Clinical and biochemical factors associated with preeclampsia in obese and lean women.

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Condensation: Obese women have a distinct phenotype associated with preeclampsia and should be considered separately in clinical prediction models.

Short title: Preeclampsia in obese and lean women.

Abstract

Background: Algorithms combining clinical factors and biomarkers have been proposed to facilitate accurate risk assessment in early pregnancy of later development of preeclampsia. The validity of these predictive tools in sub-groups of high risk women is not established. We hypothesized that clinical factors and biomarkers which predict risk of pre-eclampsia may differ between lean and obese women.

Objective: To evaluate clinical and biomarker risk factors for later development of preeclampsia in obese and lean women.

Study design: Participants from the Screening of Pregnancy Endpoints (SCOPE) study, a prospective cohort of 5690 nulliparous women were investigated. Biomarkers reflecting glucose and lipid metabolism, markers of placentation or pathogenesis of preeclampsia were measured in plasma samples at 14-16 weeks' gestation. Univariate and multivariate logistic regression was performed to identify risk factors for preeclampsia separately in obese (body mass index $\geq$30kg/m^2) and lean women (body mass index 20-25kg/m^2). Interaction between identified risk factors and body mass index was explored by combining both groups.

Results: Amongst 834 obese (median body mass index 33.1kg/m^2) and 3106 normal weight women (median body mass index 22.3kg/m^2), 77 (9.2%) and 105 (3.4%) developed preeclampsia, respectively. In obese women, risk factors included family history of thrombotic disease (Odds Ratio (OR) 2.5; 95% Confidence Interval (CI) 1.4-4.5), low placental growth factor at 15 weeks' (OR 1.8; 95% CI 1.3-2.4; per 1 log of multiple of median unit) and higher uterine artery resistance index at 20 weeks' (OR 1.3; 95% CI 1.0-1.6). In lean women, a family history of preeclampsia or gestational hypertension (OR 1.8; 95% CI 1.1-2.9), mean arterial blood pressure (OR 1.5; 95% CI 1.3-1.7), endoglin (OR 1.8; 95% CI 1.1-2.8) and cystatin C (OR 2.0; 95% CI 1.2-3.5), and uterine artery resistance index (OR 1.4; 95% CI 1.2-1.7) were associated with preeclampsia while a high fruit intake was protective (OR 0.6; 95% CI 0.4-

1.0). Interaction tests confirmed that the association between (i) placental growth factor and (ii) mean arterial blood pressure with preeclampsia differed in obese and lean women (p=0.04 and p=0.009, respectively).

Conclusion: Obese and lean women have different clinical and biomarker phenotypes in early pregnancy associated with preeclampsia. The association between placental growth factor and preeclampsia is stronger in obese women compared to lean women. Variations in the prevalence of obesity between study populations may explain the considerable heterogeneity in performance of prediction models previously reported. Obesity should be considered independently in development of prediction algorithms for preeclampsia.

Key words: Preeclampsia, Obesity, Biomarkers, Body mass index.

Introduction

Obesity amongst pregnant women has increased in parallel with global trends and is associated with adverse pregnancy outcomes for both mother and infant.[1, 2] Amongst the most prevalent of the complications is the heightened risk of preeclampsia.[2, 3] Preeclampsia, characterized by the onset of hypertension in pregnancy and proteinuria, affects 2-7% of pregnancies and occurs more frequently in nulliparous women.[4] This serious disorder can lead to multi-organ failure in the mother, eclamptic seizures and maternal death.[5] In the infant, increased mortality and morbidity is associated with fetal growth restriction and iatrogenic premature delivery.[5]

Whilst there is no known cure for preeclampsia other than delivery, early risk assessment enables heightened surveillance and pharmacological intervention with low dose aspirin or calcium supplementation.[6, 7] Major effort has therefore been directed towards risk assessment for preeclampsia, leading to description of algorithms combining known clinical risk factors with plasma biomarkers.[8, 9] Biomarkers shown to have promise include placenta derived angiogenic and anti-angiogenic factors, notably placental growth factor (PlGF) and soluble fms-like tyrosine kinase (sFlt-1), which reflect the central role of placental dysfunction in the etiology of the disease.[9, 10]

The association between obesity and preeclampsia remains incompletely understood. Differences in the metabolic profile between obese and lean pregnant women, including insulin resistance, elevated lipids, raised inflammatory mediators and related endothelial dysfunction has led to the suggestion that these maternal variables may play an integral role in the etiology of preeclampsia, potentially through an increase in the susceptibility of the maternal vasculature to placentally derived factors.[11, 12] We hypothesized that early pregnancy clinical and biochemical risk factors for later development of preeclampsia might differ between obese and normal weight pregnant women.

We have tested this hypothesis in the SCOPE cohort, a prospective study of 5690 nulliparous pregnant women of whom 15% were clinically obese.[8, 13]

Materials and Methods

SCOPE (Screening for Pregnancy Endpoints Study) is a multicenter international cohort study involving centers in Auckland, New Zealand; Adelaide, Australia; London, Manchester and Leeds, UK; and Cork, Ireland. SCOPE, described in detail elsewhere, is a prospective cohort that recruited healthy nulliparous women with singleton pregnancy.[13] Women were excluded if they were at high risk of preeclampsia, small for gestational age (SGA) or preterm birth because of underlying medical conditions including pre-gestational diabetes, gynecological history or had at least three previous miscarriages or terminations of pregnancy. In addition, pregnancies where the fetus was known to have a major anomaly or abnormal karyotype prior to recruitment, or those that received interventions that may modify pregnancy outcome were not eligible. Women were recruited between 14-16 weeks' gestation. Extensive information on socio-demographic, clinical characteristics and maternal physical examination were collected together with blood samples that were processed and stored at -80^{o}C for later analysis. At 19-21 weeks', women returned for clinical assessment and an ultrasound scan was performed, which included uterine artery Doppler waveform analysis. Women were followed until 30 days postpartum and data was recorded for any event during pregnancy, delivery or neonatal period.

Ethical approval was obtained from local ethics committees (New Zealand AKX/02/00/364, Australia REC 1712/5/2008, London, Leeds and Manchester 06/MRE01/98 and Cork ECM5 (10) 05/02/08), and all women provided written informed consent.

In the present study, miscarriage or termination of pregnancy before 20 weeks' gestation and women with unknown preeclampsia status were excluded. Using WHO criteria,

participants in SCOPE were classified into body mass index (BMI) groups of lean (BMI 20-25kg/m^2) and obese women (BMI≥30kg/m^2).[14] Underweight (BMI<20kg/m^2) or overweight women (BMI 25-30kg/m^2) were excluded from the analysis. Cases were lean or obese women who developed preeclampsia with reference groups being women who did not develop preeclampsia in the same BMI category.

The primary outcome was preeclampsia, defined as systolic blood pressure ≥140mmHg or diastolic blood pressure ≥90mmHg, or both, on at least two occasions four hours apart after 20 weeks' gestation but before the onset of labor, or postpartum, with either proteinuria (24 hour urinary protein ≥ 300mg or spot urine protein:creatinine ratio ≥30mg/mmol, or urine dipstick protein ≥++ in a midstream urine specimen) or any multisystem complication of preeclampsia.[8, 15] Multisystem complications included any of the following: acute renal insufficiency defined as a new increase in serum creatinine concentration ≥100μmol/L antepartum or >130μmol/L postpartum; liver dysfunction, defined as raised aspartate transaminase or alanine transaminase concentration, or both, >45IU/L and/or severe right upper quadrant or epigastric pain or liver rupture; neurological symptoms including eclampsia, imminent eclampsia (severe headache with hyper-reflexia and persistent visual disturbance), or cerebral hemorrhage; and hematological disorders including thrombocytopenia (platelets <100×10^9/L), disseminated intravascular coagulation, or hemolysis.[8]

For the selection of clinical factors, firstly we excluded variables that were either not easily applicable for routine practice or had missing data for more than 10% of the population.[8] Previously identified highly relevant clinical predictors of preeclampsia from this cohort were selected.[13] In addition variables with $p<0.1$ in univariate comparison of obese women with and without preeclampsia were considered. A list of 37 variables was reviewed and, based on previous knowledge of association with preeclampsia, likelihood of routine availability and applicability in clinical practice, and management of variables to avoid collinearity, 9 clinical

193 factors were selected for analysis. These variables were: education level (<12 years), family
194 history of preeclampsia or gestational hypertension, any family history of thrombotic disease
195 (venous thromboembolism or cerebrovascular accident), high fruit intake (≥ 3 pieces of fruit
196 per day at 14-16 weeks'), maternal BMI, hip circumference, arm circumference and mean
197 arterial blood pressure (MAP) at 14-16 weeks' gestation, and uterine artery resistance index
198 (RI) at 19-21 weeks' gestation (adjusted for gestational age using multiples of median (MoM)).
199 A total of 47 biomarkers were measured in plasma samples taken at 14-16 weeks'
200 gestation in the original SCOPE preeclampsia prediction study[13] based on a priori knowledge
201 of (i) an association with preeclampsia, (ii) a biological role in placentation, or (iii) a role in
202 cellular mechanisms involved in the pathogenesis of preeclampsia, for example, angiogenesis
203 and inflammation. In addition, 7 further biomarkers were measured for the purpose of this
204 study based on (i) association with obesity, (ii) biological plausibility of association with
205 preeclampsia, and (iii) a role in glucose and lipid metabolism (Table 1). Of the total 54
206 biomarkers measured between 14 to 16 weeks', 10 with ≥40% of values outside the limit of
207 detection were not considered further. The biomarkers (Table A.1) and relevant methods are
208 described in Appendix A1. Measurements for the remaining 44 biomarkers were included in
209 analyses. Data are shown in Table A.2.
210 The statistical analysis was performed separately for lean and obese women.
211 Descriptive statistics were performed using mean (SD), median (IQR) and number of
212 observations (proportion), as appropriate. Management of clinical and biomarkers were
213 previously described in the original study and the additional biomarkers measured were
214 managed accordingly.[13] In summary, no further transformation was required for clinical
215 factors; all biomarkers were log transformed and an additional 10 biomarkers were converted
216 to MoM adjusted for gestational age to account for variation by gestational age prior to log-
217 transformation (brain natriuretic peptide (BNP), fas cell surface death receptor (FAS), nephrin,

plasminogen activator inhibitor 2 (PAI-2), pregnancy associated plasma protein A (PAPP-A), PlGF, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides); for both clinical factors and biomarkers no transformation was required while assessing for linearity (Table A.1). In both the BMI categories of obese and lean women, comparison between women with and without preeclampsia was performed using t-test, Mann Whitney test, Chi squared test and Fisher exact test as appropriate. Univariate logistic regression was performed to assess clinical and biomarker risk factors for preeclampsia in both BMI groups. Factors with $p<0.05$ on univariate analysis were included in a multivariate model. Likelihood ratio tests were used to test interaction between identified risk factors from multivariate analysis and obesity and the risk of preeclampsia. Lean and obese women were combined for these interaction analyses. A sensitivity analysis was performed restricted to term preeclampsia to confirm the findings were not being largely driven by preterm disease.

All analysis was performed in STATA software, version 13.0 (StataCorp LP, College Station, Texas).

Results

Of the 5690 participants recruited to the study, 67 (1.2%) were excluded due to protocol violation or outcome data was not available for this analysis. Data from an additional 31 (0.5%) women were excluded because of miscarriage/termination of pregnancy (Figure 1). The remaining 5592 participants were classified into underweight (83; 1.5%), normal BMI (lean women) (3106; 55.5%), overweight (1536; 27.1%) and obese (834; 14.9%). The prevalence of preeclampsia was 1.2% (n=1) in underweight, 3.4% (n=105) in lean, 6.1% (n=95) in overweight and 9.2% (n=77) in obese women (chi-squared test for trend $p<0.001$). In this cohort, the proportion of term preeclampsia was similar amongst obese women (80%, n=61) and lean women (73%, n=77) ($p=0.37$).

The demographic characteristics of women who developed preeclampsia according to BMI group are shown in Table 2. Women who developed preeclampsia were more likely to have a family history of preeclampsia or gestational hypertension, irrespective of BMI group. Lean women with preeclampsia had higher MAP at recruitment (14-16 weeks' gestation) compared to women without preeclampsia (81±8 *vs.*77±7mmHg, $p<0.001$), but in obese women the difference was smaller and a non-significant trend was observed (86±8 *vs.* 84±8 mmHg, $p=0.06$) (Table 2). The severity of hypertension in lean and obese women with preeclampsia was similar and women with preeclampsia were more likely to have their labour induced (Table 3). Women with preeclampsia in both groups were also more likely to deliver earlier and by caesarean section, as compared to women without preeclampsia (Table 3). Median birthweight of infants born to women with preeclampsia was lower, independent of BMI. The neonatal SGA rate and need for NICU admission was also increased 2-3 fold in women with preeclampsia in both groups.

In univariate analysis, the clinical factors and biomarkers associated with preeclampsia in lean and obese women obtained as recorded or measured at 14-16 weeks' gestation are reported in Table 4. In lean women, factors associated with preeclampsia were family history of preeclampsia or gestational hypertension, greater maternal arm circumference, higher MAP, LDL-cholesterol, cystatin C and endoglin at 14-16 weeks', and uterine artery RI at 19-21 weeks', while a high fruit intake was protective. For obese women the factors associated with development of preeclampsia were family history of preeclampsia or gestational hypertension, family history of thrombotic disease, higher BMI and hip circumference, and lower concentration of adiponectin, HDL-cholesterol, atrial natriuretic peptide (ANP)-propeptide, brain natriuretic peptide (BNP) and PlGF. Also, uterine artery RI at 19-21 weeks' was associated with preeclampsia in obese women (Table 4).

The only risk factors identified in obese women in the multivariate model were family history of thrombotic disease (OR 2.5; 95%CI 1.4-4.5), lower PlGF (OR 1.8; 95%CI 1.3-2.4; per 1 log of MoM unit) and uterine artery RI at 19-21 weeks' (OR 1.3; 95%CI 1.0-1.6) (Table 5). These differed from risk factors identified in lean women which were maternal increase in MAP by 5 mmHg at 14-16 weeks' (OR 1.5; 95%CI 1.3-1.7), family history of preeclampsia or gestational hypertension (OR 1.8; 95%CI 1.1-2.9), higher cystatin C (OR 2.0; 95%CI 1.2-3.5) and endoglin at 14-16 weeks' (OR 1.8; 95%CI 1.1-2.8), and uterine artery RI (OR 1.4; 95%CI 1.2-1.7). High fruit intake was associated with a reduced chance of preeclampsia (OR 0.6; 95%CI 0.4-1.0). The interaction test showed that the effect of MAP (p=0.009) and PlGF (p=0.04) on preeclampsia was different in obese and lean women (Table 6). Sensitivity analysis which excluded pre-term preeclampsia, demonstrated similar associations between PlGF and risk of preeclampsia in obese women and raised MAP and risk of preeclampsia in lean women.

Comment

This study supports our hypothesis that pathways leading to preeclampsia differ in obese and lean women. Metz *et al.* have similarly shown recently that biomarker analysis in early pregnancy from sub groups of at risk women identifies different markers in each group, but a subgroup of obese women was not considered in their analysis.[16] In our study, clinical and biomarker risk factors for preeclampsia measured at 14-16 weeks' gestation differed in obese and lean women and these differences are not explained by gestational age at development of preeclampsia. Lower plasma PlGF had a stronger association with preeclampsia in obese than in lean women (Table 4), and a higher MAP at 14-16 weeks' gestation was more strongly associated with preeclampsia in lean women.

PlGF is a member of the vascular endothelial growth factors family (VEGF) of angiogenic factors, and placental expression is associated with placental growth and

development. Many previous studies have identified low PlGF in early pregnancy as a risk factor for early onset (usually <34 weeks') preeclampsia, implicating impaired placentation.[9, 13, 17] It is suggested that PlGF and its biological activity is reduced by hypoxia induced placental synthesis of the soluble sFlt-1, which binds PlGF.[18] Our unexpected result was that the strong association of PlGF in early pregnancy with preeclampsia was restricted to obese women and, on sensitivity analysis, PlGF remained associated with term preeclampsia. As the majority of cases of preeclampsia amongst obese women had term disease, this raises the possibility that the lower PlGF levels prior to preeclampsia may reflect not only defective placental angiogenesis in early pregnancy but an alternative pathological mechanism.

We are aware of only one relevant study that has assessed the relationship of PlGF and preeclampsia according to BMI. In a report confined to early onset preeclampsia, Ghosh *et al.* described that in overweight and obese women (BMI range 25.8–32.7kg/m^2) PlGF <144pg/ml measured at 20-22 weeks' gestation had a higher odds ratio for early preeclampsia (OR 7.6; 95%CI 5.3-10.1) compared to normal/underweight women (OR 2.95; 95%CI 1.7-4.3), although statistical comparison between these two estimates was not provided and the number of events was small (14 cases of early onset preeclampsia in the overweight/obese group and 15 in normal/underweight group).[19] Taken together with results of the present study where low PlGF was associated with preeclampsia in women with predominantly term disease, it appears that low PlGF in obese women may be a feature of adiposity rather than placental function. PlGF is expressed in numerous tissues other than the placenta,[20] and an effect of obesity related factors, eg adipokines, on extra-placental sites of synthesis cannot be ruled out. Previously, it has been suggested that systemic inflammation and endothelial dysfunction associated with obesity could enhance susceptibility for preeclampsia,[11, 12] and systemic inflammation was hypothesized by Gosh *et al.* as a mechanism for the stronger association of low PlGF with early onset disease in overweight/obese women.[19] It is of interest that Cindrova-Davies *et al.* have

317 reported that increased concentrations of sFlt-1 enhance the responsiveness *in vitro* of vascular
318 endothelial cells to cytokines and it could be hypothesized that a complex interaction between
319 sFlt-1 and obesity related adipokines causes vascular endothelial dysfunction including
320 reduced PlGF synthesis, and increases the risk of preeclampsia.[21]

321 Consistent with our previous reports, risk factors for preeclampsia in lean women were
322 similar to those in the analysis of the full cohort.[8, 13] Blood pressure in early pregnancy was a
323 stronger factor associated with preeclampsia in lean women than in the obese group, where it
324 was consistently raised independent of outcome (Table 3). A family history of preeclampsia,
325 high fruit intake at 15 weeks' and uterine artery Doppler resistance index at 20 weeks' were
326 previously reported to be associated with preeclampsia in the SCOPE study participants.[8, 13] In
327 the present study which addressed associations of biomarkers with preeclampsia, irrespective
328 of gestation at onset of disease, we found a weak association with low PlGF in lean women. In
329 the full SCOPE cohort PlGF was a predictor for early onset preeclampsia but not for term
330 disease.[13] Separate analysis of early onset disease was not performed in the present study
331 because of the small number of events in the lean and high BMI sub-groups.

332 In the present study, early pregnancy cystatin C was increased in lean women who
333 developed preeclampsia, in agreement with previous reports with women of unselected BMI
334 from other groups.[22, 23] Amongst other metabolic roles, cystatin C is a marker of renal function.
335 Cystatin C is also expressed in trophoblast and is suggested to play a role in placentation by
336 inhibiting cathepsins B and L.[22, 24] As it is recognized that obese non-pregnant individuals have
337 higher cystatin C than lean subjects,[25] this may explain the lack of association with
338 preeclampsia in obese women. We also found evidence of a weak association between endoglin
339 and preeclampsia in lean women. Previous studies reported endoglin to be a better marker of
340 preeclampsia when measured later than in this study, i.e. in the late second trimester (after 19
341 weeks').[26]

342 The strengths of this study are the sample size, the high quality of the data collected
343 and the low number of missing datapoints. Importantly, a wide range of biomarkers involved
344 in different biological pathways were measured which has enabled substantiation in this study,
345 and the previous report of the hypothesis that preeclampsia is a heterogeneous condition with
346 different sub-phenotypes.[13] A limitation of this study includes lack of measurement of sFlt-1,
347 however evidence suggests that the association of sFlt-1 with preeclampsia only becomes
348 evident after 20 weeks' gestation.[10] Further limitations include the small number of cases of
349 women with early onset preeclampsia, which precluded subgroup analysis. It should be noted
350 that much larger studies than SCOPE (5690 nulliparous women, 278 cases of preeclampsia)
351 would be needed to allow statistically sound distinction between early and late disease between
352 lean and obese participants. It would also be of interest in any future analyses to consider
353 overweight women in comparison to lean or obese groups.
354 In summary, the novelty of the present study is that low plasma PlGF in early pregnancy
355 was associated with the later development of preeclampsia in obese women, but not in lean
356 women. Given that most prediction models published include PlGF,[9, 13, 27] we suggest that the
357 considerable heterogeneity between studies may be explained at least in part by differences in
358 prevalence of obesity in study populations. In contrast, elevated MAP, cystatin C and endoglin
359 at 15 weeks' were associated with preeclampsia amongst lean women. We conclude that
360 obesity should be considered independently in development of prediction algorithms for
361 preeclampsia.

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439 Table 1. Biomarkers assessed in plasma from women at 14-16 week's gestation.

Biomarkers measured	
Adam-9 (Disintegrin and metalloproteinase domain-containing protein 9)	Interleukin 1 receptor antagonist (IL-1ra)
Adiponectin [a]	Kunitz type protease inhibitor 2 (HAI-2)
Angiogenin	LDL-cholesterol [a]
Arginase-1	Leptin
Arginase-2	Leptin receptor
Atrial natriuretic peptide (ANP)-propeptide	Macrophage migration inhibitory factor (MIF)
Big Endothelin-1	Matrix metalloproteinase-9 (MMP-9)
Brain natriuretic peptide (BNP)	Nephrin
C-Met	Neutrophil gelatinase-associated lipocalin (NGAL)
C-reactive protein (CRP)	Pentraxin-3
C-X-C motif chemokine 10 (CXCL 10)	Periostin
Carboxypeptidase A4 (CPA-4) precursor	Placental growth factor (PlGF)
Caspase-3	Placental growth hormone (PlGH) [a]
Chemokine (C-C motif) ligand 23 (CCL23)	Plasminogen activator inhibitor 1 (PAI-1)
Cholesterol (total) [a]	Plasminogen activator inhibitor 2 (PAI-2)
Cystatin C	Podocalyxin
Elafin	Pregnancy associated plasma protein A (PAPP-A)
Endoglin	Procalcitonin (PCT)
Endothelial cell-selective adhesion molecule (ESAM-1)	ST2
Ephrin-receptor-2	TIMP metallopeptidase inhibitor 1 (TIMP-1)
Factor inhibiting hypoxia inducible factor 1α (FIH)	Transforming growth factor (TGF) β receptor 2
Fas cell surface death receptor (FAS)	Triglycerides [a]
Fas ligand (Fas L)-soluble	Tumour necrosis factor receptor 1a (TNFR1a)
Fetal Haemoglobin (HbF)	Vascular endothelial growth factor C (VEGF-C)
HDL-cholesterol [a]	Vascular endothelial growth factor receptor 1 (VEGFR1)
Insulin [a]	Visfatin
Intercellular adhesion molecule-1 (ICAM-1)	WAP four disulfide core domain protein 2 (HE4)

440 [a] Biomarkers measured for the purpose of this study.

441 Table 2. Demographic characteristics according to BMI and PET status.

	LEAN			OBESE		
	Non-PE (n=3001)	PE (n=105)		Non-PE (n=757)	PE (n=77)	
Characteristics	**Mean ±SD or n (%)**	**Mean ±SD or n (%)**	**p**	**Mean ±SD or n (%)**	**Mean ±SD or n (%)**	**p**
Age	28.8 ±5.4	28.1 ±6.1	0.16	28.0 ±5.6	27.2 ±5.8	0.29
Ethnicity						
European	2690 (90)	98 (93)		692 (91)	69 (90)	
Asian	120 (4)	1 (1)	0.45	5 (1)	2 (3)	0.24
Indian	82 (3)	3 (3)		9 (1)	0 (0)	
Other	109 (4)	3 (3)		51 (7)	6 (8)	
Educational level (<12 years)	1089 (36)	45 (43)	0.17	325 (43)	38 (49)	0.28
Full or part time work	2583 (86)	86 (82)	0.23	615 (81)	62 (81)	0.88
Primigravida	2353 (78)	87 (83)	0.28	565 (75)	53 (69)	0.27
Previous miscarriage	380 (13)	10 (10)	0.34	111 (15)	15 (20)	0.26
Previous termination	309 (10)	7 (7)	0.23	93 (12)	9 (12)	0.88
FH of preeclampsia or GH	375 (13)	23 (22)	0.005	127 (17)	21 (27)	0.02
Smoking at 14-16 wks	288 (10)	12 (11)	0.53	118 (16)	9 (12)	0.36
BMI [a]	22.3 (21.0-23.6)	22.7 (21.3-23.5)	0.21	33.1 (31.2-36.0)	33.6 (31.6-38.2)	0.11
MAP at 14-16 wks, mmHg	77 ±7	81 ±8	<0.001	84 ±8	86 ±8	0.06

442 Abbreviations: BMI - body mass index, BP - blood pressure, DM - diabetes mellitus, FH - family history, GH - gestational hypertension, wks -

443 weeks.

444

445 [a] Median (IQR) and Mann-Whitney test shown.

446 Table 3. Pregnancy outcome according to BMI and PET status.

Outcomes	LEAN			OBESE		
	Non-PE (n=3001)	PE (n=105)		Non-PE (n=757)	PE (n=77)	
	Mean ±SD or n (%)	Mean ±SD or n (%)	p	Mean ±SD or n (%)	Mean ±SD or n (%)	p
Maternal						
Maximum systolic BP, mmHg (n=3921)	121 ±13	156 ±15	<0.001	132 ±13	161 ±15	<0.001
Maximum diastolic BP, mmHg (n=3921)	76 ±10	99 ±10	<0.001	81 ±10	103 ±9	<0.001
Induction of labour (n=3882)	876 (30)	55 (53)	<0.001	276 (37)	48 (62)	<0.001
Mode of delivery						
Spontaneous vaginal (n=3938)	1422 (47)	39 (37)	0.04	327 (43)	25 (33)	0.07
Assisted vaginal (n=3938)	875 (29)	23 (22)	0.11	131 (17)	7 (9)	0.07
Caesarean section (n=3938)	702 (23)	43 (41)	<0.001	299 (40)	45 (58)	0.001
Fetal						
GA at delivery [a]	40 (39-41)	39 (37-40)	<0.001	40 (39-41)	39 (37-40)	<0.001
Preterm delivery (GA<37w)	148 (5)	28 (27)	<0.001	42 (6)	16 (21)	<0.001
Birthweight, grams [a]	3420 (3095-3740)	2990 (2490-3470)	<0.001	3530 (3180-3865)	3270 (2725-3710)	<0.001
SGA [b] (<10th centile) (n=3937)	286 (10)	26 (25)	<0.001	97 (13)	22 (29)	<0.001
LGA [b] (>90th centile) (n=3937)	292 (10)	6 (6)	0.17	64 (9)	9 (12)	0.34
Apgar<7 at 5th minute (n=3900)	31 (1)	1 (1)	0.93	9 (1)	2 (3)	0.29
NICU admission (n=3940)	294 (10)	28 (27)	<0.001	107 (14)	22 (29)	0.001
Perinatal death (n=3940)	16 (1)	0 (0)	0.45	4 (1)	2 (3)	0.04

447 Abbreviations: BMI - body mass index, BP - blood pressure, GA - gestational age, GDM - gestational diabetes mellitus, LGA - large for gestational
448 age, NICU - neonatal intensive care unit, OGTT - Oral glucose tolerance test, PE - preeclampsia, SGA - small for gestational age.
449 [a] Median (IQR) and Mann-Whitney test shown; [b] Customized centiles used, which adjusted for ethnicity, parity, maternal weight and height, infant
450 gender and gestation at delivery.

451 Table 4. Clinical factors and plasma biomarkers associated with preeclampsia in univariate analysis.

Factors	LEAN			OBESE		
	Non-PE (n=3001)	PE (n=105)		Non-PE (n=757)	PE (n=77)	
	Mean ±SD [a] or n (%)	Mean ±SD [a] or n (%)	p	Mean ±SD [a] or n (%)	Mean ±SD [a] or n (%)	p
Clinical factors at 14-16 weeks						
Educational level (<12 years)	1089 (36)	45 (43)	0.17	325 (43)	38 (49)	0.28
FH of preeclampsia or GH	375 (13)	23 (22)	0.005	127 (17)	21 (27)	0.02
FH of thrombotic disease	317 (11)	15 (14)	0.23	85 (11)	20 (26)	<0.001
High fruit intake	1282 (43)	31 (30)	0.007	223 (30)	19 (25)	0.38
Maternal BMI, m/kg^2 [a]	22.3 (21.0-23.6)	22.7 (21.3-23.5)	0.21	33.1 (31.2-36.0)	33.6 (31.6-38.2)	0.11
Maternal Hip, cm [a]	95 (91-99)	97 (92-100)	0.08	118 (112-125)	119 (113-130)	0.07
Maternal arm circumference, cm [a]	26 (24-27)	27 (25-28)	0.008	34 (31-36)	34 (32-38)	0.16
MAP, mmHg	77 ±7	81 ±8	<0.001	84 ±8	86 ±8	0.06
Biomarkers at 14-16 weeks' [a b]						
Adiponectin, ng/ml (n=3905)	4548 (3467-5809)	4322 (3446-5527)	0.95	3782 (2892-4965)	3369 (2550-4737)	0.03
HDL-cholesterol, MoM [c] (n=3929)	1.03 (0.89-1.18)	1.00 (0.88-1.18)	0.30	0.92 (0.76-1.06)	0.89 (0.71-1.04)	0.03
ANP, ng/ml (n=3906)	0.5 (0.2-0.9)	0.4 (0.2-0.9)	0.80	0.4 (0.2-0.8)	0.3 (0.2-0.6)	0.05
BNP, MoM [c] (n=3907)	1.02 (0.73-1.46)	1.01 (0.70-1.42)	0.36	0.94 (0.63-1.34)	0.85 (0.51-1.29)	0.03
Cystatin, ng/ml (n=3906)	1813 (1454-2233)	1860 (1559-2375)	0.04	2167 (1693-2806)	2155 (1663-2818)	0.63
Endoglin, ng/ml (n=3904)	16.9 (13.3-21.8)	18.8 (14.4-25.5)	0.02	12.9 (9.8-16.9)	13.6 (9.3-19.5)	0.34
PlGF, MoM [c] (n=3905)	1.01 (0.56-1.72)	0.71 (0.41-1.68)	0.06	1.00 (0.55-1.70)	0.66 (0.31-1.31)	<0.001
Ultrasound at 19-21 weeks'						
Uterine artery RI, MoM [c] (n=3937)	1.00 ±0.17	1.07 ±0.21	<0.001	1.01 ±0.17	1.07 ±0.21	0.004

452 Abbreviations: ANP - Atrial natriuretic peptide, BMI - body mass index, BNP - Brain natriuretic preptide, FH - family history, GH - gestational
453 hypertension, MAP - mean arterial blood pressure, PlGF - placental growth factor, RI - resistance index.
454 [a] Mean (SD) or median (IQR), as appropriate; [b] Biomarkers were log transformed and t test was performed; [c] Multiple of median (MoM) for
455 gestational age.

Table 5. Risk factors for preeclampsia according to BMI.

Risk factors	Univariate OR (95%CI)	p	Multivariate [a] OR (95%CI)	p
Lean women				
FH of preeclampsia or GH	1.96 (1.22 - 3.16)	0.005	1.76 (1.08 - 2.86)	0.02
High fruit intake	0.56 (0.37 - 0.86)	0.008	0.61 (0.40 - 0.95)	0.03
Maternal arm circumference	1.12 (1.03 - 1.23)	0.01	1.09 (0.99 - 1.19)	0.08
MAP [b]	1.48 (1.31 - 1.69)	<0.001	1.45 (1.27 - 1.66)	<0.001
Cystatin [c]	1.76 (1.04 - 2.98)	0.04	2.01 (1.16 - 3.48)	0.01
Endoglin [c]	1.65 (1.07 - 2.55)	0.02	1.79 (1.14 - 2.79)	0.01
Uterine artery IR [b]	1.40 (1.16 - 1.68)	<0.001	1.40 (1.16 - 1.69)	0.001
Obese women				
FH of preeclampsia or GH	1.86 (1.09 - 3.18)	0.02	1.61 (0.90 - 2.86)	0.11
FH of thrombotic disease	2.77 (1.59 - 4.84)	<0.001	2.48 (1.38 - 4.45)	0.003
Maternal BMI	1.06 (1.02 - 1.11)	0.007	1.03 (0.94 - 1.14)	0.48
Maternal Hip	1.03 (1.00 - 1.05)	0.02	1.01 (0.97 - 1.05)	0.71
Adiponectin [c]	1.72 (1.04 - 2.86)	0.03	1.48 (0.86 - 2.55)	0.16
HDL-cholesterol [c]	3.21 (1.14 - 9.04)	0.03	1.84 (0.59 - 5.74)	0.29
ANP [c]	1.25 (1.00 - 1.57)	0.05	0.98 (0.77 - 1.26)	0.90
BNP [c]	1.54 (1.04 - 2.29)	0.03	1.41 (0.92 - 2.16)	0.11
PlGF [c]	1.82 (1.37 - 2.42)	<0.001	1.77 (1.29 - 2.42)	<0.001
Uterine artery IR [b]	1.39 (1.11 - 1.73)	0.004	1.28 (1.01 - 1.61)	0.04

Abbreviations: ANP - Atrial natriuretic peptide, BMI - body mass index, BNP - Brain natriuretic preptide, FH - family history, GH - gestational hypertension, MAP - mean arterial blood pressure, PlGF - placental growth factor, RI - resistance index.

[a] Observation per model: 3082 normal BMI and 824 obese; [b] Per 5 mmHg (MAP) and per 1 standard deviation of multiples of median (uterine artery RI); [c] Per 1 log unit. Adiponectin, HDL-cholesterol, ANP, BNP and PlGF were inverted and effect of lower value is shown.

463 Table 6. Risk factors for preeclampsia according to BMI category and interaction test.

Risk factors	LEAN OR (95% CI) [a]	OBESE OR (95% CI) [a]	Interaction [b] p
Clinical factors			
FH of preeclampsia or GH	1.96 (1.22 - 3.16)	1.86 (1.09 - 3.18)	0.88
FH of thrombotic disease	1.41 (0.81 - 2.47)	2.77 (1.59 - 4.84)	0.09
High fruit intake	0.56 (0.37 - 0.86)	0.78 (0.46 - 1.35)	0.34
MAP [c]	1.48 (1.31 - 1.69)	1.14 (0.99 - 1.32)	0.009
Biomarkers			
Cystatin [d]	1.76 (1.04 - 2.98)	0.88 (0.51 - 1.51)	0.07
Endoglin [d]	1.65 (1.07 - 2.55)	1.26 (0.79 - 1.99)	0.39
PlGF [d]	1.25 (1.00 - 1.56)	1.82 (1.37 - 2.42)	0.04
Ultrasound			
Uterine artery IR [c]	1.39 (1.11 - 1.73)	1.39 (1.11 - 1.73)	0.96

464 Abbreviations: BMI - body mass index, FH - family history, GH - gestational hypertension,
465 MAP - mean arterial blood pressure, PlGF - placental growth factor, and RI - resistance index.
466 [a] Unadjusted odds ratio per BMI category (strata specific); [b] Likelihood ratio test between a
467 model of an exposure of interest and BMI status with and without interaction; [c] Per 5 mmHg
468 (MAP) and per 1 standard deviation of multiple of median (uterine artery RI); [d] Per 1 log unit.
469 PlGF was inverted and effect of lower PlGF is shown.

Figure 1. Study population.