Impact of obesity on angiogenic and inflammatory markers in the Finnish Genetics of Pre-eclampsia Consortium (FINNPEC) cohort

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ABSTRACT

BACKGROUND: While several studies have demonstrated that obesity increases the risk of pre-eclampsia (PE), the mechanisms have yet to be elucidated. We assessed the association between maternal/paternal obesity and PE and hypothesised that maternal body mass index (BMI) would be associated with an adverse inflammatory and angiogenic profile. High-sensitivity C-reactive protein (hs-CRP) and following serum angiogenic markers were determined: soluble endoglin (sEng), soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF).

METHODS: Data on BMI were available from 1450 pregnant women with PE and 1065 without PE. Serum concentrations of hs–CRP and angiogenic markers were available from a subset at first and third trimesters.

RESULTS: Prepregnancy BMI was higher in the PE group than in controls (mean±SD) 25.3±5.2 vs. 24.1±4,4, $p<0.001$, adjusted for parity, mother's age and smoking status before pregnancy. Increased hs-CRP concentrations were observed in both PE and non-PE women similarly according to BMI category. In women with PE, a higher BMI was associated with lower sFlt-1 and sEng concentrations throughout the pregnancy ($p=0.004$, $p=0.008$, respectively). There were no differences in PlGF in PE women according to BMI.

CONCLUSIONS: We confirmed increased pre-pregnancy BMI in women with PE. Enhanced inflammatory state was confirmed in all women with overweight/obesity. Partly paradoxically we observed that PE women with obesity had less disturbed levels of angiogenic markers than normal weight women with PE. This should be taken account when angiogenic markers are used in PE prediction.

INTRODUCTION

Obesity prevalence in women of reproductive age is increasing globally at an alarming rate mainly due to sedentary lifestyles and unhealthy diet.[1] In Finland, every third (35 %) pregnant woman is overweight (body mass index, BMI ≥ 25) and 13% are obese (BMI ≥ 30).[2] The proportion of overweight women increased by 4% and proportion of obese by 2% from 2008 to 2013.

Obesity before and during pregnancy is widely recognized as an independent risk factor for several adverse pregnancy outcomes including pre-eclampsia (PE).[3-5] Meta-analyses have reported an approximately threefold increase in the risk of PE in women with obesity compared with normal weight women.[6,7] However, the underlying mechanisms between obesity and PE are not fully explained. It is also unclear whether paternal obesity affects the risk of PE. Previously Myklestad et al.[8] have found slightly greater paternal BMI in men who had fathered a PE pregnancy.

Classically PE is defined as a new-onset hypertension and proteinuria[9] although new extended guidelines have recently been published as well.[10,11] Obesity and PE share several common features of metabolic disturbances including inflammation, oxidative stress, insulin resistance and endothelial dysfunction.[12-14] These changes are also seen in a normal pregnancy but they are far stronger in PE.[15] In obese pregnancy, particularly endothelial dysfunction and inflammation have been proposed to contribute to the mechanism by which maternal adiposity may lead to abnormal vascular development of the placenta and thus, an elevated risk of PE.[12,16] In the endothelial dysfunction central feature is an adverse angiogenic profile, i.e. high soluble fms-like tyrosine kinase-1 (sFlt-1), high soluble endoglin (sEng) and low placental growth factor, PlGF. In obesity, generally, adipose tissue undergoes dynamic remodeling, including an alternation in adipogenesis and angiogenesis.[17] Furthermore, adipocyte hypertrophy induces local adipose tissue hypoxia which modifies the expression levels of angiogenic genes. A reduction in capillary density in adipose tissue accompanied by larger vessels has already been noted, and it is suggested that these changes inhibit angiogenesis

and limit the expandability of adipose tissue.[18] Whether dysregulated angiogenesis could be approached as the missed link between obesity and PE, remain to be elucidated.

In the current study, objectives were to study the association between maternal/paternal obesity and PE and to assess the association between maternal BMI and angiogenic and inflammatory profile at first and third trimesters of pregnancy. We also tested recently recommended sFlt-1/PlGF ratio rule-out and rule-in cut-off values for the different BMI categories. To assess inflammatory status we measured maternal serum levels of high sensitive C-reactive protein (hs-CRP).

METHODS

Study design and aim

Data for the present study originates from the Finnish Genetics of Pre-eclampsia Consortium (FINNPEC), a cross-sectional case-control multicentre study with a nationwide clinical and DNA database on PE and non-PE (control) women, including their partners and infants. Details of the study design, methods and procedures have been published earlier.[19] Herein, we investigated maternal and paternal obesity status and whether maternal serum concentrations of hs-CRP, sFlt-1, PlGF, sEng and sFlt-1/PlGF ratio of FINNPEC participants associate with obesity in PE and non-PE women.

Study subjects

Originally in the FINNPEC Study, 1450 patients with PE and 1065 control women without PE were recruited at the five Finnish university hospitals. Anthropometric characteristics were available from majority but the first and third trimester serum samples were available from a subset. First trimester serum samples were available from 221 PE-women and 239 non-PE women. Third trimester serum samples were available from 175 PE- and 55 non-PE women. All participants provided written informed consent, and the FINNPEC study protocol was approved by the coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa.

Inclusion criteria

Nulliparous or multiparous women with a singleton pregnancy were eligible for the study. PE was defined as hypertension and proteinuria occurring after 20 weeks of gestation. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, and proteinuria as the urinary excretion of ≥0.3 g protein in a 24-hour specimen, or 0.3g/l, or two ≥1+ dipstick readings in a random urine determination with no evidence of a urinary tract infection. Each diagnosis was ascertained from hospital records and independently confirmed by a research nurse and a study physician. PE subtypes were defined by the time of delivery: early-onset PE (<34 weeks of gestation) and late-onset PE (≥34 weeks of gestation).

Exclusion criteria

Exclusion criteria were multiple pregnancy, maternal age less than 18 years and an inability to provide informed consent based on information being offered in Finnish or Swedish.

Background and obstetric data

Extensive information on medical history, obstetric history, pregnancy complications, pregnancy outcome, proteinuria, blood pressure, laboratory measurements, delivery and newborn was obtained from the hospital records and maternity cards. Data on smoking status were collected from the maternity cards and complemented from the background information questionnaires if needed. Data on pre-pregnancy weight and height were obtained from maternity cards. Women were further divided into three categories based on their pre-pregnancy BMI: normal weight as a BMI less than 25 kg/m^2, overweight as a BMI from 25 to 29.99 kg/m^2, and obesity as a BMI of 30 kg/m^2 or greater.

Weight gain during pregnancy was determined as the difference between pre-pregnancy weight and the weight at the last measurement at the maternity clinic. Last measurement occurred on average at 35.2±3.8 and 38.3±3.0 weeks of gestation for PE and non-PE women, respectively. Weight cycling in BMI units was calculated based on height and self-reported maximum and minimum weight during adulthood reported in the background information questionnaires. In addition, own birth weight of women were self-reported. Data on paternal BMI was calculated based on self-reported height and weight at the recruitment.

Serum samples and angiogenic markers

First and third trimester serum samples were collected from women receiving care from the Hospital District of Helsinki and Uusimaa. First trimester serum samples were obtained via first trimester biochemical screening for fetal chromosome abnormalities (range 9-15 weeks of gestation), and serum samples from the third trimesters (range 20-42 weeks of gestation) were collected at hospitals for study protocol.

Data on fasting blood glucose and oral glucose tolerance test (OGTT) were available from a subset (n=178 for PE and n=99 for non-PE women). OGTT was performed on average at 25,5±5.9 and 26.4±4.7 weeks of gestation for PE and non-PE, respectively.

Maternal serum sFlt-1 and PlGF concentrations were measured using sFlt-1 and PlGF electro-chemiluminescence immunoassays (ECLIA; Roche Diagnostics GmbH, Mannheim, Germany) on a cobas e601 analyser (Hitachi High Technology Co, Tokyo, Japan). Serum concentrations of sEng (CD105) were measured using the human Quantikine Endoglin ELISA kit (R&D Systems, UK) according to the manufacturer's instructions. The intra-assay and inter-assay coefficients of variation for PlGF were <0.8% and <2.3% in the concentration range 96–1020 pg/ml, for sFlt-1 <1.4% and <1.7% in the range 97–5390 pg/ml.

Recently recommended rule-out cut-off values[20] of 33 (20 weeks to delivery), rule-in cut-offs of 85 (until 33 weeks 6 days) and 110 (34 weeks to delivery) were tested for the Elecsys immunoassay sFlt-1/PlGF ratio. Furthermore, we tested a cut-off for the sFlt-1/PlGF ratio that was presented very recently in the PROGNOSIS study.[21] Zeisler et al.[21] derived a single cut-off value independent of the weeks gestation: values below 38 were considered negative and were used to rule-out PE within 1 week after assessment of the ratio. The clinical characteristics and outcomes of PE women presenting a normal angiogenic profile (sFlt1/PlGF ratio <85) with those women who are characterized by an abnormal angiogenic profile (sFlt1/PlGF ratio ≥85) were also compared according to Rana et al.[22]
Serum CRP was measured with the CRP-latex method using a highly sensitive immunoturbidometric method application (Hs-CRP, Cardiac-neonatal, Beckman-Coulter) and AU680 analyzer (Beckman-Coulter Inc., CA, USA). The intra-assay variation of the method was less than 5,0 % (CV%) and the sensitivity 0,06 mg/l (CV % less than 10%).

Statistical analysis

Statistical tests were performed with IBM SPSS statistics 22 software (IBM Corp). The normality of distributions of the variables was verified with the Kolmogorov–Smirnov test. Logarithmic transformation was used when appropriate. Each biomarker was ln-transformed to handle right-skewness, and estimated means were back-transformed as geometric means and 95% confidence intervals for purposes of presentation. For the continuous variables, comparisons between groups were analysed with general linear model univariate ANOVA at baseline and with linear mixed models during the pregnancy. Selected co-variables [smoking status, maternal age, gestational weeks at sampling and parity] were included in the models as covariates. Normality was assessed by plotting the residuals. Women with angiogenic and non-angiogenic PE were compared using the Mann-Whitney-U test. Multiple linear regression analysis was used separately to assess the influence of smoking status, maternal age, gestational weeks at sampling and parity on the variation of hs-CRP at first and third trimester (dependent variable).

For the categorical variables, the comparisons were performed with the Fisher's exact test. With skewed distributions, comparisons between continuous variables were performed by the Mann Whitney U-test.

RESULTS

Anthropometric characteristics

Women with PE had higher pre-pregnancy BMI and they gained more weight during pregnancy than non-PE controls (**Table 1**). PE women also reported to have greatest difference in maximum and minimum BMI during adulthood. There was no difference in self-reported paternal BMI or in self-reported own birth weight between PE and non-PE groups.

The majority in both PE and non-PE groups were women with normal weight, whereas the proportion of women with obesity (BMI ≥ 30) was greater in the PE group **(Table 2**). Women with overweight and obesity were more likely to have higher systolic and diastolic blood pressure in the PE and control groups. They also suffered more often from chronic hypertension and gestational diabetes in both groups. Women with overweight and obesity had higher fasting, 1-hour and 2-hour glucose concentrations than women with normal weight but only in the PE group. In the non-PE group, women with overweight and obesity had more often gestational hypertension than women with normal weight. In the PE group, women with overweight and obesity were more often diagnosed with placental insufficiency. In both PE and control groups, women with overweight and obesity delivered larger infants than women with normal weight (**Table 2**).

There was no difference in prepregnancy BMI between early-onset PE group (delivery < 34 weeks of gestation, n=262) and late-onset group (delivery ≥ 34 weeks of gestation, n=1185). Early PE 25.6±5.1 vs. late 25.2±5.3, p=0.450 adjusted for maternal age and parity.

PE multiparous women had higher BMI than nulliparous women (26.5±5.8 vs. 24.8±5.0, p<0.001, respectively). In addition, there was no difference among non-PE multiparous and nulliparous women (24.1±4.5 vs. 24.1±4.3), respectively.

Hs-CRP

There was no difference in hs-CRP at first or third trimester between PE and non-PE women, (**Table 3**). PE women had higher first trimester concentrations of hs-CRP than non-PE women when analyses were adjusted for parity, mother's age, smoking status and gestational weeks at sampling. However, the difference was not significant after further adjustment for BMI (**Table 3**).

In PE and non-PE women, highest hs-CRP concentrations at first and third trimesters were detected in women with BMI ≥ 30 compared to women with BMI<25 or 25≤ BMI ≤29.99 **(Table 4).** Furthermore, when multiple linear regression analysis was performed to determine which variables independently associated with maternal serum hs-CRP at first or third trimester, BMI explained the variation of both first and third trimester concentrations of hs-CRP in both PE (R^2=0.178, B=0.349, p<0.001 and R^2=0.070, B=0.265, p=0.001, respectively) and non-PE groups (R^2=0.198, B=0.346, p<0.001 and R^2=0.152, B=0.299, p=0.035, respectively).

Angiogenic markers according to BMI categories

Concentrations of angiogenic markers during pregnancy in PE and non-PE women according to the BMI categories are presented in **Table 5** and in **Figure 1**. In PE women, lower sFlt-1 and sEng concentrations at first and third trimesters were detected in women with BMI ≥ 30 compared to women with BMI<25 or 25≤ BMI ≤29.99 (**Table 5**). Similar difference was observed for sFlt-1 among controls during first trimester. There were no differences in PlGF concentrations between BMI category groups. In PE group, obese women also had lowest sFlt-1/PlGF ratio at third trimester. Furthermore, the differences sEng and sFlt-1/PlGF ratio between PE and non-PE groups were apparent in the normal weight category but not in the obese category (**Table 5**). Correlation plots for BMI and angiogenic markers are illustrated in **Supplementary figure 1**. In PE women sFlt-1 and sEng concentrations correlated negatively with BMI at first and third trimester.

In the analyses within time, in women with PE, a higher BMI was associated with lower sFlt-1 and sEng concentrations throughout the pregnancy (**Figure 1**) and a trend for a lower sFlt-1/PlGF ratio was also observed. BMI was not associated with PlGF among PE women, but among non-PE women the higher BMI associated with lower concentrations throughout the pregnancy.

The proportion of PE women whose sFlt-1/PlGF ratio was below the cut-off of 33 (rule-out according to the NICE guideline) was higher in the group of PE women with BMI ≥30. Similar trend was observed for the cut-off 38. Furthermore, among PE women, there were less women with BMI≥30 who exceeded cut-off 110 (rule-in between 34 weeks 0 days and delivery).

The women with non-angiogenic PE showed a trend for higher BMI (**Supplementary table 1**).

DISCUSSION

The wide range of risk factors reflects the heterogeneity of PE. Obesity, which is increasing at an alarming rate, is one of the risk factors for PE but the underlying mechanisms are not yet understood. We assessed the association between obesity and PE and hypothesized that maternal BMI is associated with a pro-inflammatory profile (i.e. increased hs-CRP concentration) and an adverse angiogenic profile (i.e. high sFlt-1, high endoglin and low PlGF concentrations). Enhanced inflammatory state was confirmed in all women with obesity. Furthermore, we observed that imbalance of angiogenic factors in PE differ between women with obesity and normal weight. PE women with obesity had less disturbed levels of angiogenic markers than PE women with normal weight.

Obesity and clinical characteristics

It is known that obesity increases the overall risk of PE by approximately two- to threefold.[23,24] In the FINNPEC study, PE women had higher BMI compared with non-PE women. There was no difference in BMI between early- and late-onset PE groups. In addition, there was no difference in BMI between nulli- and multiparous non-PE women but the multiparous PE women had clearly higher BMI than nulliparous PE-women. It has been shown previously that multiparous non-PE women having at least two or more children compared to primiparous or nulliparous women have later greater increase in BMI.[25,26] However, the association between PE and future weight gain is to our knowledge, less studied. Callaway et al.[27] have shown that hypertensive disorders of pregnancy are associated with increased weight gain over two decades. These findings are important since the propensity to weight gain might partly contribute to the burden of later-life chronic diseases of PE women. Furthermore, it highlights the importance of postpartum care of these women.

Interestingly, PE women also reported to have greatest difference in maximum and minimum BMI during adulthood. In line with our findings, Frederick et al.[28] have earlier found that adult weight gain is associated with an increased risk of PE. The association between adult weight gain and PE risk appeared to be strongest particularly among women with a history of weight cycling.[28] This further highlights the public health perspective and lifelong promotion of healthy lifestyle.

Women with BMI ≥ 30 in both PE and non-PE group were oldest, had highest blood pressure and proportions of chronic hypertension and gestational diabetes. In non-PE group these women with BMI ≥ 30 also suffered more from gestational hypertension. It is notable that approximately every third woman with BMI ≥ 30 in both PE and non-PE groups suffered from gestational diabetes.

Women with overweight and obesity had higher fasting, 1-hour and 2-hour glucose concentrations than women with normal weight but only in the PE group. This might highlight the pronounced role of insulin resistance in PE accompanied with dyslipidaemia and endothelial dysfunction.[29] It is unknown whether insulin resistance has a role in the aetiology of PE or is it secondary to the disease.

PE women reported to have lower own birth weight compared with non-PE women which is in line with the previous literature.[30,31] Interestingly, the increased risk of PE weight appears to be restricted to those women who were born small and became overweight as adults.[30]

Paternal obesity has been shown to represent an independent risk factor for pregnancies resulting in small-for–gestational age (SGA) infants independently of maternal factors associated with fetal growth restriction.[32] However, it is still unclear whether paternal obesity affects the risk of PE. Paternal obesity might not only reflect the couple's lifestyle and dietary choices, but also the specific paternal genetic background. However, we could not observe differences in self-reported paternal BMI or in self-reported own birth weight between PE and non-PE groups.

Angiogenic markers

In women with PE, a higher BMI was associated with lower concentrations of sFlt-1 and sEng throughout the pregnancy. It was noteworthy that in a cross-sectional setting the differences in sEng between PE and non-PE groups were apparent in the normal weight category but not in the obese categories. Moreover, our findings suggest that the different sFlt-1/PlGF ratio cut-offs might be needed for women with obesity when utilized in PE prediction.

The mechanisms underlying the association between maternal obesity and PE are not well understood. It has been suggested that maternal obesity is associated with endothelial damage and impaired angiogenesis leading to abnormal placental function.[33] The results on BMI and angiogenic markers in PE are contradictory. In accordance with our results, Zera et al.[34] have demonstrated inverse association between sFlt-1 concentration and BMI across gestation in pregnancies affected by placental dysfunction. In addition, Suwaki et al.[35] reported lower sFlt-1 levels in overweight PE group compared with the normal weight group. Similarly as in the current study, they did not detect difference in the levels of sFlt-1 in the normotensive control group.

In non-PE women, Mijal et al.[36] have demonstrated that levels of both sFlt-1 and PlGF are lower in obese pregnant women while Faupel-Badger et al.[37] have shown that higher BMI is associated with higher sFlt-1 concentrations and a higher sFlt-1/PlGF ratio. Moreover, Straughen et al.[38] found that mean sFlt1 levels in second and third trimester were higher in women with normal weight compared to women with overweight/obesity. In the current study, there was no difference in PlGF concentrations between BMI categories in PE-women. Very recently, Vieira et al.[39] have demonstrated that lower PlGF in early pregnancy was restricted to women with PE and obesity.

To our knowledge, sEng is less studied in PE and obesity. Lower levels of sEng are found in obese non-PE women compared with normal weight controls in early pregnancy.[40] Lappas et al.[41] have found that in placenta, pre-existing maternal obesity had no effect on the endoglin expression. On the other hand, in omental adipose tissue, maternal obesity was associated with increased gene expression of endoglin and increased secretion sEng.

It could be speculated that the lower levels of sFlt-1 and sEng represent differences in volume of distribution rather than actual differences over time. However, we could not detect similar differences according to BMI categories in PlGF concentration and sFlt-1/PlGF ratio and thus, we conclude that the differences cannot be explained by the increased volume observed in obesity.

Instead, we suggest that the role of expanded adipose tissue and its vascular bed in obesity needs further investigation. Adipose tissue is probably the most highly vascularised tissue in the body, and each adipocyte is surrounded by an extensive capillary network.[42] Multiple angiogenic factors have been recognised in adipose tissue including sFlt-1, sEng and PlGF.[41,43,44]

Interestingly, Herse et al.[44] have shown that lean non-PE women have higher sFlt-1 levels in adipose tissue and that TNF-α could downregulate sFlt-1. Therefore high levels of sFlt-1 might restrain adipose tissue growth via inhibition of local angiogenesis. The concept that leaner women have higher sFlt-1 levels in adipose tissue that may prevent angiogenesis and thus minimize obesity represents a novel potential molecular mechanism. Very recently, Huda et al.[45] studied adipose tissue of PE women and demonstrated that visceral adipose tissue had a higher activated macrophage content and higher mRNA expression of TNFα in PE than in controls. In the current study, however, we were not able to examine adipose tissue of these women although it is possible that numerous pro- and anti-angiogenic factors secreted by adipose tissue could control systemic angiogenesis and might explain observed differences between normal weight and overweight/obese women.

hs-CRP

Previously, hs-CRP has been suggested to be a predictive marker for PE[46-48] but not all have confirmed this.[49] Qui et al.[47] reported that statistical significance of hs-CRP in PE is lost after adjustment for maternal BMI. Accordingly, we found slightly increased hs-CRP concentrations in PE women at first trimester but the difference was attenuated after adjustment for BMI. Hs-CRP levels increase during pregnancy[50] which we confirmed by observing a trend for higher concentrations in non-PE at third trimester (serum samples were taken on average 2.5 weeks later in non-PE group).

In the current study, hs-CRP levels associated with obesity similarly in women with and without PE. Furthermore, linear regression identified BMI as a most significant factor for hs-CRP concentrations at first and third trimester in both groups when other factors known to affect hs-CRP concentration were also taken account.[51]

Hs-CRP is mainly produced by liver but human placenta is also a source.[52] Moreover, very recently Huda et al.[45] demonstrated that subcutaneous adipose tissue is an important determinant of circulating CRP at the third trimester of pregnancy in both healthy and PE pregnancies.

Increased hs-CRP concentrations were observed both in PE and non-PE women similarly according to BMI category and thus, it highlights low-grade inflammation generally observed in obesity. However, based on our findings, it could not serve as a specific marker of inflammation associated with PE.

Strengths and limitations

One of the strengths of the current study are that the diagnostic criteria for PE were well defined and detailed clinical information allows us to accurately define the phenotypes. Furthermore, its prospective cohort design makes possible to analyse changes over time. However, one of the major limitations in the current study is that only BMI was used as the measure of obesity, which may not be the most adequate measure of adiposity. Furthermore, maternal and paternal information on BMI was self-reported. One of the limitations is also that the inter-individual variations in serum concentrations were relatively large and the sample size was small, especially when further dividing into subcategories according to BMI.

Conclusions

In the current study, we confirmed increased pre-pregnancy BMI in PE women when compared with non-PE women. Levels of angiogenic factors were less disturbed in PE women with obesity and thus, these women appear to present a distinct subphenotype compared with normal weight PE women. We suggest that obesity status should be taken account when sFlt-1/PlGF ratio cut-offs are used in PE as a prognostic and diagnostic marker. Future studies should address the question whether angiogenic markers released from the adipose tissue affects maternal and placental vascular function.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGENDS

Fig. 1 Angiogenic markers (mean ± s.e.m.) according the BMI categories. Linear mixed model (BMI as continuous variable) adjusted for smoking status (prepregnancy and during pregnancy), age, gestational weeks at sampling, gestational weeks and parity.