Early Pregnancy-Associated Plasma Protein A Concentrations are Associated with Third Trimester Insulin Sensitivity

Short title: PAPP-A associations with insulin sensitivity

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Précis: Pregnancy-associated plasma protein A concentrations, measured at the start of the second trimester, were associated with subsequent insulin sensitivity, blood pressure and gestational diabetes. Petry *et al.*

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Abstract

Context: First or early second trimester pregnancy-associated plasma protein-A (PAPP-A) concentrations have previously been shown to be lower in women who subsequently develop gestational diabetes (GDM) and gestational hypertension. Objective: We therefore sought to investigate why circulating PAPP-A concentrations are related to the subsequent risk of GDM and gestational hypertension. **Patients, Design and Setting:** We measured serum PAPP-A concentrations around week 15 of pregnancy and related these to indices derived from week 28 oral glucose tolerance tests (OGTT) and blood pressures across pregnancy in the Cambridge Baby Growth Study cohort. **Results:** Increased PAPP-A concentrations were associated with reduced GDM risk (odds ratio 0.623 (0.453, 0.856), P=3.5 x 10^{-3}, n=777) and reduced mean arterial blood pressures (β=-0.202– -0.177, P=1.7–6.9 x 10^{-3}, n=347–355). They were also negatively associated with week 28 fasting (β=-0.149, P=6.6 x 10^{-4}, n=777) and 60 minute (β=-0.188, P=1.5 x 10^{-5}, n=777) OGTT glucose concentrations. These associations were underpinned by the strong associations between increased week 15 PAPP-A concentrations and decreased week 28 insulin resistance (HOMA IR: β=-0.319, P=1.7 x 10^{-13}, n=768), and increased insulin secretion relative to insulin sensitivity (insulin disposition index: β=0.202, P=6.5 x 10^{-6}, n=731). **Conclusions:** These results suggest that links between PAPP-A concentrations in early pregnancy and subsequent glucose concentrations and blood pressures may be mediated by changes in insulin sensitivity (and secretion).

Keywords: gestational diabetes, gestational hypertension, pregnancy, placental hormones.

Introduction

Pregnancy associated plasma protein A (PAPP-A) is produced by the placenta in pregnancy and is one of only four placentally-derived proteins found in the maternal circulation in high concentrations. (1) It largely circulates as a heterotetramer consisting of two PAPP-A subunits covalently bound to two subunits of the preform of eosinophil major basic protein. (2) PAPP-A's principal function as a metalloproteinase of the metzincin superfamily, appears to be the cleavage of circulating insulin-like growth factor binding protein 4 (IGFBP-4), although there are suggestions that PAPP-A may also be involved in the cleavage of IGFBP -2 (3) and -5. (4) It is only the uncomplexed, dimeric form of PAPP-A, the proportion of which varies throughout pregnancy but is around 1 % of circulating concentrations, which displays this proteolytic activity. (5) Whichever binding protein uncomplexed PAPP-A cleaves it would appear to have a role in regulating IGF bioavailability in pregnancy. (6) This is important as the IGF axis plays a critical role in fetal growth, and placental growth and function during pregnancy. (7)

Measurement of circulating PAPP-A concentrations is used, along with nuchal translucency scanning and free β-human chorionic gonadotrophin concentration measurement, in first trimester screening for fetal chromosomal abnormalities including Down Syndrome, Patau Syndrome and Edward Syndrome (trisomies 21, 13 and 18, respectively). In pregnancy circulating PAPP-A concentrations are also influenced by such factors as gestational age (increasing in a curvilinear fashion with gestational age), maternal weight (decreasing with increasing weight) and height (increased with increasing height), ethnicity (higher in women of Afro-Caribbean, East Asian and South Asian racial origin), method of conception (decreased in first trimester and raised in the third trimester with *in vitro* fertilisation), parity (decreased in parous women) and smoking status (decreased in smokers). (8) In addition they appear to be decreased by pre-existing type 1 and type 2 (8) diabetes. A number of studies have found associations between reduced circulating PAPP-A concentrations early in pregnancy and the subsequent development of gestational diabetes (GDM) (9-16), although these are not uniform occurrences. (17, 18) Where such associations have been found, studies have tended not to investigate mechanisms that may be underpinning these associations. Other studies have found associations between reduced circulating PAPP-A concentrations early in pregnancy and the

development of gestational hypertension (13, 19) or pre-eclampsia later in pregnancy (20, 21), conditions that are commonly linked with GDM. As both GDM and gestational hypertension can impact upon the baby's birth weight, early pregnancy circulating PAPP-A concentrations have been related to birth weight in several studies. (16, 18, 22-29) In this study we attempted to extend the relationships of early pregnancy PAPP-A concentrations with the adverse conditions of pregnancy to other markers of impaired glucose tolerance derived from the oral glucose tolerance test (OGTT) and high blood pressures. We hypothesised that the relationships were mediated by changes in IGF bioactivity.

Research Design and Methods

Cambridge Baby Growth Study

The Cambridge Baby Growth Study (prospective and longitudinal) recruited mothers attending ultrasound clinics during early pregnancy at the Rosie Maternity Hospital, Cambridge, U.K., between 2001 and the present. Blood samples were drawn at recruitment (and centrifuged, the serum separated and stored at -80 ^{o}C until analysis). There were two main phases of recruitment for the Cambridge Baby Growth Study, phase I running from 2001 until 2009, from which the samples used in this study were obtained. Blood samples were collected on average at week 15 of pregnancy at the booking clinic (n=821). Table 1 shows the clinical characteristics of the study participants who had blood samples taken. At 28 weeks of gestation, 1,074 of the mothers underwent a 75-g OGTT after an overnight fast. Venous blood was collected after fasting and at 60 min. after the glucose load for the measurement of plasma glucose concentrations (using a routine glucose oxidase–based method). Capillary whole blood glucose concentrations were measured at 0, 30, 60, 90 and 120 mins. using an Abbott Freestyle Mini (Abbott Diagnostics, Maidenhead, U.K.). Routine blood pressure measurements during three time points in pregnancy were documented from hospital notes. (30) The baby's birth weights were recorded by hospital midwives and retrieved from the hospital notes. In this cohort, 96.9 % of the offspring were of Caucasian ethnicity, 0.8 % were of mixed race, 0.6 % were black (African or Caribbean), 0.8 % were Oriental, and 0.9 % were Indo-Asian.

Disease Diagnostic Thresholds

The Cambridge Baby Growth Study participants were divided into cases and controls for GDM according to the International Association of Diabetes in Pregnancy Study Group criteria (31) using the OGTT fasting and 60 min. plasma glucose concentrations. Its prevalence in the Cambridge Baby Growth Study was 10.2 %. Evidence of gestational hypertension was sought from the hospital notes (defined using the inclusion of a diagnosis of pre-eclampsia, gestational hypertension or pregnancy-induced hypertension). Alternatively the National Institute for Health and Care Excellence criteria for defining gestational hypertension (blood pressure measurements in the second half of pregnancy >= 140 mmHg systolic or 90 mmHg diastolic blood pressure in women without chronic hypertension) (32) were used, with the exception that for our study evidence of gestational hypertension was accepted if the blood pressure cut offs were exceeded at one reading rather than at least two. Its prevalence in the Cambridge Baby Growth Study was 6.1 %. (30)

Cohort Subgroups

The 821 serum samples collected early in the second trimester of pregnancy were ordered according to their PAPP-A concentrations. The 48 samples with the lowest PAPP-A concentrations formed the "low PAPP-A" group and the 48 samples with the highest PAPP-A concentrations formed the "high PAPP-A" group. Bioactive IGF was estimated in these subgroups.

Ethical Approval

The Cambridge Baby Growth Study was approved by the local ethics committee, Addenbrooke's Hospital, Cambridge, U.K. Written informed consent was obtained from all the mothers who took part in this study.

Circulating Hormone Concentration Measurements

PAPP-A was measured by time-resolve fluoroimmunoassay (autoDELFIA, Perkin Elmer Ltd., Seer Green, U.K.). The minimum detection limit of this assay was 22.5 mg/L (5 mU/L). The intra-assay

coefficient of variation (CV) was less than 8 % and the inter-assay CV was less than 10 % throughout. Insulin was measured by enzyme-linked immunosorbent assay using a commercial kit (DSL, London, U.K.). Sensitivity was 0.26 mU/L (1.6 pmol/L). Intra-assay CVs were 4.4 and 5.1 % at 10.4 mU/L (62 pmol/L) and 35.9 mU/L (215 pmol/L), and equivalent inter-assay CVs were 8.7 and 2.9 %; this assay has no cross-reactivity with proinsulin at levels up to 9.1 µg/L (1,000 pmol/L). Bioactive IGF is a cell-based measurement, which assesses the ability of serum IGF-I and IGF-II to phosphorylate the IGF-I receptor (IGF1R) *in vitro*, using human embryonic cells transfected with cDNA of the human *IGF1R* gene (33). The serum signal is compared with a serial IGF-I dilution and expressed in µg/L. The detection of phosphorylated IGF1R in crude cell lysates was performed using a commercial kit (catalogue #DYC 1770E) from R & D Systems (Abingdon, U.K.). Sensitivity was < 0.08 µg/L. The intra-assay CV averages 6 % for the signals and 12 % for the corresponding concentrations; the long-term inter-assay CV is 20 %.

Calculations

Mean arterial blood pressure was calculated as the sum of the systolic and twice the diastolic blood pressures, all divided by three. Insulin sensitivity was estimated using the Homeostasis Model Assessment (HOMA), calculated using the week 28 circulating glucose and insulin concentrations and the online HOMA calculator (available at https://www.dtu.ox.ac.uk/homacalculator/). Insulin secretion was assessed in terms of the insulinogenic index, calculated as (insulin 60 min. − insulin 0 min.)/ (glucose 60 min. − glucose 0 min.). The insulin secretion for the given insulin sensitivity was assessed in terms of the insulin disposition index, calculated as the insulinogenic index divided by the reciprocal of the fasting insulin concentration. The areas under the whole capillary blood glucose curve (AUC) of the OGTT were calculated using the trapezoid rule. Those under receiver operating characteristic (ROC) curves were calculated using the “lroc” function of Stata. The body mass index (BMI) was calculated as the pre-pregnancy weight (kg) divided by the height (m) squared. A BMI < 25 kg/m^2 was considered lean, 25-30 was considered overweight and > 30 was considered obese.

Statistical Analysis

Associations were tested by linear regression (for continuous dependent variables) or logistic regression (for binary dependent variables). All the analyses involving PAPP-A were adjusted for the number of weeks of pregnancy when the blood samples was taken. Log-transformed data were analysed in this study when required to normalise the distribution of the residuals, as required for the linear regression. ROC curve equality was tested using Stata's "roccomp" function. Data are mean (95 % confidence interval) unless stated otherwise. $P < 0.05$ was considered statistically significant throughout. Statistical analyses were performed using Stata 13 (StataCorp LP, College Station, Texas, U.S.A.).

Results

Association with GDM and Gestational Hypertension

Maternal serum PAPP-A concentrations at week 15 were significantly associated with a protective effect on the development of GDM by week 28 of pregnancy (odds ratio (OR) 0.623 (0.453, 0.856), $p=3.5x10^{-3}$, McFadden's pseudo r^2=1.7%, n=777). The ROC curve AUC was 0.592. Adding PAPP-A to an established model for predicting GDM containing BMI and age did not improve the AUCs (going to 0.639 from 0.647 without PAPP-A; n=581; p=0.3; Fig. 1). After adjusting for pre-pregnancy BMI the statistical significance of the association between GDM and PAPP-A concentrations was lost (p=0.5). Analysing associations in lean (OR 1.164 (0.677, 1.999), p=0.6, n=434 of which 24 developed GDM), overweight (OR 0.629 (0.322, 1.228), p=0.18, n=140 of which 20 developed GDM) and obese (OR 0.545 (0.370, 0.804), $p=2.2x10^{-3}$, n=241 of which 11 developed GDM) women separately, the significant relationship was only seen in obese women. Adjusting the model for fetal sex did not alter the significance of the association between GDM and PAPP-A concentrations (OR 0.617 (0.448, 0.850), $p=3.1x10^{-3}$, pseudo r^2=1.8%, n=775), although the relationship was stronger in male (OR 0.575 (0.381, 0.867), $p=8.2x10^{-3}$, pseudo r^2=2.8%, n=407) than female (OR 0.670 (0.399, 1.123), p=0.1, pseudo r^2=1.8%, n=368) fetus pregnancies. Associations of maternal serum PAPP-A concentrations at week 15 with the subsequent development of gestational hypertension did not quite reach statistical significance in the number of participants studied (odds ratio 0.613 (0.365, 1.030), p=0.065, McFadden's pseudo r^2=2.1 %, n=361).

Association with Maternal Late Pregnancy Glucose Concentrations and Indices of Insulin Secretion and Sensitivity in the OGTT

Maternal serum PAPP-A concentrations at week 15 were significantly negatively associated with week 28 fasting plasma glucose concentrations (standardised β=-0.149, r^2=1.2 %, p=6.6x10^{-4}, n=777) and areas under the capillary blood glucose curve (standardised β=-0.144, r^2=1.1 %, p=3.2x10^{-3}, n=610) (Fig. 2a and 2b). It was also negatively associated with week 28 HOMA IR (standardised β=-0.319, r^2=6.6 %, p=1.7x10^{-13}, n=768), an association which had the largest effect size (Fig. 2c). This relationship upheld when the model was adjusted for fetal sex (standardised β=-0.324, r^2=6.8%, p=8.2x10^{-14}, n=766), and when analysing male fetus (standardised β=-0.377, r^2=9.0%, p=4.5x10^{-10}, n=402) and female fetus (standardised β=-0.268, r^2=4.5%, p=1.8x10^{-5}, n=364) pregnancies separately. Additional negative associations were found between PAPP-A concentrations and plasma glucose concentrations 60 minutes after the 75 g glucose load and the insulin disposition index (Table 2).

Association with Mean Arterial Blood Pressure in Pregnancy

Maternal serum PAPP-A concentrations at week 15 were significantly negatively associated with maternal mean arterial blood pressure at the readings taken around weeks 12, 31 and 37 of pregnancy (Table 3). Maternal mean arterial blood pressures were also significantly negatively associated with week 28 HOMA IR at weeks 12 (standardised β-coefficient=-0.219, p=1.9x10^{-6}, n=465), 31 (standardised β-coefficient=-0.264, p=7.0x10^{-9}, n=468) and 37 (standardised β-coefficient=-0.318, p=5.7x10^{-12}, n=458).

Association of Maternal PAPP-A Status with Circulating IGF Bioactivity

Those women with the highest serum PAPP-A concentrations had lower pre-pregnancy BMIs, at week 15 were more insulin sensitive at week 28 than those with the lowest PAPP-A concentrations, had slightly lower fasting glucose concentrations at week 28 and lower mean arterial blood pressures

in the second half of pregnancy (Table 4). There was no detectable difference in circulating bioactive IGF activity however.

Association with Baby's Birth Weight

Maternal serum PAPP-A concentrations at week 15 were not significantly associated with either the baby's unadjusted birth weight (standardised β=-0.005, p=0.9, n=772) or with birth weight adjusted for gestational age at birth, sex and parity (standardised β=0.009, p=0.8, n=768). However there was a significant positive association when adjustment was made for gestational age at birth, sex, maternal weight prior to pregnancy, parity, smoking and gestational age when the blood samples were taken (standardised β=0.112, p=$8.4x10^{-3}$, n=642).

Discussion

In this study we have confirmed negative associations between early pregnancy serum PAPP-A concentrations and subsequent development of GDM and high blood glucose concentrations. Our ROC curve analyses suggest that such PAPP-A measurements do not offer an advantage over established risk factors for the prediction of GDM development however. Sub-group analysis revealed that the negative association between GDM and PAPP-A was only detectable in obese women and was stronger in male fetus pregnancies. It is difficult to know if this reflects a physiological relationship in obese women/male fetus pregnancies or just a lack of statistical power in the other groups. The negative association between PAPP-A concentrations and gestational hypertension, as observed in other studies (13, 19), almost reached statistical significance in our study (presumably the reason that it did not was due to insufficient statistical power in the lower number of pregnancies studied). Negative associations with mean arterial blood pressures were, however, observed. Uniquely this study showed that the strongest association between early pregnancy PAPP-A concentrations and an index from the week 28 OGTT was with HOMA IR, suggesting that the principal reason PAPP-A is related to the future development of GDM and high blood pressures is via regulation of insulin sensitivities (high circulating glucose concentrations and blood pressures both being related to reduced insulin sensitivity). As GDM is related to relative reductions in both insulin secretion and

insulin sensitivity, it is also not surprising that another relationship was observed between the early pregnancy PAPP-A concentrations and the insulin disposition index, although the strongest relationship by far remained the one with insulin sensitivity.

The role of circulating PAPP-A in pregnancy appears to be cleavage of certain IGFBPs. Given that circulating free (35) as well as total (36) IGF-I appears to influence insulin sensitivity, we hypothesised that the strong associations observed with GDM, high blood pressures and insulin sensitivity are underpinned by PAPP-A regulating IGF bioavailability in pregnancy which in turn regulates insulin sensitivity and ultimately contributes to protection against or the development of certain adverse conditions of pregnancy. However we could not detect a difference in IGF bioactivity between women with some of the highest week 15 circulating PAPP-A concentrations and women with some of the lowest, despite having large differences in week 15 PAPP-A concentrations and week 28 insulin sensitivities. One possible explanation of this is the lack of statistical power available to us in trying to detect a difference in IGF bioactivity in only 96 samples. Alternatively PAPP-A may affect localised rather than circulating IGF-I and –II concentrations. (2) The physiology of the IGF axes and insulin sensitivity is complicated due to the presence of IGF binding proteins and differences between localised and circulating IGF concentrations. One previous pregnancy study failed to find a relationship around week 28 between either serum total IGF-I or –II concentrations and insulin sensitivity (37), whilst another more recent study found a negative association between week 28 serum total IGF-I concentrations and insulin sensitivity (38). A further study found higher weeks 24-28 total IGF-I concentrations in women with GDM (39), possibly resulting from these women having higher placental growth hormone drive which could lead to reduced insulin sensitivity despite higher circulating IGF-I concentrations (40).

A number of other studies (16, 22-28) have found positive associations between early PAPP-A and the baby's birth weight, although this finding is not universal. (18, 29) Indeed we failed to find an association when analysing raw or minimally adjusted data. However close inspection of the other studies reveals that finding such an association depends on whether PAPP-A levels were analysed in terms of multiples of the median or just unadjusted concentrations. When we adjusted our PAPP-A

concentrations for as many of the factors as that are used in the calculation of multiples of the median as we had available to us, the association with baby birth weights emerged. This was not surprising given that in our cohort the week 28 mother's insulin sensitivity and glucose concentrations, both of which were associated with week 15 PAPP-A concentrations, were related to the baby's birth weight.

Although reasonably sized, this study has a number of limitations. Firstly the number of blood pressure readings available to us was relatively modest and we could not confirm the association between early pregnancy circulating PAPP-A concentrations and the development of gestational hypertension. The fact that circulating PAPP-A concentrations had significant associations with blood pressures throughout pregnancy suggests that the study was underpowered to find such an association with gestational hypertension (dichotomous variable analyses having less statistical power than analyses using continuous variables). However the association with blood pressures tempers this limitation somewhat, and there was sufficient power to detect an association between gestational hypertension and insulin sensitivity. Secondly the PAPP-A and bioactive IGF concentration measurements were not done in the same sample as those used for the insulin and glucose concentrations. We do not know if the week 28 PAPP-A and bioactive IGF concentrations track those at week 15. However the study was designed to enhance previous findings showing first trimester PAPP-A concentrations are negatively related to the subsequent development of GDM, which has been achieved. Given the strength of the various associations it is possible that the week 15 and 28 results would be related albeit probably imperfectly (8). The final limitation is that the study was designed to replicate first trimester findings and technically the stage of pregnancy when the blood samples were adjusted to, at week 15, was in the second trimester. Although PAPP-A values would probably have been higher at week 15 than earlier in pregnancy (8) the factors that affect circulating PAPP-A concentrations in pregnancy, apart from gestational age and maternal weight and height, tend to be fixed rather than fluid (8) so the association trends are unlikely to have been affected.

In summary we confirmed associations between circulating PAPP-A concentrations in week 15 of pregnancy and the future development of GDM and high blood pressure. Underpinning these

associations was an even stronger association with insulin sensitivity, which may relate to the cleavage of IGFBPs by PAPP-A leading to increases in bioactive IGF which regulates insulin sensitivity, at least outside pregnancy (35, 36). Further studies on the effect of IGFs on insulin sensitivity in pregnancy would therefore appear warranted.

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References

1. Lin TM, Galbert SP, Kiefer D, Spellacy WN, Gall S. Characterization of four human pregnancy-associated plasma proteins. *Am J Obstet Gynecol.* 1974;118:223-236.

2. Oxvig C. The role of PAPP-A in the IGF system: location, location, location. *J Cell Commun Signal.* 2015;9:177-187.
3. Monget P, Mazerbourg S, Delpuech T, Maurel MC, Maniere S, Zapf J, Lalmanach G, Oxvig C, Overgaard MT. Pregnancy-associated plasma protein-A is involved in insulin-like growth factor binding protein-2 (IGFBP-2) proteolytic degradation in bovine and porcine preovulatory follicles: identification of cleavage site and characterization of IGFBP-2 degradation. *Biol Reprod*. 2003;68:77–86.
4. Laursen LS, Overgaard MT, Soe R, Boldt HB, Sottrup-Jensen L, Giudice LC, Conover CA, Oxvig C. Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. *FEBS Lett*. 2001;504:36–40.
5. Gyrup C, Christiansen M, Oxvig C. Quantification of proteolytically active pregnancy-associated plasma protein-A with an assay based on quenched fluorescence. *Clin Chem*. 2007;53:947-954.
6. Conover CA. Key questions and answers about pregnancy-associated plasma protein-A. *Trends Endocrinol Metab.* 2012;23:242-249.
7. Bowman CJ, Streck RD, Chapin RE. Maternal-placental insulin-like growth factor (IGF) signaling and its importance to normal embryo-fetal development. *Birth Defects Res B Dev Reprod Toxicol*. 2010;89:339-349.
8. Wright D, Silva M, Papadopoulos S, Wright A, Nicolaides KH. Serum pregnancy-associated plasma protein-A in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol*. 2015;46:42-50.
9. Beneventi F, Simonetta M, Locatelli E, Cavagnoli C, Badulli C, Lovati E, Garbin G, Genini E, Albertini R, Tinelli C, Martinetti M, Spinillo A. Temporal variation in soluble human leukocyte antigen-G (sHLA-G) and pregnancy-associated plasma protein A (PAPP-A) in pregnancies complicated by gestational diabetes mellitus and in controls. *Am J Reprod Immunol*. 2014;72:413-421.
10. Husslein H, Lausegger F, Leipold H, Worda C. Association between pregnancy-associated plasma protein-A and gestational diabetes requiring insulin treatment at 11-14 weeks of gestation. *J Matern Fetal Neonatal Med*. 2012;25:2230-2233.

11. Kulaksizoglu S, Kulaksizoglu M, Kebapcilar AG, Torun AN, Ozcimen E, Turkoglu S. Can first-trimester screening program detect women at high risk for gestational diabetes mellitus? *Gynecol Endocrinol.* 2013;29:137-140.
12. Lovati E, Beneventi F, Simonetta M, Laneri M, Quarleri L, Scudeller L, Albonico G, Locatelli E, Cavagnoli C, Tinelli C, Spinillo A, Corazza GR. Gestational diabetes mellitus: including serum pregnancy-associated plasma protein-A testing in the clinical management of primiparous women? A case-control study. *Diabetes Res Clin Pract*. 2013;100:340-347.
13. Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG*. 2000;107:1265-1270.
14. Spencer K, Cowans NJ. The association between gestational diabetes mellitus and first trimester aneuploidy screening markers. *Ann Clin Biochem*. 2013;50:603-610.
15. Syngelaki A, Kotecha R, Pastides A, Wright A, Nicolaides KH. First-trimester biochemical markers of placentation in screening for gestational diabetes mellitus. *Metabolism*. 2015;64:1485-1489.
16. Wells G, Bleicher K, Han X, McShane M, Chan YF, Bartlett A, White C, Lau SM. Maternal Diabetes, Large-for-Gestational-Age Births, and First Trimester Pregnancy-Associated Plasma Protein-A. *J Clin Endocrinol Metab*. 2015;100:2372-2379.
17. Cheuk QK, Lo TK, Wong SF, Lee CP. Association between pregnancy-associated plasma protein-A levels in the first trimester and gestational diabetes mellitus in Chinese women. *Hong Kong Med J*. 2016;22:30-38.
18. Saruhan Z, Ozekinci M, Simsek M, Mendilcioglu I. Association of first trimester low PAPP-A levels with adverse pregnancy outcomes. *Clin Exp Obstet Gynecol*. 2012;39:225-228.
19. Kumar M, Sharma K, Singh R, Singh S, Ravi V, Singh K, Gupta U, Bhattacharya J. Role of maternal factors, PAPP-A, and Doppler in screening for early- and late-onset pregnancy hypertension in Asian population. *Hypertens Pregnancy*. 2016;35:382-393.
20. Odibo AO, Zhong Y, Longtine M, Tuuli M, Odibo L, Cahill AG, Macones GA, Nelson DM. First-trimester serum analytes, biophysical tests and the association with pathological morphometry in the placenta of pregnancies with preeclampsia and fetal growth restriction. *Placenta*. 2011;32:333–338

21. Poon LC, Maiz N, Valencia C, Plasencia W, Nicolaides KH. First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. *Ultrasound Obstet Gynecol*. 2009;33:23–33.
22. Canini S, Prefumo F, Pastorino D, Crocetti L, Afflitto CG, Venturini PL, De Biasio P. Association between birth weight and first-trimester free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Fertil Steril.* 2008;89:174-178.
23. Gabbay-Benziv R, Esin S, Baschat AA. Incorporating first trimester analytes to predict delivery of a large for gestational infant in women with impaired glucose tolerance. *J Perinat Med*. 2015;43:299-303.
24. González González NL, Plasencia W, González Dávila E, Padrón E, di Renzo GC, Bartha JL. First and second trimester screening for large for gestational age infants. *J Matern Fetal Neonatal Med.* 2013;26:1635-1640.
25. Parlakgumus HA, Aytac PC, Kalaycı H, Tarim E. First trimester maternal lipid levels and serum markers of small- and large-for-gestational age infants. *J Matern Fetal Neonatal Med*. 2014;27:48-51.
26. Habayeb O, Daemen A, Timmerman D, De Moor B, Hackett GA, Bourne T, Lees CC. The relationship between first trimester fetal growth, pregnancy-associated plasma protein A levels and birthweight. *Prenat Diagn*. 2010;30:873-878.
27. Leung TY, Sahota DS, Chan LW, Law LW, Fung TY, Leung TN, Lau TK. Prediction of birth weight by fetal crown-rump length and maternal serum levels of pregnancy-associated plasma protein-A in the first trimester. *Ultrasound Obstet Gynecol*. 2008;31:10-14.
28. Gentile, M., Schifano, M., Lunardi, S., Nanini, C., Moscuzza, F., Sergiampietri, C., Ciantelli, M., Monacci, F., Boldrini, A., Luchi, C. and Ghirri, P. Maternal PAPP-A Levels at 11 - 13 Weeks of Gestation Predict Foetal and Neonatal Growth. *Open J Obstet Gynecol*. 2015;5:365-372.
29. Tarim E, Hacivelioğlu SÖ, ÇOK T, Bağiş HT. First trimester maternal serum PAPP-A levels and macrosomia in nondiabetic mothers. *Turk J Med Sci*. 2011;41:581-586.
30. Petry CJ, Sanz Marcos N, Pimentel G, Hayes MG, Nodzenski M, Scholtens DM, Hughes IA, Acerini CL, Ong KK, Lowe WL Jr, Dunger DB. Associations between fetal imprinted genes and maternal blood pressure in pregnancy. *Hypertension*. 2016;68:1459-1466.

31. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva Ad, Hod M, Kitzmiler JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y, Schmidt MI. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care*. 2010;33:676–682.
32. National Institute for Health and Care Excellence. Hypertension in pregnancy: diagnosis and management. NICE guidelines [CG107]. August 2010. National Institute for Health and Care Excellence web site http://www.nice.org.uk/guidance/cg107. Accessed July 28, 2016.
33. Chen JW, Ledet T, Orskov H, Jessen N, Lund S, Whittaker J, De Meyts P, Larsen MB, Christiansen JS & Frystyk J. A highly sensitive and specific assay for determination of IGF-I bioactivity in human serum. *Am J Physiol*. 2003;284:E1149–1155.
34. Ong KK, Diderholm B, Salzano G, Wingate D, Hughes IA, MacDougall J, Acerini CL, Dunger DB. Pregnancy insulin, glucose, and BMI contribute to birth outcomes in nondiabetic mothers. *Diabetes Care*. 2008;31:2193-2197.
35. Nyomba BL, Berard L, Murphy LJ. Free insulin-like growth factor I (IGF-I) in healthy subjects: relationship with IGF-binding proteins and insulin sensitivity. *J Clin Endocrinol Metab*. 1997;82:2177-2181.
36. Sesti G, Sciacqua A, Cardellini M, Marini MA, Maio R, Vatrano M, Succurro E, Lauro R, Federici M, Perticone F. Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. *Diabetes Care*. 2005;28:120-125.
37. McIntyre HD, Chang AM, Callaway LK, Cowley DM, Dyer AR, Radaelli T, Farrell KA, Huston-Presley L, Amini SB, Kirwan JP, Catalano PM; Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study Cooperative Research Group. Hormonal and metabolic factors associated with variations in insulin sensitivity in human pregnancy. *Diabetes Care*. 2010;33:356-360.
38. Zhu Y, Mendola P, Albert PS, Bao W, Hinkle SN, Tsai MY, Zhang C. Insulin-like growth factor axis and gestational diabetes: A longitudinal study in a multiracial cohort. *Diabetes*. 2016;65:3495-3504.
39. Matuszek B, Lenart-Lipińska M, Burska A, Paszkowski T, Smoleń A, Nowakowski A. Increased serum insulin-like growth factor-1 levels in women with gestational diabetes. *Adv Med Sci*. 2011;56:200-206.

40. McIntyre HD, Zeck W, Russell A. Placental growth hormone, fetal growth and the IGF axis in normal and diabetic pregnancy. *Curr Diabetes Rev*. 2009;5:185-189.

Table 1 Clinical characteristics of the Cambridge Baby Growth Study participants who had week 15 serum PAPP-A concentrations measured.

Demographic	
N	821
Maternal age at birth of baby (years)	33.2 (32.9, 33.5) (n=689)
Parity	1.7 (1.6, 1.8) (n=779)
Pre-pregnancy BMI (kg/m^2)	24.1 (23.9, 24.5) (n=643)
Unadjusted birth weight of baby (kg)	3.498 (3.462, 3.535) (n=779)
Percentage giving birth to males (%)	52.6
Gestational age of offspring at delivery (decimal weeks)	39.9 (39.8, 40.0) (n=782)
Percentage that smoked at any point during pregnancy (%)	4.1
Percentage that developed GDM (%)	8.9
Percentage that developed gestational hypertension (%)	6.0

Data are mean (95 % confidence intervals) or percentages.

Table 2 Association between maternal PAPP-A concentrations at week 15 (15.0 (14.8, 15.1) weeks) of pregnancy and week 28 OGTT indices of maternal glucose tolerance and insulin resistance and secretion.

Index	**Standardised Coefficient (β)**	**p-value**	**r^2 (%)**	**n**
Plasma glucose concentration 60 min. after a 75 g glucose load	-0.188	1.5×10^{-5}	2.4	777
HOMA S	0.319	1.8×10^{-13}	6.6	768
Insulinogenic index	-0.024	0.6	0	731
Insulin disposition index	0.202	6.5×10^{-6}	2.6	731

Table 3 Association between maternal PAPP-A concentrations around week 15 of pregnancy and mean arterial blood pressure across pregnancy.

Pregnancy Stage (weeks)	Standardised Coefficient (β)	p-value	r^2 (%)	n
11.8 (11.5, 12.0)	-0.187	4.1×10^{-3}	1.6	352
31.4 (31.3, 31.5)	-0.202	1.7×10^{-3}	2.3	355
37.0 (36.9, 37.0)	-0.177	6.9×10^{-3}	2.5	347

Table 4 Comparison of the groups selected as having the highest and lowest unadjusted week 15 serum PAPP-A concentrations in terms of clinical characteristics, week 28 OGTT indices of maternal glucose tolerance, insulin secretion and insulin sensitivity plus blood pressures.

Index	Pregnancy Stage (weeks)	Lowest PAPP-A concentrations	Highest PAPP-A concentrations	Standardised Coefficient (β)	p-value
Maternal Age (decimal years)	Birth of baby	32.7 (31.4, 34.1) (n=48)	33.0 (31.6, 34.3) (n=48)	0.027	0.8
Parity	Throughout	1.8 (1.5, 2.1) (n=48)	1.6 (1.3, 1.8) (n=48)	-0.130	0.2
Pre-pregnancy BMI (kg/m^2)	0	27.1 (25.4, 28.7) (n=39)	22.0 (20.2, 23.8) (n=33)	-0.442	1.0×10^{-4}
Percentage giving birth to males (%)	Birth of baby	54.5	51.1	N/A	0.7
Percentage smoking (%)	At any time during pregnancy	8.9	4.5	N/A	0.4
Percentage who develop GDM (%)	> 20	16.7	4.2	N/A	0.09
PAPP-A (mU/L)	15	1096 (906, 1326) (n=48)	18126 (14983, 21929) (n=48)	0.836	2.0×10^{-31}
Bioactive IGF (IGF-IR Activation) (µg/L)	15	2.57 (2.32, 2.81) (n=44)	2.68 (2.44, 2.92) (n=46)	0.070	0.5

Fasting plasma glucose (mg/dL)	28	79 (77, 83) (n=48)	76 (74, 79) (n=48)	-0.234	0.02
Plasma glucose 60 min. after a 75 g glucose load (mg/dL)	28	132 (123, 141) (n=48)	124 (115, 133) (n=48)	-0.103	0.3
Area under the curve of capillary whole blood glucose concentrations (g.min/dL)	28	15.77 (15.03, 16.49) (n=48)	15.32 (14.53, 16.09) (n=48)	-0.100	0.4
Insulinogenic index ($\Delta ins_{60}/\Delta gluc_{60}$)	28	148 (122, 179) (n=48)	144 (118, 174) (n=48)	-0.024	0.8
Insulin disposition index (L/mmol)	28	13135 (10518, 16403) (n=48)	17456 (13978, 21798) (n=48)	0.188	0.08
HOMA IR	28	1.14 (1.00, 1.30) (n=48)	0.83 (0.73, 0.99) (n=47)	-0.331	1.0 x 10^{-3}
Mean arterial blood pressure (mmHg)	12	84 (78, 90) (n=16)	79 (75, 84) (n=26)	-0.214	0.4
	31	89 (85, 94) (n=16)	78 (75, 82) (n=24)	-0.522	5.9 x 10^{-4}
	37	91 (86, 96) (n=16)	83 (79, 87) (n=24)	-0.398	0.01

Data are mean (95 % confidence intervals). Δins_{60} is the change in plasma insulin concentrations over the first hour of the OGTT. Similarly $\Delta gluc_{60}$ is the change in plasma glucose concentrations over the first hour of the OGTT. N/A = not applicable.

Figure Legends

Figure 1 ROC curves for the prediction of GDM development based on: (1) week 15 serum PAPP-A concentrations (AUC=0.592 ± 0.041), (2) maternal BMI before pregnancy and age (AUC=0.647 ± 0.042), and (3) a combination of (1) and (2) (AUC=0.639 ± 0.041). N=581 per model, AUC data are mean ± SEM.

Figure 2 Scatter diagrams that illustrate the association between week 15 PAPP-A concentrations and week 28 (a) fasting plasma glucose concentrations, (b) areas under the capillary glucose curve from the OGTT and (c) insulin resistance (HOMA IR). Also shown are the regression lines and their 95 % confidence intervals. All PAPP-A concentrations are natural log-transformed throughout.