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Title: Ultrasound And Endocrinological Markers Of First Trimester Placentation And Subsequent Fetal Size

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Abstract: Introduction: To study the relationship between 2-dimensional placental ultrasound measurements and maternal serum (MS) levels of biomarkers of placentation and in pregnancies presenting with an isolated abnormally high or low birthweight at term, without evidence of placental insufficiency.
Method: We performed a population based cohort study of 306 pregnancies delivered at term including 30 presenting with large-for-gestational age (LGA, birthweight > 90th centile) and 17 small-for-gestational age (SGA; birthweight < 10th centile). Antenatal measurements included placental thickness and 2D-volume and MS levels of pregnancy-associated plasma protein A (PAPP-A) and free-beta human chorionic gonadotrophin (fβhCG) at 11 - 13+6 weeks of gestation and mid-trimester MS α-fetoprotein (AFP), unconjugated estriol (uE3) and inhibin A levels.
Results: In the subgroup with a normal birthweight (10th-90th centile), there was a significant positive correlation between birthweight and the basal plate surface area ($p<0.001$) and 2D placental volume ($p< 0.01$). In the LGA subgroup, MS PAPP-A was significantly ($p<0.05$) higher than in normal controls and there was a significant ($p<0.01$) positive correlation with birthweight. There was no significant difference for any of the ultrasound and biomarkers parameters between SGA and the normally grown controls.
Discussion: In uncomplicated singleton pregnancies with a normal birthweight, 2D measurements of placentation are related with fetal size but are not related to subsequent excessive or slow fetal growth. LGA at birth is associated with increased MS PAPP-A at 11-14 weeks of gestation supporting the association between PAPP-A synthesis and early placental growth and development.

Dear Graham

As requested, I have added in the Supplementary Figures 1 and 2 (Scatter plot illustrating the relationship between first trimester placental surface area or 2D volume and birthweight for the whole cohort),
Eric has added the statistical data for these results into the results section on page 10. He also corrected 2 typos and added 2 decimals in all the F numbers for consistency.

Yours sincerely
Anna David

Conflict of Interest Statement

The authors state that no conflict of interest exists.

Ultrasound And Endocrinological Markers Of First Trimester Placentation And Subsequent Fetal Size

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ABSTRACT (243 words)

Introduction: To study the relationship between 2-dimensional placental ultrasound measurements and maternal serum (MS) levels of biomarkers of placentation and in pregnancies presenting with an isolated abnormally high or low birthweight at term, without evidence of placental insufficiency.

Method: We performed a population based cohort study of 306 pregnancies delivered at term including 30 presenting with large-for-gestational age (LGA, birthweight > 90^{th} centile) and 17 small-for-gestational age (SGA; birthweight < 10^{th} centile). Antenatal measurements included placental thickness and 2D-volume and MS levels of pregnancy-associated plasma protein A (PAPP-A) and free-beta human chorionic gonadotrophin (fβhCG) at 11 - 13^{+6} weeks of gestation and mid-trimester MS α-fetoprotein (AFP), unconjugated estriol (uE3) and inhibin A levels.

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Highlights

- ~~In a population based cohort study of 306 uncomplicated singleton pregnancies delivered at term, 2D placental measurements correlate with fetal size in the total study group and To study the relationship between 2D placental measurements, maternal placental biomarkers and fetal growth complications~~
- ~~in uncomplicated singleton~~ pregnancies with a normal birthweight ~~2D placental measurements correlate with fetal size~~
- Placental basal plate surface area and 2D placental volume do not predict large or small fetal size and these measurements cannot be used to predict birthweight in individual cases..
- ~~Large (LGA) and small for gestational age (SGA) at birth are not associated with 2D ultrasound measurements of the placenta~~
- ~~LGA but not SGA is associated with raised first trimester maternal serum pregnancy-associated plasma protein~~First trimester maternal serum pregnancy-associated plasma protein concentration is higher in LGA pregnancies than compared with normal and AGA pregnancies.

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1. Introduction

Major changes take place in the uterine environment between the first trimester and the rest of pregnancy [1]. These changes are characterised by the establishment of the intervillous circulation, a switch from histiotrophic to haemotrophic fetal nutrition and the remodelling of the primitive placenta [2]. In early pregnancy, the oxygen concentration within the chorionic sac is maintained at a low value to protect the primitive placenta and the developing fetus from the teratogenic effect of oxygen free radicals [1,2]. The onset of the maternal arterial circulations at the end of the first trimester leads to a three-fold rise in the intraplacental oxygen concentration [1,3]. The haemodynamics of the utero-placental circulation and distribution of oxygen around and inside the placenta play an important role in shaping the topography of the villous tree of the definitive placenta and the formation of the free placental membranes [1-3].

Fetal size is dependent on nutrient availability, which in turn is related to villous development and the capacity of the villous trophoblast to transport these nutrients [4]. Sufficient dilation of the utero-placental circulation together with rapid villous angiogenesis are the key factors necessary for adequate placental development and function, and subsequent fetal growth. Placental and fetal development is related and placental growth kinetics are important features predicting post-natal health and in particular cardiovascular adaptations in childhood, even when fetal size is in the normal range [5]. At birth, there is a correlation between fetal and placental weight and the ratio of these weights i.e. the feto-placental weight ratio (FPR) gives retrospectively

an indication of the appropriateness of fetal growth during pregnancy and estimates the potential risks for chronic diseases in later life [6].

The placenta actively produces a large number of hormones that serve to regulate and balance maternal and fetal physiology and in particular fetal growth via the production and metabolism of growth-regulating hormones [7]. The expression of numerous signaling molecules is altered in the placentas from pregnancies affected by the fetal growth complications of fetal growth restriction and macrosomia. Many maternal serum (MS) biomarkers have been proposed for the detection of abnormal fetal growth but when used alone in the first trimester they have a limited role as screening tests. For example, pregnancy-associated plasma protein-A (PAPP-A) during the first trimester cannot be used alone as a marker of excessive fetal growth, large [8] or of small-for-gestational age (SGA) [9,11]. Similarly new biomarkers such as adiponectin, a hormone that regulates glucose levels and fatty acid oxidation, have been recently tested in the first trimester to predict fetal size later in pregnancy with variable results [12,13].

For decades, ultrasound remains the best method for the screening diagnosis, characterization and follow-up of abnormal fetal growth. The advent of Doppler ultrasound has enabled the study of the development of the uterine and umbilical circulations *in utero* from early in normal pregnancy [14]. Further studies have shown an association between uterine circulation, abnormal placental features including size, abnormal level of biomarkers in MS and abnormal fetal growth [15,16]. More recently 3-dimensional (3D) ultrasound has enabled the study of placental volume *in utero* throughout pregnancy. These studies have shown an association between placental

volume at 11-13 weeks and birth weight in pregnancies with a normal and complicated outcome [17-22].

The combination of the assessment of placental size and shape with 3D ultrasound and MS biomarkers can serve as the foundation upon which to build a multivariate model for the early prediction of abnormal fetal size. The broad variability in placental volume 3D measurements and current time-consuming approach compared to 2D ultrasound has made its clinical use difficult [23]. In this study, we investigated the relationship between first trimester (11 - 13^{+6} weeks of gestation) 2D ultrasound measurements of placentation and MS levels of commonly measured placental proteins PAPP-A and free-beta human chorionic gonadotrophin (fβhCG), and mid-trimester (15-22^{+0} weeks of gestation) inhibin and subsequent fetal size.

2. Patients and methods

A group of 476 women booked for antenatal care at University College London Hospital (UCLH) were recruited prospectively over a 40-month period. All pregnant women at UCLH are offered a screening test for Trisomy 21 which combines nuchal translucency (NT) measurement and MS levels of PAPP-A and fβhCG at 11–13^{+6} weeks (combined test) and/or of AFP, uE3 and inhibin-A at 15–22^{+0} weeks (integrated test). All women were recruited at the time of the NT ultrasound examination between 11^{+0} and 13^{+6} weeks of gestation (77-97 gestational days) as determined either by menstrual age, or

when there was a discrepancy of more than 5 days by the ultrasound measurement of the fetal crown-rump length (CRL).

Demographic data including maternal age, ethnicity, parity, cigarette smoke exposure, age and body mass index (BMI) were collected from questionnaires completed at the time of the first appointment. All women underwent routine screening for gestational diabetes with a random glucose at booking and at 28 weeks of gestation. In addition women with specific risk factors (previous LGA, family history of diabetes) were offered a formal 2 hour glucose tolerance test at 28 weeks. Pregnancy outcome information was collected from the medical case notes and hospital electronic patient records. The birthweight percentile was calculated using the customized gestational age related optimal growth chart (GROW) (www.gestation.net), which takes into account ethnicity, maternal height, weight, age and fetal birthweight and gender.

Women diagnosed with a first trimester miscarriage, fetal abnormality, medical condition, multiple pregnancy and abnormal pregnancy outcome including, late miscarriage, intrauterine death, stillbirth, preterm (<37 weeks of gestation) or post-term delivery (≥41+6 weeks of gestation), smokers or lost to follow-up were excluded from the analysis. Placental pathology results were available for cases of SGA and IUGR identified at birth.

2.1 Ultrasound examination

All examinations were performed using a 3.5-5 MHz ultrasound probe (Voluson 730 and E8 Expert, GE, USA). In all cases, the fetal NT, CRL and basic anatomy were obtained. The placental basal plate dimensions and

thickness were measured by viewing the whole placenta as previously described [25,26]. Three measurements of each parameter were taken and they were subsequently averaged for analysis. In brief the longest sagittal and transverse diameters of the placental basal plate were measured at the level of the utero-placental interface and the placental thickness was measured underneath the cord insertion. The basal plate surface area of the placenta was estimated using the following formula: *Sagittal length x transverse length x π/4.* The placental volume was calculated as being the equation for half the volume of an ellipsoid: *½ (sagittal length x transverse length x thickness x 4/3π), as previously described* [24]. The placental measurements were entered into the prospective study database at the time of the ultrasound scan. Four sonographers were trained by the study authors prior to the study commencement so as to perform the placental ultrasound measurements in a reproducible and systematic way.

2.2 Bioassays

MS PAPP-A and fβhCG levels were measured using the AutoDELFIA PAPP-A, time-resolved fluoro-immunoassay (PerkinElmer, Turku, Finland). Inhibin A MS levels were measured using a commercial ELISA. The measured proteins were converted to multiple of the median (MoM) for a pregnancy of the same gestational age and adjusted for maternal age and ethnicity.

2.3 Study group and statistical analysis

The study group included 306 non-smoking women with a spontaneous singleton pregnancy delivering after an uncomplicated pregnancy between 37^{+1} weeks (259 days of pregnancy) and 41^{+6} weeks (286 days of pregnancy).

The study group was divided into 3 subgroups according to the neonatal weight percentile at birth adjusted for neonatal gender [24]. Cases in the large-for-gestatonal age (LGA, birth weight > 90th centile) and SGA (birth weight <10th centile with no evidence of fetal growth restriction, as defined as abnormal umbilical artery and uterine artery Doppler or abnormal cerebroplacental ratio) subgroup were individually matched for maternal BMI, parity and for the CRL with three cases from the normal (10-90th centile) subgroup. The study was approved by the Joint UCL/UCLH Committees on the Ethics of Human Research (Reference Number: 05/Q0505/82). All women received information about the study and written consent was obtained prior to the ultrasound examination.

The data were analyzed using the StatGraphic data analysis and statistical software package (Station, TX). Standard Kurtosis analysis indicated that some values were not normally distributed and are therefore presented as median and interquartile range (IQR). The median values of the different variable investigated in the macrosomic and SGA subgroups and controls were compared using a Mann-Whitney (Wilcoxon) W test. Individual correlations between the different ultrasound, endocrinologic and clinical variables were calculated by the least square method and their slopes tested for significance by the *F* ratio test. A p value of <0.05 was considered significant.

3. Results

There were 259 (84.6%) pregnancies with a birthweight between 10th and 90th centile (normal), 30 (9.8%) with a birthweight >90th centile (LGA) and 17 (5.6%) with a birthweight below the 10th centile (SGA).

The regression analysis between birthweight and the ultrasound and endocrinologic parameters in the entire study group indicated a significant positive correlation with placental basal surface area (F= 11.03; r=0.41: P<0.005) and 2D volume (F= 7.60; r=0.29, p<0.01), (Supplementary Figures 1 and 2) and MS PAPP-A concentration (F= 5.10; r=0.14, p<0.05), but no such relationship existed for MS fβhCG.

3.1 Normal birth weight

At the time of the ultrasound examination, the median CRL was 62.3 mm (IQR 56.1;70). Using the NIH BMI calculator (www.nhlbi.nih.gov/ guidelines/obesity/ BMI/ bmicalc.htm), 12 women were classified at the antenatal booking appointment (8-10 weeks of gestation) as underweight (BMI < 18.5), 181 as normal weight (BMI 18.5-24.9), 52 as overweight (BMI 25-29.9 and 14 as obese (≥ 30).

Table 1 displays and compares the clinical, ultrasound and endocrinological data of women with a BMI < 25 with those with a BMI ≥ 25. The MS level of fβhCG at 11-13^{+6} weeks was significantly (p<0.05) higher in women with a BMI ≥ 25. There was no significant difference for the other parameters between the two subgroups.

There was a significant positive correlation between the basal plate surface area (F= 10.30; r=0.20, p<0.001) and placental volume (F= 7.60; r=0.17, p< 0.01) at 11-13^{+6} weeks and subsequent fetal size. There was no significant correlation between MS fβhCG or PAPP-A at 11-13^{+6} weeks and mid-trimester AFP, uE3 and inhibin MS levels and fetal size.

3.2 LGA

The median birthweight was 4128g in the LGA subgroup (IQR 4000;4350) compared to 3289 g in the AGA subgroup (IQR 3121;3487, p<0.001). The concentration of MS PAPP-A at 11-13^{+6} weeks was significantly (p<0.05) higher in the serum of the women delivering an LGA neonate than in controls with a normal birthweight at term (Table 2). There was no significant difference for the ultrasound parameters in this subgroup. There was a significant positive correlation (F= 8.03; r=0.20, p<0.01) between MS PAPP-A concentration and increased birthweight at term (Figure 1), but no such relationship existed for MS fβhCG AFP, uE3 and inhibin.

3.4 SGA

The median birthweight was 2850g in the SGA subgroup (IQR 2800;2922) compared to 3462 g in the AGA control subgroup (IQR 3206;3632, p<0.001). There was no significant difference for the ultrasound and endocrinologic parameters between SGA and control pregnancies (Table 3). There was no significant correlation between MS-hormonal levels at 11-13^{+6} weeks and mid-trimester and SGA.

4. Discussion

Our results indicate a relationship between 2D measurements of the placentation area and volume, MS PAPP-A at 11-13^{+6} weeks of gestation and birthweight in uncomplicated singleton pregnancies that deliver at term. In otherwise uncomplicated pregnancies, presenting with LGA or SGA at term, 2D first-trimester ultrasound measurements of placentation are not related to abnormal fetal size and only MS PAPP-A are related to subsequent excessive fetal size.

There are well-documented associations between the levels of first and second trimester MS biomarkers and abnormal placental development in pregnancies subsequently complicated by pre-eclampsia and intrauterine growth restriction (IUGR). A longitudinal study has shown that in cases of IUGR, with or without accompanying pre-eclampsia, the placenta is already smaller at 12-18 weeks of gestation on ultrasound than in healthy controls [26]. In the most severe cases of deficient arterial conversion the intervillous circulation is abnormal from the beginning of the second trimester, resulting in pronounced ultrasonographic changes in placental texture [14,27,28], that are associated with IUGR, pre-eclampsia and high MS alpha-fetoprotein levels at 18-28 weeks [14]. In these pregnancies, there is a narrow implantation basis or basal plate diameter, increased thickness and patchy decrease in placental echogenicity secondary to the fetal plate being pushed up by jet-like blood streams from the spiral arteries [14]. We have previously shown that in pregnancies subsequently complicated by pre-eclampsia, the levels of MS PAPP-A are lower at the end of the first trimester and the basal plate surface area is smaller [24] reflecting indirectly the development of the definitive

267 placenta [24,25]. These findings support the concept that placental-related
268 complications of the second-half of gestation have their pathophysiological
269 origin in abnormal placentation and utero-placental development during the
270 first trimester of pregnancy [3].

271 In the present study, we have excluded pregnancies with an existing or
272 subsequent medical complications such as pre-eclampsia or gestational
273 diabetes and those that delivered pre or post-term. We observed that in the
274 subgroup presenting with a normal birthweight at term, there was a significant
275 positive correlation between birthweight and the basal plate surface area
276 ($p<0.001$) and PV ($p< 0.01$). In this subgroup, we also found that the MS level
277 of βhCG at 11-13^{+6} weeks was significantly higher ($p<0.05$) in women with a
278 BMI $\geq$ 25 than in women with a BMI < 25. First-trimester 3D placental volume
279 is closely associated with fetal and placental size [18] and hCG synthesis is
280 related to trophoblast proliferation and promotes development and growth of
281 the spiral arteries [29]. These findings highlight the pivotal interaction between
282 normal placentation, placental biosynthesis functions and normal fetal size,
283 and confirms that maternal BMI is a confounding factor that needs to be
284 adjusted for the first-trimester biochemical screening of trisomy 21.

285 In the LGA subgroup, we found that the MS PAPP-A at 11-13^{+6} weeks
286 of gestation was significantly higher ($p<0.05$) than in normal controls and was
287 positively correlated with birthweight at term. PAPP-A is mainly produced by
288 the villous trophoblast and during pregnancy its synthesis is up-regulated by
289 progesterone, which promotes the adhesion and proliferation potential of
290 trophoblastic cells [30]. It is also a key regulator of insulin-like growth factor
291 bioavailability essential for normal fetal development. This can explain why at

11-13^{+6} weeks of gestation, low MS levels of PAPP-A have been associated with a higher risk of pre-eclampsia and poor fetal growth during the second half of pregnancy [8-11,25,31]. Conversely, high MS PAPP-A levels as in the present study have been associated with increased fetal size [21,32]. However, the sensitivity of MS PAPP-A at 11-13^{+6} weeks of gestation is too low to be solely used as a screening method for the prediction of abnormal birth weight and perinatal complications [8,9,11].

We found no difference in the ultrasound and biomarker parameters in the present study between SGA and normal birthweight controls. It is possible that our small sample size in the SGA group could explain the lack of relationship between the parameters and fetal size in the SGA group. The conflicting results from studies using first and second trimester 3D placental volume measurements to predict fetal size in the third trimester [18,33] are probably because of the difficulties with manual placental segmentation. In the future innovative automatic segmentation approaches for 3D ultrasound may be find placental measurements to be clinically applicable [34]. Variation in placental volume is greater in the first trimester before 10 weeks i.e. before the definitive placenta is fully formed [35], with a fundal or low placental position being more difficult to assess than the fully anterior or posterior position [33]. We have previously found that in pregnancies with a normal outcome, there is no significant relationship between placental shape at 11-13^{+6} weeks of gestation and birthweight or centile, suggesting that the placental shape is not completely determined during the early phases of placentation and is not directly related to fetal size during the first half of pregnancy [24,25].

A distinct relationship exists between the uterine spiral arteries and the fetal or placental lobules and the finding of a smaller basal plate surface area at 11-13^{+6} weeks of gestation in pregnancies subsequently complicated by pre-eclampsia [24]. This also suggests that ultrasound measurements of the basal surface area reflects placentation indirectly and that this parameter is less likely to be affected by the physiological morphological changes in the placental structure between 8 and 12 weeks of gestation than placental volume evaluated with 3D ultrasound.

Conclusion

In uncomplicated singleton pregnancies with a normal birthweight, 2D measurements of placentation are related with fetal size but are not related to subsequent excessive or slow fetal growth. LGA at birth is associated with increased MS PAPP-A at 11-14 weeks of gestation supporting the association between PAPP-A synthesis and early placental growth and development. The clinical use of routine first trimester placental measurements in uncomplicated pregnancies remains to be evaluated by large prospective cohorts.

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Table 1. Comparison (W test) of the birthweight, crown rump length (CRL), ultrasound and endocrinological data in women with a booking BMI < 25 (n= 193) with those with a BMI ≥ 25 (n= 66). BMI was measured at 8-10 weeks of gestation

Variables	BMI < 25		BMI ≥25		
	Median	LQ;UQ	Median	LQ;UQ	p
CRL (mm)	63	56;70	62	57;70	0.80
Birthweight (g)	3475	3226;3689	3454	3251;3622	0.89
Placental thickness (mm)	18.1	15.6;21.6	18.4	16.1;21.8	0.81
Basal plate surface (mm^2)	442	319;531	398	313;517	0.23
Placental volume (mm^3)	67.2	45.6;94.7	61.9	47.7;77.8	0.35
fβhCG (MoM)	0.98	0.69;1.42	1.20	0.76;1.84	<0.05
Inhibin A (MoM)	0.95	0.74;1.29	1.10	0.80;1.27	0.23
PAPP-A (MoM)	1.08	0.74;1.51	0.95	0.70;1.30	0.19

Data are presented as median and lower quartile (LQ); upper quartile (UQ).

Table 2. Comparison of the median for the ultrasound and endocrinological measurements of placental development in LGA neonates (birthweight >90th centile, n= 30) and controls (birthweight 10th-90th centile, n=90).

Variables	LGA		Controls		
	Median	LQ;UQ	Median	LQ;UQ	p
Placental thickness (mm)	18.2	15.2;21.7	17.9	15.3;22.0	0.91
Basal plate surface (mm^2)	446	366;522	453	346;556	0.36
Placental volume (mm^3)	63.4	47.7;87.1	68.4	49.9;87.5	0.71
fβhCG (MoM)	1.13	0.69;1.59	1.09	0.72;1.46	0.78
Inhibin A (MoM)	0.88	0.67;1.18	1.02	0.78;1.26	0.17
PAPP-A (MoM)	1.27	0.98;1.73	1.03	0.74;1.46	<0.05

Data are presented as median and lower quartile (LQ); upper quartile (UQ).

Table 3. Comparison of the median for the ultrasound and endocrinological measurements of placental development in SGA neonates (birthweight $<10^{th}$ centile, n= 17) and controls (birthweight 10^{th}-90^{th} centile, n=56).

Variables	SGA		Controls		
	Median	LQ;UQ	Median	LQ;UQ	p
Placental thickness (mm)	17.5	14.7;19.3	18.5	17.5;21.5	0.11
Basal plate surface (mm^2)	458	368;586	443	318;519	0.91
Placental volume (mm^3)	64.5	52.7;82.4	67.6	47.1;88.6	0.27
fβhCG (MoM)	1.09	0.91;1.58	1.11	0.75;1.58	0.87
Inhibin A (MoM)	0.98	0.80;1.08	1.09	0.78;1.37	0.45
PAPP-A (MoM)	1.15	0.91;1.26	1.08	0.74;1.49	0.91

Data are presented as median and lower quartile (LQ); upper quartile (UQ).

Figure 1 Legend

Scatter plot illustrating the relationship between first trimester maternal serum PAPP-A Multiple of the Median (MoM) and birthweight in the Large for Gestational Age group. The regression line is indicated in blue, the red and the purple lines indicate the confidence intervals.

Supplementary Figure 1 Legend

Scatter plot illustrating the relationship between first trimester placental surface area and birthweight for the whole cohort. The regression line is indicated in blue, the red and the purple lines indicate the confidence intervals.

Supplementary Figure 2 Legend

Scatter plot illustrating the relationship between first trimester placental 2D volume and birthweight for the whole cohort. The regression line is indicated in blue, the red and the purple lines indicate the confidence intervals.

Figure

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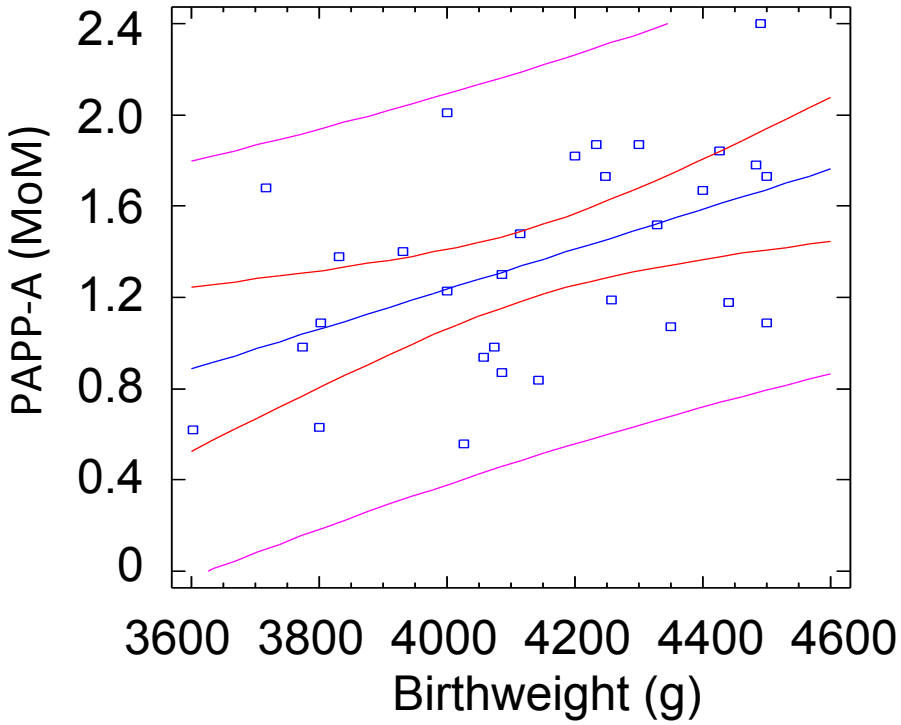

Supplementary File

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Supplementary File

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