multiple groups were made using one-way ANOVA for
parametrically distributed data and Kruskal-Wallis for non-parametrically distributed data. Categorical variables are presented in terms of frequencies and percentages. Proportions of categorical variables were compared between groups using chi-squared tests. Linear regression was used to assess associations between baseline characteristics and plasma kisspeptin levels. Logistic regression was used to assess associations between plasma kisspeptin levels and antenatal complication diagnoses. Multivariable logistic regression models were used to account for gestational age at the time of sampling and adjust for maternal age, ethnicity, BMI, smoking status and parity. A P-value of <0.05 was considered statistically significant.

Results

Study Population

Amongst 105 women with complicated pregnancies there was a total of 117 diagnoses of pregnancy complications (24 PET, 14 PIH, 24 FGR, 41 GDM and 14 PTB). This larger number of diagnoses was because 10 of these 105 women with pregnancy complications had more than one pregnancy complication diagnosed (e.g. both PET and FGR) and these ten women were excluded from analyses of the specific pregnancy complication (e.g. PET alone). Thus, 95 women were included in analyses of individual pregnancy complications: PET (n=20), PIH (n=12), FGR (n=17), GDM (n=35) and PTB (n=11). In total, 370 patients provided 1273 plasma kisspeptin samples (26). No significant differences in baseline characteristics of maternal age, BMI, smoking status, gravidity or parity were observed between control and antenatal complication group (Table 1).
Plasma kisspeptin levels in healthy control pregnancies

Multivariable linear regression was used to determine baseline factors that contributed to the variability in plasma kisspeptin levels in healthy pregnancies. Gestational age was positively associated with plasma kisspeptin levels, whereas maternal BMI and Afro-Caribbean ethnicity were negatively associated with plasma kisspeptin levels (26).

Plasma kisspeptin levels in patients with hypertensive disorders of pregnancy (HDP)

MoM kisspeptin levels were higher in HDP-affected pregnancies than in healthy control pregnancies (p=0.014) (Figure 1A). The rate of rise in plasma kisspeptin with gestation throughout pregnancy was higher in pregnancies affected by HDP than in control pregnancies (p<0.0001) (26).

We compared unadjusted kisspeptin levels stratified by trimester at the time of measurement; unadjusted plasma kisspeptin levels were higher in HDP pregnancies in the third trimester (28-40 weeks) than in control pregnancies (p=0.0097), whereas levels in earlier gestations did not differ (Figure 1B). The odds of HDP, adjusted for maternal age, ethnicity, BMI, smoking status and parity, were increased by 30% (95% CI 16-47%) for every 1 nmol/L increase in plasma kisspeptin (p<0.0001) (Table 2).

Interestingly, the rate of increase in kisspeptin levels with gestation was higher in women with late-onset PET (i.e. onset of PET at 34 weeks of gestation or later) compared to controls and women with early-onset PET (p=0.0040) (Figure 2 A). Late first trimester kisspeptin levels (9-13 weeks) in women with late-onset PET were higher than in controls, whereas early onset PET levels were lower than in controls (p=0.0029) (Figure 2B). In an exploratory analysis, women with PET and IUGR had lower kisspeptin levels than those who had PET alone, but higher levels than women with IUGR alone (p=0.0004) (Figure 2C). Moreover, the rate of increase in kisspeptin levels with gestation did not significantly differ by the severity of PET (Figure 2D).
Plasma kisspeptin levels in patients with fetal growth restriction (FGR)

MoM kisspeptin was lower in FGR-affected pregnancies than in healthy pregnancies (p=0.0047) (Figure 1C). The rate of increase in plasma kisspeptin with gestation throughout pregnancy was lower in pregnancies affected by FGR than in control pregnancies (p=0.0040) (26). Plasma kisspeptin levels in the late first trimester (9-13 weeks) and third trimester (28-40 weeks) were lower in FGR than in control pregnancies (p=0.040 and p=0.025, respectively) (Figure 1D). The odds of FGR adjusted for maternal age, ethnicity, BMI, smoking status and parity were decreased by 28% (95%CI 4-46%) for every 1 nmol/L increase in plasma kisspeptin (p=0.025) (Table 3). There was no significant association between birthweight and plasma kisspeptin in women with healthy pregnancies (data not presented).

Plasma kisspeptin levels in patients with gestational diabetes mellitus (GDM)

Overall, MoM kisspeptin was lower in GDM-affected pregnancies than in healthy control pregnancies (p=0.020) (Figure 3A). However, there was no difference in the rate of increase in plasma kisspeptin with gestation throughout pregnancy between GDM and healthy control pregnancies (p=0.90) (26). When unadjusted kisspeptin levels were analysed by each trimester, there were no differences between plasma kisspeptin levels between GDM and healthy control pregnancies in any trimester (Figure 3B). Plasma kisspeptin levels were not significantly altered in GDM pregnancies, both in univariable analysis and after adjustment for maternal age, ethnicity, BMI, smoking status and parity (26).
Plasma kisspeptin levels in women with preterm birth (PTB)

Of the eleven women with PTB, only one had an emergency caesarean section for fetal distress, whereas the remainder delivered by spontaneous vaginal delivery. MoM kisspeptin at all gestations was higher in PTB-affected pregnancies than in control pregnancies (p=0.014) (Figure 3C). However, there was no difference in the rate of increase in plasma kisspeptin levels with gestation between PTB and control pregnancies (p=0.39) (26). When unadjusted kisspeptin levels were analysed per trimester, plasma kisspeptin levels in the late first trimester (9-13 weeks) were higher in PTB than in control pregnancies (p=0.026) (Figure 3D). The odds of PTB adjusted for maternal age, ethnicity, BMI, smoking status and parity were increased by 20% (95%CI 1.14-2%) for every 1 nmol/L increase in plasma kisspeptin (p=0.036) (26).

Discussion

In the present study, we investigated whether circulating kisspeptin levels are altered in women affected by antenatal complications and report the specific trimester at which changes in plasma kisspeptin levels occur. We found that unadjusted plasma kisspeptin levels were principally abnormal in the third trimester of pregnancies affected by HDP and FGR. By comparison, there were no significant changes in βhCG levels at any gestation in HDP or FGR pregnancies. Multiple of gestation-specific median values of kisspeptin were higher in pregnancies affected by PTB (p=0.014), and lower in those affected by GDM (p=0.020). These data are consistent with the suggestion that changes in plasma kisspeptin levels could reflect placental dysfunction manifested clinically as pregnancy complications.

Plasma kisspeptin levels were increased during the third trimester in pregnancies affected by HDP. Several studies have reported increased placental KISS1 expression in the third trimester of pregnancies affected by HDP in comparison to uncomplicated pregnancies (21,37–40). However,
most studies have reported lower circulating kisspeptin levels in women with PET than in normotensive controls during the first (1.554 vs 1.995 nmol/L) (20), second (4.46 vs 10.3 nmol/L) (17), (1109 vs 1188 pg/ml) (22), and third trimesters (0.58 vs 1.66 ng/ml) (21). The constraint of plasma kisspeptin alterations to occurring only during later trimesters in our study is not consistent with the hypothesis that these complications are routed in abnormalities of placentation, which occurs during the first trimester. However, it is noteworthy that circulating kisspeptin levels do not necessarily proportionately reflect placental KISS1 expression; i.e. whilst circulating kisspeptin levels rise throughout pregnancy (16), placental KISS1 expression peaks in the first trimester and subsequently declines (13,41). In keeping with this, it has been noted that circulating kisspeptin levels increase in multiple pregnancy in proportion to the number of chorions (15). Consequently, circulating kisspeptin levels are likely to reflect changes in both KISS1 expression and placental mass.

Several large studies have reported subset-specific associations between PET and placental mass (42–44). Early-onset (<34 weeks) PET, arising due to defective trophoblast invasion, incomplete spiral artery remodelling and subsequent placental insufficiency, is associated with low placental mass (42,43). Late-onset (≥ 34 weeks) PET is believed to arise from hypoxic stress in term placentae as they outgrow their circulation, restricting intervillous perfusion, and is associated with increased placental mass (44,45). However, Qiao et al. reported that third-trimester placental KISS1 overexpression was limited to early-onset PET (40). Furthermore, low placental mass is associated with HDP severity (46), i.e. placental mass decreases from PIH, through late-onset PET, to early-onset PET. In the present study, late onset PET was associated with an increased trajectory of plasma kisspeptin rise with gestation than in early onset PET or controls, predominantly driven by higher levels in the second trimester.

Previous reports have identified a negative association between circulating kisspeptin levels and severity of HDP; Cetokovic observed lower kisspeptin levels in PET than in controls, but not in PIH (17). Likewise, Ziyaraa reported lower kisspeptin levels in severe PET (1.59 ± 0.26 ng/ml), but not in mild PET (2.18 ± 0.76 ng/ml), in comparison to normotensive controls (2.30 ± 0.51 ng/ml) during the second trimester (although not during the third trimester) (18). However, Adali reported a trend
towards even lower third trimester kisspeptin levels in severe PET (1.17 ± 0.24 ng/ml) than in mild PET (2.61 ± 0.40 ng/ml), as compared with controls (9.69 ± 1.35 ng/ml) (19). Thus, an increasing severity of PET may be associated with reductions in circulating kisspeptin levels. However, we did not find significant alterations in plasma kisspeptin with severity of PET in the present study. Furthermore, we were careful to distinguish participants with more than one pregnancy complication that could have disparate effects on plasma kisspeptin levels; which, to our knowledge, was only done in one previous study (21). For example, we observed that PET complicated by IUGR was associated with an attenuation of the increase in kisspeptin levels observed in women with PET alone.

Another potential confounder relevant to HDP is body mass index (BMI); we observed a negative association between BMI and kisspeptin levels in healthy control pregnancies. Notably, obesity is also a known risk factor for PET (47). Logie et al. reported lower kisspeptin levels during the second trimester in obese normotensive women than in lean normotensive controls, and even lower levels in obese women who subsequently developed PET (48). Such complexities in the categorisation, severity and onset of PET, and correction for possible confounders such as BMI and gestational age, could explain differences between kisspeptin levels observed in the present study and in other reports.

In FGR in the absence of PET, we observed lower third-trimester plasma kisspeptin levels than in controls. This is consistent with the existing literature; three studies have previously reported reduced circulating kisspeptin levels in FGR compared to controls, during the first (1376±1317 vs 2035±1260 pmol/L) (23), second (1164 vs 1188 pg/mL) (22) and third (1.6±0.3 vs 2.9±0.6 ng/mL) trimesters (24). FGR is hypothesised to arise from abnormal trophoblast invasion and spiral artery conversion that limits oxygen supply to the placenta (49,50). The resulting ischaemic injury generates reactive oxygen species (51), leading to apoptosis and restriction of placental and fetal growth (52–54). Thus, low circulating kisspeptin levels could reflect low placental mass (55,56) in pregnancies affected by FGR (22,23,57).

While we observed no significant differences in gestation-specific kisspeptin levels in pregnancies affected by GDM, however the gestation adjusted kisspeptin levels from all trimesters were lower.
than in control pregnancies. Previous data thus far are inconclusive, with two studies reporting lower second and third-trimester plasma kisspeptin levels in women with GDM than in controls (17,25), whilst another reported no differences (58). Nonetheless, kisspeptin has been proposed to play a significant role in beta-cell adaptation to the pregnant state (25). Bowe et al. reported impaired insulin response to glucose in pregnant mice following pharmacological kisspeptin blockade (25), and a glucose intolerant phenotype in beta-cell Kiss1R knockout pregnant mice (25).

Recently, a putative role for kisspeptin in the initiation of labor has emerged (69). Intracerebroventricular administration of kisspeptin to late trimester pregnant rats has been demonstrated to increase firing of oxytocin neurons (69). RT-PCR studies have also identified higher placental KISS1 mRNA expression in preterm placentae than in those of term Caesarean sections, which, in turn, demonstrated higher levels of KISS1 mRNA expression than placentas delivered vaginally at term (70). However, no alterations in circulating kisspeptin have been observed during the third trimester in those studies (70), in keeping with the results of the present study.

Kisspeptin exists in several physiological isoforms, produced through splicing of a 154-amino acid pre-propeptide encoded by the KISS1 gene (16). Whilst only kisspeptin -13, -14 and -54 isoforms have been isolated from placental trophoblasts (71), matrix assisted laser desorption / ionisation – time of flight analysis (MALDI-TOF) (a form of mass spectrometry) of trophoblast culture medium has yielded kisspeptin-10, -13, -14 and -54 (13). The major circulating kisspeptin isoform in pregnancy is believed to be kisspeptin-54 (16). However, only kisspeptin-10 has been shown to increase intracellular Ca^{2+} levels (via Kiss1R binding) in trophoblast cells expressing physiological levels of Kiss1R in vitro (13,72). It is possible that kisspeptin-10 is spliced from kisspeptin-54 to act at a cellular level, before reaching the circulation (9). Thus, further data assaying specific kisspeptin isoforms would be of interest (73).

In conclusion, this is the first study to directly compare circulating kisspeptin levels between healthy pregnancies and those complicated by HDP, FGR, GDM and PTB throughout all three trimesters. We identified higher circulating levels of kisspeptin in HDP and lower kisspeptin levels in FGR-affected
pregnancies than in uncomplicated control pregnancies. Our data on circulating kisspeptin levels in HDP are in keeping with published data reporting increased expression of kisspeptin in women affected by HDP (21,37–40) and with data reporting reduced circulating kisspeptin levels in pregnancies complicated by FGR. Although differences were observed in plasma kisspeptin levels of women with pregnancy complications compared to healthy controls, there was sufficient heterogeneity in the perturbations, to limit clinical application of plasma kisspeptin for diagnosis of these complications. However, these data provide insight into kisspeptin’s potential to reflect placental dysfunction associated with these placental complications throughout pregnancy and highlight the potential of circulating kisspeptin to reflect the risk of pregnancy complications.
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Author Contribution Statement

AA, MAM, MP, ED, BP, PCE, RN, CI-E, SAC, EGM, TH, EP, LY, PB, ANC, TWK, CK, HF, TB, and WSD designed the study, analysed the data, prepared the manuscript, and designed the figures and tables. AA, MAM, MP, PCE, CK, HF conducted data collection. AA, MAM, MP, ED, BP, PCE, RN, CK, TWK performed statistical analysis. TB and WD were the project supervisors, reviewed and edited the manuscript and are the guarantors of this research project. All authors have made a substantial, direct and intellectual contribution to the work and approved the manuscript prior to its submission.
Data Availability

All data generated or analysed during this study are included in this published article or in the data repositories listed in References.
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Figure Legends

Figure 1: Kisspeptin levels throughout pregnancy in women with hypertensive diseases of pregnancy (HDP) compared to control pregnancies and in women with fetal growth restriction (FGR) compared to control pregnancies

1A. Scatterplot of medians (IQR) of multiples of gestation specific median kisspeptin levels in healthy controls (red) (n=265 women providing 898 samples) and women with hypertensive diseases of pregnancy (HDP) (blue) (n=32 women providing 133 samples); groups were compared by Mann Whitney U Test.

1B. Medians (IQR) of plasma kisspeptin levels in healthy controls (red) (n=265 women providing 898 samples) and women with hypertensive diseases of pregnancy (HDP) (blue) (n=32 women providing 133 samples) over the early and late first trimester, and second and third trimesters. Data were analysed by Mann Whitney U Test.

1C. Scatterplot of medians (IQR) of multiples of gestation specific median kisspeptin levels in healthy controls (red) (n=265 women providing 898 samples) and women with fetal growth restriction (FGR) (blue) (n=17 women providing 56 samples); groups were compared by Mann Whitney U Test.

1D. Medians (IQR) of plasma kisspeptin levels in healthy controls (red) (n=265 women providing 898 samples) and women with fetal growth restriction (FGR) (blue) (n=17 women providing 56 samples) over the early and late first trimester, and second and third trimesters. Data were analysed by Mann Whitney U Test.

Figure 2: Variation in plasma kisspeptin levels according to severity, onset and complications of pre-eclampsia

2A. Scatterplot of plasma kisspeptin levels in healthy controls (blue) (n=265 women providing 898 samples), women with early onset pre-eclampsia (PET <34 weeks) (red) (n=8 women providing 27 samples) and women with late onset pre-eclampsia (PET> 34 weeks) (black) (n= 16 women providing 65 samples) over weeks of gestational age estimated by Crown Rump Length (CRL) throughout pregnancy. Data were analysed by simple linear regression ($r^2$=0.55 in controls, 0.87 in early PET and
0.54 in late PET); p=0.0040 by ANCOVA. Analysis of subgroups of HDP are presented only as exploratory analyses and should be interpreted with caution as they may be subject to type 2 error.

2B. Medians (IQR) of plasma kisspeptin levels in healthy controls (blue) (n=265 women providing 898 samples), women with early onset pre-eclampsia (PET <34 weeks) (red) (n=8 women providing 27 samples) and women with late onset pre-eclampsia (PET >34 weeks) (black) (n=16 women providing 65 samples) over the early and late first trimester, and second and third trimesters. Data were analysed by Kruskall Wallis test. Analysis of subgroups of HDP are presented only as exploratory analyses and should be interpreted with caution as they may be subject to type 2 error.

2C. Scatterplot of plasma kisspeptin levels in women with pre-eclampsia (PET) (red) (n=20 women providing 82 samples), women with intra-uterine growth restriction (IUGR) (blue) (n=17 women providing 56 samples) and women with both pre-eclampsia and intra-uterine growth restriction (PET & IUGR) (purple) (n=3 women providing 8 samples) over weeks of gestational age estimated by Crown Rump Length (CRL) throughout pregnancy. Data were analysed by simple linear regression (\(r^2=0.55\) in PET, 0.69 in IUGR and 0.92 in PET & IUGR); p=0.0004 by ANCOVA. Analysis of subgroups of HDP are presented only as exploratory analyses and should be interpreted with caution as they may be subject to type 2 error.

2D. Scatterplot of plasma kisspeptin levels in women with severe PET (red) (n=13 women providing 39 samples) and women with non-severe PET (blue) (n=7 women providing 38 samples) over weeks of gestational age estimated by Crown Rump Length (CRL) throughout pregnancy. Data were analysed by simple linear regression (\(r^2=0.76\) in severe PET and 0.47 in non-severe PET); p=0.73 by ANCOVA. Analysis of subgroups of HDP are presented only as exploratory analyses and should be interpreted with caution as they may be subject to type 2 error.
**Figure 3: Kisspeptin levels throughout pregnancy in women with Gestational Diabetes Mellitus (GDM) compared to control pregnancies and in women with Preterm birth (PTB) compared to control pregnancies**

**3A.** Scatterplot of medians (IQR) of multiples of gestation specific median kisspeptin levels in healthy controls (red) (n=265 women providing 898 samples) and women with gestational diabetes mellitus (GDM) (blue) (n=35 women providing 122 samples); groups were compared by Mann Whitney U Test.

**3B.** Medians (IQR) of plasma kisspeptin levels in healthy controls (red) (n=265 women providing 898 samples) and women with gestational diabetes mellitus (GDM) (blue) (n=35 women providing 133 samples) over the early and late first trimester, and second and third trimesters. Data were analysed by Mann Whitney U Test.

**3C.** Scatterplot of medians (IQR) of multiples of gestation specific median kisspeptin levels in healthy controls (red) (n=265 women providing 898 samples) and women with preterm birth (PTB) (blue) (n=11 women providing 38 samples); groups were compared by Mann Whitney U Test.

**3D.** Medians (IQR) of plasma kisspeptin levels in healthy controls (red) (n=265 women providing 898 samples) and women with preterm birth (PTB) (blue) (n=11 women providing 38 samples) over the early and late first trimester, and second and third trimesters. Data were analysed by Mann Whitney U Test.
### Table 1. Baseline characteristics and pregnancy outcomes of the total sample (N) and pregnancies grouped by antenatal complications.

|                      | Controls N=265 | PE N=20 | Severe PE N=13 | Non-Severe PE N=7 | PHN=12 | FGR N=17 | GDM N=35 | PTB N=11 | Pts with >1 complication N=10 | All pregnancies N=370 | p-value |
|----------------------|----------------|---------|----------------|-------------------|---------|----------|----------|----------|-------------------------------|----------------------|---------|
| Maternal age (years)| 32.2±5.3       | 33.2±5.5| 33.7±6.0       | 32.0±5.2          | 32.5±6.1| 28.7±6.8 | 33.8±4.8 | 30.0±5.7 | 33.8±8.4                      | 32.2±5.5             | 0.217   |
| BMI (kg/m²)         | 24.3±4.8       | 27.4±5.1| 28.6±7.3       | 29.1±4.7          | 27.7±7.3| 26.0±4.8 | 26.9±4.1 | 24.2±4.2 | 29.6±8.9                      | 25.1±5.1             | 0.143   |
| Gravida             | 3.0±1.9        | 3.0±2.1 | 2.6±2.1        | 3.0±2.2           | 3.0±1.9 | 2.3±1.5  | 2.5±1.7  | 3.4±1.4  | 3.0±2.4                       | 2.9±1.9              | 0.528   |
| Parity              | 0.7±1.0        | 0.6±0.9 | 0.5±1.1        | 0.6±0.8           | 0.8±1.3 | 0.5±0.9  | 0.5±0.7  | 0.7±0.9  | 0.5±1.0                       | 0.7±0.9              | 0.268   |
| Maternal Ethnicity  |                      |         |                |                   |         |          |          |          |                               |                     |         |
| White                | 184 (69.4%)     | 11 (55.0%)| 6 (46.2%)      | 3 (42.9%)         | 8 (66.7%)| 7 (41.2%)| 13 (37.1%)| 9 (81.8%)| 3 (30.0%)                      | 235 (63.5%)          | <0.0005 |
| Black                | 31 (11.7%)      | 5 (25.0%) | 5 (38.5%)   | 2 (28.6%)         | 2 (16.7%)| 8 (47.1%)| 6 (17.1%)| 0 (0.0%) | 4 (40.0%)                      | 56 (15.1%)           |         |
| Asian                | 27 (10.2%)      | 3 (15.0%) | 1 (7.7%)     | 1 (14.3%)         | 1 (8.3%) | 1 (5.9%) | 12 (34.3%)| 1 (9.1%) | 1 (10.0%)                      | 46 (12.4%)           |         |
| Other                | 23 (8.7%)       | 1 (5.0%)  | 1 (7.7%)     | 1 (14.3%)         | 1 (8.3%) | 1 (5.9%) | 4 (11.4%)| 1 (9.1%) | 2 (20.0%)                      | 33 (8.9%)            |         |
| Smoking              | 30 (11.3%)      | 2 (10.0%) | 0 (0.0%)     | 1 (14.3%)         | 1 (8.3%) | 3 (17.6%)| 2 (5.7%) | 1 (9.1%) | 1 (10.0%)                      | 40 (10.8%)           | 0.929   |
| Alcohol consumption  | 165 (62.3%)     | 12 (60.0%)| 7 (53.8%)    | 5 (71.4%)         | 9 (75.0%)| 9 (52.9%)| 22 (62.9%)| 3 (27.3%)| 7 (70%)                        | 227 (61.4%)          | 0.007   |
| Baby weight          | 339±441         | 312±560 | 289±763      | 327±560           | 321±533 | 209±511 | 311±596 | 261±589 | 207±813                       | 322±605              | <0.0005 |
| Baby weight centile  | 60.6±27.6       | 62.6±30.8| 41.9±35.7 | 68.6±28.5         | 56.6±24.0| 5.2±7.5  | 58.4±31.6| 68.5±16.1| 28.9±34.5                      | 57.0±30.2            | 0.750   |
| Baby gender          |                      |         |                |                   |         |          |          |          |                               |                     |         |
| Male                 | 109 (41.1%)     | 9 (45.0%) | 9 (69.2%)    | 3 (42.9%)         | 7 (58.3%)| 9 (52.9%)| 19 (54.3%)| 5 (45.5%)| 4 (40.0%)                      | 162 (43.8%)          |         |
| Female               | 145 (54.7%)     | 11 (55.0%)| 4 (30.7%)    | 4 (57.1%)         | 5 (41.7%)| 8 (47.1%)| 15 (42.9%)| 6 (54.5%)| 6 (60.0%)                      | 196 (53.0%)          |         |
Mean±SD for continuous, normally distributed variables, and numbers of patients (percentages) for categorical variables are presented. Differences in mean and proportion were tested with ANOVA and Chi², respectively. A p-value of <0.05 was classified as statistically significant. Bold numbers indicate statistically significant differences in baseline characteristics between groups. **Abbreviations:** N, total sample size; PET, pre-eclamptic toxaemia; PIH, pregnancy induced hypertension; FGR, fetal growth restriction; GDM, gestational diabetes mellitus; PTB, preterm birth; pts, patients; kg, kilograms; m, metres; BMI, body mass index, Smoking, indicates maternal smoking during pregnancy, Alcohol consumption, indicates maternal alcohol consumption at all during pregnancy. Severe and non-severe PE were presented as subgroups and were not compared statistically.
Table 2. Association between plasma kisspeptin and Hypertensive Diseases of Pregnancy (HDP).

Logistic regression was used to assess the association between (1) kisspeptin with HDP diagnosis in univariable analysis and (2) after adjustment for gestational age (estimated using CRL), maternal age, ethnicity, BMI, smoking status and parity. Odds ratios denote odds of HDP diagnosis for every 1 nmol/L increase in plasma kisspeptin. A p-value of <0.05 was classified as significant. Bold numbers indicate statistically significant predictors of HDP after adjustment. Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval. BMI, body mass index; kg, kilograms; m², metres squared; and CRL, crown-rump length.

|                          | Crude OR (95% CI) | P     | Adjusted OR (95% CI) | P     |
|--------------------------|-------------------|-------|----------------------|-------|
| Plasma kisspeptin (nmol/L) | 1.12 (1.04-1.20)  | 0.004 | 1.30 (1.16-1.47)     | <0.0001 |
| Gestational age (weeks)  | 1.00 (0.98-1.02)  | 0.972 | 0.95 (0.92-0.98)     | 0.003  |
| Maternal age (years)     | 1.04 (1.01-1.8)   | 0.018 | 1.03 (0.99-1.08)     | 0.089  |
| Maternal Ethnicity (vs Caucasian) |            |       |                      |       |
| Afro-Caribbean           | 1.70 (1.06-2.71)  | 0.027 | 1.28 (0.75-2.18)     | 0.374  |
| Asian Other              | 1.23 (0.72-2.12)  | 0.452 | 0.82 (0.46-1.45)     | 0.493  |
| Maternal BMI (kg/m²)     | 1.11 (0.08-1.15)  | <0.0001 | 1.14 (1.10-1.18) | <0.0001 |
| Cigarette smoker         | 1.00 (0.99-1.00)  | 0.215 | 0.99 (0.99-1.00)     | 0.121  |
| Parity                   | 0.96 (0.79-1.18)  | 0.721 | 0.78 (0.63-0.97)     | 0.025  |
Table 3. Association between plasma kisspeptin and Fetal Growth Restriction (FGR). Logistic regression was used to assess the association between (1) kisspeptin with FGR diagnosis in univariable analysis and (2) after adjustment for gestational age (estimated using CRL), maternal age, ethnicity, BMI, smoking status and parity. Odds ratios denote odds of FGR diagnosis for every 1nmol/L increase in plasma kisspeptin. A p-value of <0.05 was classified as significant. Bold numbers indicate statistically significant predictors of FGR after adjustment. Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; BMI; body mass index; kg, kilograms; m², metres squared; and CRL, crown-rump length.

|                          | Crude          | Adjusted       |
|--------------------------|----------------|----------------|
|                          | OR (95% CI)    | P              | OR (95% CI)    | P              |
| Plasma kisspeptin (nmol/L) | 0.89 (0.74-1.06) | 0.188          | 0.72 (0.54-0.96) | 0.025          |
| Gestational age (weeks)   | 1.00 (0.98-1.03) | 0.757          | 1.04 (1.00-1.09) | 0.053          |
| Maternal age (years)      | 0.86 (0.82-0.90) | <0.0001        | 0.90 (0.85-0.95) | <0.0001        |
| Maternal Ethnicity (vs Caucasian) |               |                |                |
| Afro-Caribbean            | 5.50 (3.05-9.95) | <0.0001        | 4.74 (2.39-9.41) | <0.0001        |
| Asian                     | 0.46 (0.11-1.95) | 0.289          | 0.55 (0.13-2.40) | 0.427          |
| Other                     | 1.44 (0.49-4.25) | 0.506          | 1.59 (0.52-4.85) | 0.416          |
| Maternal BMI (kg/m²)      | 1.02 (0.96-1.06) | 0.562          | 0.98 (0.92-1.04) | 0.461          |
| Cigarette smoker          | 1.01 (1.00-1.02) | 0.006          | 1.01 (1.00-1.01) | 0.154          |
| Parity                    | 0.77 (0.54-1.10) | 0.157          | 0.75 (0.51-1.11) | 0.155          |
