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**Decreased Maternal Hypothalamic-Pituitary-Adrenal Axis Activity in Very Severely Obese
Pregnancy: associations with birthweight and gestation at delivery**

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ABSTRACT

Background: The maternal hypothalamic-pituitary-adrenal-axis (HPAA) undergoes dramatic activation during pregnancy. Increased cortisol and corticotrophin-releasing-hormone (CRH) associate with low birthweight and preterm labour. In non-pregnant obesity, the HPAA is activated but circulating cortisol levels are normal or lower than in lean women. We hypothesized that maternal cortisol levels would be lower in obese pregnancy, and would associate with increased fetal size and length of gestation.

Method: Fasting serum cortisol was measured at 16, 28 and 36 weeks gestation and at 3-6 months postpartum in 276 severely obese and 135 lean women. In a subset of obese (n=20) and lean (n=20) we measured CRH, hormones that regulate bioavailable cortisol (corticosteroid-binding-globulin, estradiol, estriol, progesterone). Urinary glucocorticoid metabolites were measured in pregnant (obese n=6, lean n=5) and non-pregnant (obese n=7, lean n=7) subjects.

Results: Maternal cortisol and HPAA hormones were lower in obese pregnancy. Total urinary glucocorticoid metabolites increased significantly in lean pregnancy, but not in obese. Lower maternal cortisol in obese tended to be associated with increased birthweight ($r=-0.13$, $p=0.066$). In obese, CRH at 28 weeks correlated inversely with gestational length ($r=-0.49$, $p=0.04$), and independently predicted gestational length after adjustment for confounding factors (mean decrease in CRH of -0.25 pmol/L (95% CI -0.45 to -0.043 pmol/L) per/day increase in gestation).

Conclusion: In obese pregnancy, lower maternal cortisol without an increase in urinary glucocorticoid clearance may indicate a lesser activation of the HPAA than in lean pregnancy. This may offer a novel mechanism underlying increased birthweight and longer gestation in obese pregnancy.

1. INTRODUCTION

There has been a global rise in obesity in the last three decades(1). Around 64% of women of reproductive age in the United States are overweight and 35% are obese(2) with a similar pattern in Europe(3,4). There is a strong temporal gradient of increasing risk of adverse outcomes with increasing maternal body mass index (BMI; kg/m²)(5,6) including increased birthweight(7) with associated cardiovascular disease in adulthood(8), prolonged pregnancy(9) and stillbirth(10). Mechanisms underlying the links between maternal obesity and adverse pregnancy outcomes are unknown.

Glucocorticoids are vital for fetal growth and organ maturation, but fetal over-exposure to cortisol is associated with intrauterine growth restriction(11), and increased cardiovascular risk in later life(12). During pregnancy, the maternal hypothalamic-pituitary-adrenal-axis (HPAA) is activated, resulting in an exponential increase in the maternal circulating levels of cortisol and corticotrophin-releasing-hormone (CRH)(13). A number of endocrine changes contribute to the increase in total cortisol levels. The placenta releases CRH from the second trimester (~16 weeks gestation) onwards leading to a complex feed-forward loop, with placental CRH activating the maternal HPAA, which generates cortisol, and maternal cortisol simultaneously stimulates placental CRH synthesis, thus further increasing cortisol levels(14). Increased hepatic synthesis of corticosteroid-binding-globulin (CBG; the predominant binding protein of cortisol) during pregnancy, driven by estrogen stimulation(15), is considered to contribute to the rise in maternal total cortisol(14). Rising levels of progesterone, which displaces cortisol from CBG, may also contribute to excess of free, bioavailable cortisol(16). Pregnancy is associated with increased cortisol clearance (via hepatic metabolism and urinary excretion), suggesting that in addition to elevated CRH and CBG, an overall increase in maternal HPAA activity contributes to the increase in total maternal cortisol with advancing gestation(14). The HPAA is thought to play a key role in governing the length of gestation and triggering the onset of parturition. High maternal circulating CRH at 31-33 weeks gestation is associated with preterm labour (<37 weeks), and low levels with post-term pregnancy (>41 weeks)(17,18). It is not known whether maternal CRH is altered in obese pregnancy, or whether these alterations influence the duration of pregnancy.

Non-pregnant obesity is also associated with activation of the HPAA, and also with increased clearance of cortisol(19) leading to normal or lower levels of total circulating cortisol(20). Further, increased BMI has been negatively correlated with awakening cortisol levels(21). Preliminary evidence from two recent studies of obese pregnant women suggests that the HPAA changes in non-pregnant obesity may be maintained during obese pregnancy. Obese women ($\text{BMI} \geq 30 \text{ kg/m}^2$) in early pregnancy were found to have lower morning salivary cortisol(22), and lower total cortisol(23) than pregnant women with a normal BMI.

To our knowledge, there are no reports of HPAA hormones and their association with clinical outcomes in very severely obese ($\text{BMI} \geq 40 \text{ kg/m}^2$) pregnant women. We therefore aimed to measure total morning cortisol levels in very severely obese (grade 3 obesity, $\text{BMI} \geq 40 \text{ kg/m}^2$) pregnant women and a lean control group ($\text{BMI} < 25 \text{ kg/m}^2$). In sub-studies we further characterized the HPAA in obese pregnancy to identify potential underlying mechanisms for the predicted lower maternal cortisol in obese pregnancy. We hypothesized that maternal CRH and factors that may reduce (CBG, estradiol) or increase (progesterone) free cortisol levels would be altered in obese pregnancy, in favour of lowering maternal cortisol, and that urinary glucocorticoid metabolites would be increased in obese pregnant women, in-keeping with increased HPAA activity, similar to non-pregnant obesity. Finally to test the hypothesis that reduced fetal glucocorticoid exposure in obese pregnancy would contribute to higher birthweight and longer gestation we explored associations with clinical outcomes related to fetal growth and pregnancy duration.

2. METHODS

2.1 Participants

We recruited 286 severely obese and 137 lean pregnant women with singleton pregnancies to a longitudinal study of obesity in pregnancy (Hormones in Pregnancy; HIP). Eligible women had a singleton pregnancy and a normal booking ultrasound scan. Women who used regular glucocorticoid medication during pregnancy (obese $n=7$, lean $n=1$) or received antenatal steroids for suspected preterm

delivery were excluded (obese n=3, lean n=1), resulting in a final dataset of 276 obese and 135 lean women. Ethical approval and written informed consent were obtained. Participants reported demographic information on questionnaires and attended for study visits at 16 weeks, 28 weeks, and 36 weeks gestation and between 3-6 months postpartum. At each study visit, fasting morning blood samples were obtained between 08.30h and 09.30h. At the first study visit, a subset of women (obese n=14, lean n=38) collected three saliva samples for analysis of cortisol, to assess the cortisol awakening response, using a Salivette® collector (Sarstedt, Numbrecht, Germany); samples were collected at bedtime, at waking, and 30-minutes after waking, and were stored at -80°C until analysis.

A separate cohort of women provided 24-hour urine collections for analysis of glucocorticoid metabolites. Obese and lean pregnant women provided collections at 19 weeks and 36 weeks gestation, and non-pregnant obese and lean controls provided one collection (obese pregnant n=6, obese non-pregnant n=7, lean pregnant n=5, lean non-pregnant n=7). Groups are referred to as LNP (lean non-pregnant), ONP (obese non-pregnant), LP1 (lean pregnant visit 1, approximately 19 weeks gestation), LP2 (lean pregnant visit 2, approximately 36 weeks gestation), OP1 (obese pregnant visit 1, approximately 19 weeks gestation) and OP2 (obese pregnant visit 2, approximately 36 weeks gestation). Total urine volume was recorded and stored in 20ml aliquots at -80°C until analysis. Eligible women for both cohorts were aged between 18-45 years and were Caucasian. Exclusion criteria for both cohorts were diabetes, active endocrine disorders, use of glucocorticoid medications or multiple pregnancy.

Clinical outcomes were extracted from medical records. Macrosomia was defined as a birthweight ≥ 4000 g at term (≥ 37 weeks). Low birthweight was defined as ≤ 2500 g.

2. 2 Laboratory Methods

Commercially available radioimmunoassay kits were used for the analysis of total serum cortisol, cord blood cortisol (ImmuChem Cortisol ¹²⁵I; ICN Biomedicals, CA, USA; inter-assay CV 7.6%). We measured CBG, CRH, estradiol, estriol, progesterone and sex hormone binding globulin (SHBG) in

plasma samples from a randomly selected subset of the HIP study cohort (obese n=20, lean n=20). CBG was measured by ELISA as described by Lewis et al.,(24); this included ‘total’ CBG and ‘intact’ CBG (which has binding affinity for cortisol). ‘Cleaved’ CBG (which lacks binding affinity for cortisol) was then calculated as a ratio of total:intact CBG. Free cortisol was calculated when both cortisol and CBG measurements were available using Coolens’ equation(25), which uses total cortisol and CBG levels. CRH was measured by extracted radioimmunoassay as described by Smith et al.,(26). ELISA kits were used to measure total estradiol (Demeditec DE2693; inter-assay CV 6.72-9.39%), total estriol (Demeditec DE3717; inter-assay CV $\leq 10\%$) total progesterone (Demeditec DE1561; inter-assay CV 5.4-6.99%), and SHBG (Demeditec DE2996; inter-assay CV 3.1-8.0%).

Urine glucocorticoid metabolites were quantified in 24-hour urine collections by gas chromatography electron impact tandem mass spectrometry following solid phase extraction hydrolysis of conjugates and formation of their methoxime-trimethylsilyl derivatives, as previously described(27). Metabolites included urinary free cortisol and its metabolites (5 β -tetrahydrocortisol (THF), 5 α -tetrahydrocortisol (α -THF), α -cortol, and β -cortol); and urinary cortisone and its metabolites (tetrahydrocortisone (THE), α -cortolone, β -cortolone). We measured the sum of total glucocorticoid metabolites (THF, α -THF, THE, α -cortol, β -cortol, α -cortolone, β -cortolone), as a measure of total cortisol production. The ratio of cortisol:cortisone were examined as a marker of 11 β -hydroxysteroid-dehydrogenase-type 2 (11 β -HSD2) enzyme activity, which converts active cortisol into inactive cortisone; the ratio of α -THF:cortisol as a measure of 5 α -reductase activity, which metabolises cortisol to α -THF; and the ratio of (THF+ α -THF):THE as an indicator of the balance of whole-body 11 β -hydroxysteroid dehydrogenase enzyme activities (11 β -HSD1 and 11 β -HSD2 activity(28).

2.3 Statistical analysis

Data distribution was assessed for normality by visually assessing histograms. Data that were not normally distributed were normalized using the natural-log transformation. The independent t-test was used to test for differences between continuous variables and the chi-squared test for categorical variables. Repeated measures analysis of variance (ANOVA) was used to compare the pattern of change

in hormones with advancing gestation between obese and lean. Correlations between HPAA hormones with birthweight and gestation at delivery were tested and regression analysis used to adjust for confounding factors, including smoking status, baby gender, and gestational age at delivery. Analysis was performed using SPSS v20 (IBM). Data in text are mean $\pm$ (sd), and data in figures are mean $\pm$ SEM. Statistical significance was considered at $p < 0.05$.

Power calculations: A formal sample size calculation was not performed for either cohort. We aimed to generate data for over 200 very severely obese and 100 lean control women for the HIP study. Post-hoc power calculations using G*Power3.1 software(29) showed that a sample size of $n=462$ was required for 80% power to demonstrate an inverse correlation between cortisol and birthweight at the 5% significance level.

3. RESULTS

3.1 Demographics

Table 1 shows characteristics of the 276 obese and 135 lean women and their babies. Obese women were younger than lean, had greater social deprivation(30), were of higher parity and were more likely to smoke. Supplementary table 1 shows characteristics of women included in the subset of obese ($n=20$) and lean ($n=20$) for analysis of CBG and sex steroids; and supplementary table 2 shows characteristics of women who provided 24-hour urine collections.

3.2 Gestational Changes in HPAA Hormones

3.2.1 Cortisol and CBG levels are lower in obese pregnancy

Total serum cortisol concentrations were higher in pregnancy than post-partum in obese and lean women but were significantly lower in obese than in lean at each time-point during pregnancy and postpartum (figure 1a). The diurnal pattern of cortisol measured from saliva cortisol (peak cortisol levels on waking) was maintained in both obese and lean women (figure 1b). Obese women had apparent ‘blunting’ of the cortisol awakening response with significantly lower cortisol levels in samples collected at 30-minutes after waking than in lean women. Bedtime and waking saliva cortisol did not differ between obese and lean.

Mean total cortisol at 16 weeks gestation did not differ according to maternal age, social class, parity or smoking status during pregnancy (data not shown). There were no differences in cortisol levels at any time according to gestational diabetes or pre-eclampsia status (data not shown).

Total and intact CBG rose similarly throughout pregnancy in both lean and obese women (figure 2). Total CBG levels were significantly lower in obese compared with lean women at 28 weeks and postpartum. Intact CBG did not differ between obese and lean at any time-point, and cleaved CBG was significantly lower in obese postpartum. Free cortisol levels calculated using intact CBG values were significantly lower in obese than in lean at 28 and 36 weeks, while free cortisol calculated using total CBG was lower in obese compared with lean at 36 weeks (table 2). Although absolute levels of free cortisol calculated with intact CBG levels appeared higher than levels calculated using total CBG values, this was not statistically significant.

3.2.2 In obese pregnancy, hormones regulating cortisol levels are lower

CRH concentrations rose throughout pregnancy. The greatest increase was between 28 and 36 weeks, but the rise was less marked in obese women (figure 3). CRH levels were significantly lower in obese women at 28 weeks with a similar trend at 36 weeks. Estradiol, estriol and progesterone concentrations all rose throughout pregnancy, but the rise was less marked in obese women (figure 3). Estradiol and estriol were significantly lower in obese compared with lean at 28 and 36 weeks while progesterone was significantly lower in obese throughout pregnancy. SHBG concentrations rose throughout pregnancy, but did not differ in lean and obese pregnant women (figure 3).

3.2.3 Urine glucocorticoid metabolites are not increased in obese pregnancy compared with non-pregnancy

Total urinary glucocorticoid metabolites were significantly higher in obese non-pregnant than in lean non-pregnant women (consistent with increased HPA axis activity and cortisol production; figure 4a). In lean, total glucocorticoid metabolites were significantly higher at LP1 and LP2 compared to LNP. In contrast in obese, total glucocorticoid metabolites were similar during pregnancy and in non-pregnancy.

There was a similar pattern of findings with urinary free cortisol (figure 4b) free cortisone (figure 4c), total cortisol metabolites (figure 4d) and total cortisone metabolites (figure 4e), with higher levels in lean pregnant compared with LNP and no significant change in obese pregnant compared to ONP.

The ratios of cortisol:cortisone (figure 4f) and THFs:THE (figure 4g) decreased with advancing gestation in lean pregnant compared to LNP consistent with increasing conversion of cortisol to cortisone (by whole-body 11β -HSD2), and/or decreasing regeneration of cortisol from cortisone (by 11β -HSD1). Neither ratio changed significantly during pregnancy in obese women.

The ratio of α -THF:cortisol was higher in ONP compared to LNP women (figure 4h). In keeping with this, levels of α THF were significantly higher in ONP compared with LNP, and were higher at LP1 and LP2 compared to LNP women (data not shown). Taken together, these data suggest that metabolism of cortisol to α -THF (by 5α -reductase) is higher in LNP compared to ONP women, and that such metabolism increases in lean women with advancing gestation. The opposite pattern was observed in obese; with a lower ratio of α -THF:cortisol and lower levels of α -THF in obese pregnant compared to ONP, suggesting that metabolism of cortisol to α -THF (by 5α -reductase) is blunted in obese pregnancy. The ratio of THE:cortisone was lower in LNP compared to ONP women, and was lower at LP1 and LP2 compared to LNP women (figure 4i). There were no significant changes in the obese with advancing gestation. This suggests metabolism of cortisone to THE (by 5β -reductase) increases with advancing gestation in lean women, but may be blunted in obese women during pregnancy.

3.3 Associations of HPAA hormones with birthweight, gestation at delivery and infant cortisol levels

Birthweight did not differ between lean and obese in the unadjusted analysis (table 1), but was significantly higher in obese after adjustment for gestation at delivery, sex of baby, maternal age and smoking status ($\beta=0.102$, $p=0.03$). In lean women, increased cortisol at 16 weeks was positively associated with birthweight in unadjusted analysis though this was not significant after adjusting for smoking status, DepCat score, gestational age at delivery and baby gender ($r=0.19$, $p=0.032$, $p_a=0.10$). In contrast, in the obese there was a weak negative correlation between lower cortisol at 16 weeks and

higher birthweight, though this was not significant in adjusted analyses ($r=-0.13$, $p=0.066$, $p_a=0.33$). Cortisol at 28 weeks was lower in lean women with macrosomic babies compared to appropriate for gestational age babies (1760 ± 729 nmol/L vs 2551 ± 4228 nmol/L; $p=0.048$), but did not differ in obese women with macrosomic babies, or in women with low birthweight babies. CRH did not correlate with birthweight in lean or obese.

Obese women delivered at an earlier gestation than lean women (Table 1) due to a higher proportion of elective caesarean deliveries (obese 17.8% vs. lean 5.9%; $p<0.0001$), as there were more previous caesarean sections (obese 49.4% vs lean 28.6%; $p=0.01$). A higher rate of medical complications in obese women meant that there was a higher rate of induction of labour before 40 weeks (obese 30.2% vs. lean 17.6%; $p<0.0001$). In women who did not undergo early iatrogenic delivery before 40 weeks the mean gestation was not significantly different between lean and obese (lean 283 ± 8 days, obese 282 ± 11 days; $p=0.53$). In obese, there was an inverse correlation between CRH at 28 weeks and gestational length ($r=-0.49$, $p=0.04$). After adjusting for maternal smoking and mode of delivery, this remained significant as an independent predictor of gestation at delivery (mean decrease in CRH of -0.25 pmol/L (95% CI -0.45 to -0.043 pmol/L) per/day increase in gestation). Cortisol did not associate with gestational age at delivery in lean or obese.

4. DISCUSSION

Our findings demonstrated lower fasting morning cortisol levels in very severely obese women throughout pregnancy compared with a lean control group; a pattern similar to non-pregnant severe obesity(21). The lack of an increase in urinary clearance of glucocorticoids during pregnancy in obese women suggests that in contrast to non-pregnant obesity(19,31), the activation of the HPAA, which normally occurs during pregnancy(13,32) was blunted in these very severely obese pregnant women. Further, the apparent 'blunting' of the cortisol awakening response we observed in obese pregnant women would also support the notion that obese pregnant women have lower production of cortisol. Our exploratory analysis suggests this HPAA dysregulation may contribute to complications of obese pregnancy including higher birthweight and prolonged gestation.

Our observation that hormones regulating circulating cortisol levels including CBG, CRH and estrogens were also lower in obese pregnancy suggests these may contribute to the lower maternal cortisol in obese pregnant compared to lean pregnant women. CRH was lower in obese pregnant compared to lean pregnant women. The positive feedback loop between glucocorticoids and placental CRH(33) raises the question of whether lower cortisol in obese was a cause or consequence of lower CRH. Elevated maternal total cortisol at 15 weeks is predictive of a subsequent surge in placental CRH at 31 weeks in women who deliver preterm(17). This would support lower cortisol levels in very severely obese pregnancy at an earlier gestation as the driving factor for the lower CRH we observed later in pregnancy in obese.

Our observations of lower progesterone and estrogen levels in very severe obese pregnancy may not be due to altered SHBG levels, which did not differ between lean and obese during pregnancy. Further, our data are consistent with recent observations in less severely obese pregnant women(34), where it was proposed that impaired mitochondrial function in the placentas of obese pregnant women limits placental sex steroid biosynthesis. Cortisol is not considered to be synthesized in the placenta, so it is unlikely that this contributes to the lower cortisol we found in very severely obese pregnancy. In contrast to our findings of lower cortisol and lower estrogen in very severely obese pregnant women, a small study in postmenopausal women suggested that higher estrogen levels may magnify the negative feedback of cortisol on the HPAA in postmenopausal women, leading to lower cortisol levels(35). To the best of our knowledge, there are no other data describing the effects of estrogen on cortisol in severely obese pregnant women. Evidence from *in vitro* studies would suggest that lower progesterone and estrogen may limit CRH production(36,37), which in turn could reduce total cortisol levels.

The measurements of total and intact CBG allowed us to calculate free, bioavailable cortisol levels, which were also lower in obese pregnant women. The reason for the lack of rise in total CBG in obese women is unknown, but likely reflects the lower estrogen levels we observed(14). Other factors that may alter CBG levels include altered metabolic, inflammatory or genetic factors. The very severely

obese women in our cohort had higher levels of insulin, inflammatory markers and liver enzymes than the lean women(38); all factors which would be anticipated to increase CBG levels. However, as we have previously demonstrated that very severely obese women failed to accumulate hepatic fat during pregnancy, we can speculate that the associated improvement in hepatic insulin sensitivity may also drive a reduction in CBG synthesis in very severely obese pregnancy. Although it has been suggested that the placenta synthesises CBG(39), whether this differs in obese and lean placental tissue is not known. Our recent observation that morning cortisol levels are genetically determined through cortisol binding to CBG(40) implies a further mechanism for regulation of maternal and fetal glucocorticoid exposure, which requires further exploration. Alternatively, if CRH is the primary driving force mediating cortisol levels, the lower CBG may be a mechanism by which the maternal liver balances regulation of fetal cortisol exposure and the normal physiological roles of cortisol in the mother.

The lack of rise in urinary total glucocorticoid metabolites, free cortisol, free cortisone, total cortisol metabolites and total cortisone metabolites in obese pregnant compared to lean pregnant women is consistent with decreased activity of the HPAA in obese pregnancy, resulting in lower production of cortisol. This contrasts to the findings in the lean where total urinary glucocorticoid metabolite excretion increased with advancing gestation, paralleling the rising circulating cortisol levels, and also contrasts to non-pregnant obesity where urinary glucocorticoid clearance is increased compared to lean controls. Further, our data suggests that the changes in activity of whole-body 11β -HSD2, 11β -HSD1, 5α -reductase and 5β -reductase enzymes may be blunted in obese pregnant compared to lean pregnant women. These preliminary data would suggest that the lower circulating levels of cortisol in obese cannot be explained by increased metabolism or clearance of cortisol. Further studies are needed to explore this, and to investigate possible alternative explanations for the apparent lack of increased clearance of cortisol which has been reported in non-pregnant obesity(19).

Our data add to the emerging literature in humans that exposure to endogenous cortisol is a critical factor regulating growth and length of gestation(13). In exploratory analyses we observed a weak inverse trend between lower cortisol at 16 weeks and higher birthweight in obese, as well as lower

cortisol levels in lean women with macrosomic babies. Further, lower CRH at 28 weeks gestation in obese was also significant as an independent predictor of longer gestation. Although our study was under-powered to look at birth outcomes this is the first study to show that dysregulation of the HPAA may contribute to the complications of obese pregnancy including increased fetal size and prolonged gestation.

Strengths of this study include the large sample size, which allowed for repeated measures of cortisol from blood in the same individual including in pregnancy and postpartum, the careful timing of morning samples and the detailed assessment of hormones controlling cortisol release. Further, the marked differences in BMI levels between the severely obese pregnant and lean pregnant groups meant our two groups were very different for comparison. We do acknowledge that the sample size of the cohort of women with 24 hour urine collections was small and larger studies are needed to confirm these results. A limitation is that total cortisol was measured by immunoassay, which has been reported to detect lower levels of cortisol during pregnancy, compared with liquid chromatography-tandem mass spectrometry (LCMSMS)(14), however, we were still able to detect significant differences between the lean and obese study groups. Further limitations of the study are that we used Coolens' equation(25) to estimate free cortisol levels; this has been used in other studies of cortisol in pregnancy(41) but not validated. The single time-point sampling would not account for variation in cortisol levels caused by hormone oscillations. Studies investigating the ultradian rhythm generated by cortisol pulsatility are on-going. Finally placental cortisol metabolism and transfer in obese pregnancy remain unknown and further studies are needed to understand more about the contribution of the placenta to maternal and fetal glucocorticoid exposure.

In conclusion, our study suggests that there is dysregulation of the HPAA in obese pregnancy resulting in lower circulating levels of cortisol and its regulatory hormones consistent with decreased HPAA activity. Our preliminary data suggests this may contribute to some of the adverse complications of obese pregnancy, increased fetal size and prolonged pregnancy. A better understanding of the role of

HPAA dysregulation in adverse pregnancy outcomes may inform us which high-risk pregnancies should be targeted to improve the health of the pregnant woman and the developing baby.

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TABLES

Table 1

Maternal Characteristics			
	Obese (n=276)	Lean (n=135)	p-value
Age (years)	31.2 (5.3)	33.0 (4.6)	<0.001
BMI (kg/m ²)	44.0 (4.0)	22.7 (1.7)	< 0.001
Parity n (%)			
- Prim	137 (49.6)	84 (62.2)	0.002
- Para 1	82 (29.7)	41 (30.4)	
- Para ≥ 2	57 (20.7)	10 (7.4)	
Ethnicity n (%)			
- Caucasian	263 (95.3)	133 (98.5)	0.10
- Non-Caucasian	13 (4.7)	2 (1.5)	
DepCat Score n (%)			
- 1-3	80 (29.0)	90 (66.7)	<0.001
- 4+	196 (71.0)	45 (33.3)	
Smoking Status n (%)			
- Never Smoked	161 (58.8)	81 (60.0)	0.005
- Ex-smoker	79 (28.8)	50 (37.0)	
- Current smoker	34 (12.4)	4 (3.0)	
Labour onset Mode n (%)			
- Spontaneous	111 (40.9)	96 (71.1)	<0.0001
- Induced	105 (38.6)	30 (22.2)	
- No labour	56 (20.5)	9 (6.7)	
Mode of Delivery n (%)			
- Spontaneous vertex delivery	121 (44.5)	75 (55.5)	<0.0001
- Assisted vaginal delivery	36 (13.2)	31 (23.0)	
- Emergency caesarean section	67 (24.5)	21 (15.6)	
- Elective caesarean section	49 (17.8)	8 (5.9)	

Medical Complications n (%)			
- Gestational diabetes	43 (15.6)	2 (1.5)	<0.0001
- Pre-eclampsia	5 (1.8)	3 (2.2)	0.80
- Pregnancy induced hypertension	14 (5.1)	2 (1.5)	0.07
Neonatal Characteristics – Term babies			
	Obese n=262	Lean (n=132)	p-value
Baby Sex			
- Male n (%)	123 (46.9)	63 (47.7)	0.88
- Female n (%)	139 (53.1)	69 (52.3)	
Gestational age at delivery (days)	280.4 (8.6)	282.5 (8.3)	0.02
Birthweight (g)	3629.1 (531.8)	3571.6 (498.4)	0.30
Macrosomia (>4000g)			
- Yes n (%)	64 (24.4)	25 (18.9)	
- No n (%)	198 (75.6)	107 (81.1)	0.22
Low birth weight (<2500g)			
- Yes n (%)	4 (1.5)	0 (0)	0.15
Weight at 3months of age (kg)	3583.0 (580.6)	3554.0 (507.6)	0.62
Preterm babies (<37 weeks)			
	Obese n=14	Lean n=3	p-value
Birthweight (g)	2721.1 (780.6)	2776.7 (257.9)	0.91
Gender n (%)			
- Male	10 (71.4)	1 (33.3)	0.21
- Female	4 (28.6)	2 (66.7)	
Gestational age at delivery (days)	250.4 (14.5)	256.3 (1.5)	0.91

Table 1. Maternal and neonatal characteristics. Parity was defined as ‘prim’ (no previous pregnancies delivered after 24 weeks), ‘para 1’ (one previous delivery after 24 weeks gestation) and

‘para ≥ 2 ’ (two or more previous deliveries after 24 weeks gestation). Socioeconomic status was measured using deprivation category (DepCat) scores based on postcode(30). DepCat score 1-3 represents the least deprived; score 4+ represents the most deprived. DepCat information was unavailable for 1 obese woman. ‘Smokers’ were defined as those who considered themselves as current smokers. Missing data: smoking status (obese n=2), labour onset mode (obese n=4), mode of delivery (obese n=3). Data are mean (sd) or N(%).

Table 2

Free Cortisol (nmol/L)	Obese (n=20)	Lean (n=20)	p-value
Calculated with Total CBG			
16 weeks	482.6 (484.1)	473.9 (342.8)	0.63
28 weeks	392.4 (225.9)	431.4 (156.5)	0.32
36 weeks	326.1 (177.9)	617.0 (330.0)	0.001
Postpartum	293.6 (180.2)	329.8 (211.5)	0.628
Calculated with Intact CBG			
16 weeks	552.8 (521.0)	729.4 (337.3)	0.22
28 weeks	403.2 (227.8)	508.9 (123.7)	0.005
36 weeks	349.6 (164.1)	646.8 (324.4)	0.003
Postpartum	314.0 (205.3)	370.6 (208.8)	0.403

Table 2. Free Cortisol calculated using total and intact CBG levels. Data are mean (sd).

FIGURES

Figure 1

Figure 1a-b. Total Serum Cortisol (1a) and Saliva Cortisol (1b).

Data are mean (sem); * $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.0001$. Sample size obese (n=276), lean (n=135).

Figure 2

Figure 2a-c. Total corticosteroid binding globulin (CBG) (2a), Intact CBG (2b) and Cleaved CBG (2c).

Data are mean (sem); *p<0.05, **p≤0.01. Samples size obese (n=20), lean (n=20).

Figure 3

Figure 3a-d. Corticotrophin releasing hormone (CRH) (3a), estradiol (3b), estriol (3c) and progesterone (3d) and sex-hormone-binding-globulin (SHBG) (3e).

Data are mean (sem); *p<0.05, **p<0.01, ***p<0.0001. Sample size obese (n=20), lean (n=20).

Figure 4

Figure 4a-i. Urine glucocorticoid metabolites.

Key: LNP (lean non-pregnant), ONP (obese non-pregnant), LP1 (lean pregnant visit 1, approximately 19 weeks gestation), LP2 (lean pregnant visit 2, approximately 36 weeks gestation), OP1 (obese pregnant visit 1, approximately 19 weeks gestation), OP2 (obese pregnant visit 2, approximately 36 weeks gestation), THF (5β-tetrahydrocortisol), α-THF (5α-tetrahydrocortisol), THE (tetrahydrocortisone), 11β-HSD1 (11beta-hydroxysteroid-dehydrogenase-type 1) and 11β-HSD2 (11beta-hydroxysteroid-dehydrogenase-type 2).

Data are mean (sem); *p<0.05, **p≤0.01, ***p≤0.0001. Sample size LNP (n=7), ONP (n=7), LP (n=5) and OP (n=7).

SUPPLEMENTARY TABLES

Supplementary Table 1

Maternal Characteristics			
	Obese (n=20)	Lean (n=20)	P-value
Age (years)	32.6 (5.2)	33.5 (3.0)	0.53
BMI (kg/m²)	43.3 (4.1)	22.4 (1.8)	<0.0001
Parity n (%)			
- Primiparous	11 (55)	10 (50)	0.88
- Para 1	7 (35)	7 (35)	
- Para ≥ 2	2 (10)	3 (15)	
Smoking status n (%)			
- smoker	1 (5)	0 (0)	0.40
- non-smoker	19 (95)	20 (100)	

Supplementary table 1. Maternal characteristics of subset of obese (n=20) and lean (n=20) with analysis of CBG and sex steroids

Parity was defined as ‘nulliparous’ (no previous pregnancies), ‘primiparous’ (no previous pregnancies delivered after 24 weeks), ‘para 1’ (one previous delivery after 24 weeks gestation) and ‘para ≥ 2 ’ (two or more previous deliveries after 24 weeks gestation). ‘Smokers’ were defined as those who considered themselves as current smokers. Data are mean (sd) or N(%).

Supplementary table 2

Maternal Characteristics					
	ONP (n=7)	LNP (n=7)	OP (n=6)	LP (n=5)	P-value
Age (years)	35.2 (5.3)	25.9 (2.0)	27.1 (4.1)	35.2 (1.9)	<0.0001
BMI (kg/m²)	40.8 (4.2)	22.6 (1.8)	42.1 (5.8)	27.1 (4.1)	<0.0001
Parity n (%)					
- Nulliparous	2	7	-	-	<0.0001
- Primiparous	2	-	2	1	
- Para 1	2	-	2	4	
- Para ≥ 2	-	-	2	0	
Smoking status n (%)					
- smoker	1	0	0	0	0.34
- non-smoker	6	7	6	5	

Supplementary table 2. Maternal characteristics of subset of women who completed 24 hour urine collections.

Parity was defined as ‘nulliparous’ (no previous pregnancies), ‘primiparous’ (no previous pregnancies delivered after 24 weeks), ‘para 1’ (one previous delivery after 24 weeks gestation) and ‘para ≥ 2 ’ (two or more previous deliveries after 24 weeks gestation). ‘Smokers’ were defined as those who considered themselves as current smokers. Data are mean (sd) or N(%).

Key: LNP (lean non-pregnant), ONP (obese non-pregnant), LP1 (lean pregnant visit 1, approximately 19 weeks gestation), LP2 (lean pregnant visit 2, approximately 36 weeks gestation), OP1 (obese pregnant visit 1, approximately 19 weeks gestation) and OP2 (obese pregnant visit 2, approximately 36 weeks gestation).