The association between higher maternal pre-pregnancy body mass index and increased birth weight, adiposity, and inflammation in the newborn

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Running Title: Pre-pregnancy BMI and cord blood inflammation

What is already known:

- Obesity is associated with chronic inflammation in adults
- Increased maternal BMI is associated with increased neonatal birth weight
- Cardiometabolic diseases have fetal origins

What this study adds:

- Increased maternal BMI is associated with increased neonatal adiposity
- Increased maternal BMI is associated with increased cord blood inflammatory markers
- These associations may be partially mediated by maternal inflammation in pregnancy.

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ABSTRACT

Background
Excess adiposity and adiposity-related inflammation are known risk factors for cardiovascular disease in adults, however little is known regarding the determinants of adiposity-related inflammation at birth.

Objectives
The aim of this study was to investigate the association between maternal pre-pregnancy BMI and newborn adiposity and inflammation.

Methods
Paired maternal (28-weeks gestation) and infant (umbilical cord) blood samples were collected from a population-derived birth cohort (Barwon Infant Study, n=1074). Data on maternal co-morbidities and infant birth anthropomorphic measures were compiled, and infant aIMT was measured by trans-abdominal ultrasound. In a selected subgroup of term infants (n=161), matched maternal and cord lipids, high sensitivity C-reactive protein (hsCRP) and maternal soluble CD14 were measured. Analysis was completed using pair-wise correlation and linear regression. Because of their non-normal distribution, pathology blood measures were log transformed prior to analysis.

Results
Maternal pre-pregnancy BMI was positively associated with increased birth weight (mean difference 17.8g per kg/m^2, 95%CI 6.6 to 28.9; p=0.002), newborn mean skinfold thickness, (mean difference 0.1 mm per kg/m^2, 95%CI 0.0 to 0.1; p<0.001), and cord blood hsCRP (mean difference of 4.2% increase in hsCRP per kg/m^2 increase in pre-pregnancy BMI, 95%CI 0.6% to 7.7%, p=0.02), but not cord blood sCD14. Inclusion of maternal hsCRP as a covariate attenuated the associations between pre-pregnancy BMI and both newborn skinfold thickness and cord blood hsCRP.

Conclusion
Higher maternal pre-pregnancy BMI is associated with increased newborn adiposity and inflammation. These associations may be partially mediated by maternal inflammation during pregnancy.

INTRODUCTION

The incidence of obesity, dyslipidemia, and cardiometabolic disease is increasing worldwide.[1] The underlying pathogeneses of these diseases begin in early life, therefore pregnancy and infancy are increasingly recognised as key periods for potential intervention. In the US, almost two thirds of pregnant women are obese or overweight (body mass index (BMI) >25kg/m^2)[2], and pre-pregnancy obesity is associated with increased newborn birth weight[3, 4], which in turn is associated with both early childhood obesity and metabolic syndrome[3]. The mechanisms of the association between infant adiposity and later life metabolic syndrome are unknown, however in adults chronic inflammation is important in the development of the cardio-metabolic consequences of obesity[5]. Maternal obesity has been associated with inflammation during pregnancy[6], but the relationship between maternal obesity and offspring adiposity and inflammation is less well understood.

Pregnancy is a pro-inflammatory state[7], and the systemic inflammation is more marked in overweight and obese pregnant women[8]. In obesity, inflammatory cytokine production is promoted by adipose tissue, dysbiosis and microbial translocation. Accordingly, obesity is associated with chronic elevation of inflammatory biomarkers. Animal models demonstrate that maternal obesity is associated with inflammatory changes in offspring adipose tissue[9], as well as hypomethylation-induced increased expression of inflammatory cytokines in adipose tissue[10]. Moreover, in animal models, these inflammatory changes increase with successive generations, with the highest expression of inflammatory genes in the third generation of offspring whose mothers were fed high fat diets[10]. Human studies are needed to understand the relationship between maternal obesity and newborn inflammation and adiposity, and to evaluate the possible contribution of maternal inflammation during pregnancy.

High-sensitivity C-reactive protein (hsCRP) is a widely-employed marker of inflammation that is used in adult clinical algorithms to stratify risk of cardiovascular events[11], and as an end-point in cardiovascular intervention trials[12]. In adults increased hsCRP is associated with adiposity and metabolic syndrome, but it is unknown if this relationship is present in infancy. Other inflammatory markers include soluble CD14 (sCD14), which is indicative of monocyte activation and correlates with translocation of bacterial lipopolysaccharide from the gastrointestinal tract[13]. Circulating sCD14 is increased in obesity and metabolic syndrome, suggesting chronic translocation of bacterial ligands from the gastrointestinal tract may contribute to the inflammatory milieu ('metabolic endotoxemia')[13]. Previous studies suggest that neither hsCRP nor sCD14 cross the healthy placenta[14, 15]. It is therefore considered likely that cord blood levels of hsCRP and sCD14 reflect endogenous production of these inflammatory markers[14, 15].

Using a population-derived birth cohort, we investigated the prospective association between maternal pre-pregnancy BMI and each of the following perinatal outcomes: birth weight, newborn adiposity and cord blood inflammation. We then evaluated whether the relationships between maternal pre-pregnancy BMI and offspring adiposity and inflammation were mediated by maternal inflammation during pregnancy.

MATERIALS AND METHODS

Study design and participants

The Barwon Infant Study is a pre-birth cohort (n=1074 mother-newborn pairs) from the Barwon region in the south east of Australia[16]. Women were recruited using an unselected sampling frame at their first antenatal hospital

visit and were subsequently excluded if their newborns were delivered prior to 32 weeks, developed a serious illness in the first week of life, or had significant congenital or genetic abnormalities. Inflammatory markers were measured in maternal serum and cord blood in a sub-sample of 161 participants. This subsample was not a random sub-sample, but rather comprised all participants that had adequate maternal and cord blood specimen availability, complete antenatal data (including maternal pre-pregnancy BMI) as well as complete 6 week data collection.

Ethics approval for this study was granted by the Barwon Health Human Research and Ethics Committee (HREC 10/24). Participating parents provided written informed consent.

Study measurements

Data on maternal age, parity, prenatal weight, and antenatal co-morbidities were collected from questionnaires and hospital records. Maternal pre-pregnancy BMI was calculated from self-reported pre-pregnancy weight and directly measured maternal height at the first study visit (28-32 weeks gestation). Maternal pre-pregnancy BMI was primarily analyzed as a continuous variable, however for some mediation analyses a dichotomous variable was created, classifying women as overweight if they had a maternal pre-pregnancy BMI >25 kg/m^2, in keeping with international definitions of overweight and obesity[17]. The Socio-Economic Indexes for Areas (SEIFA), a validated measure of socio-economic status, was calculated participant's residential address at recruitment[18].

Birth anthropometry measures (birth weight, length, head circumference) were collected within the first two days of life. Skinfold thickness measurements were obtained at standardised anatomical locations (triceps and subscapular) using Holtain calipers, by one of two trained examiners. Each site was measured two to

three times and the average taken and the result. Newborn skinfold measures have a coefficient of reliability of 75-93%[19]. Mean skinfold thickness, a measure of infant adiposity, was calculated as the average of the subscapular and triceps skinfold measurement[20]. Z-scores for birth weight adjusted for gestational age and sex were calculated using Australian normative data[21]. Z scores were not routinely adjusted for maternal BMI because BMI is a component of the putative causal pathway of interest. Neither did we retain ethnicity or parity in the final model, as adjustment for these variables had minimal effect on Z-score point estimates.

Blood sample processing & biomarker assays

Non-fasting blood samples were collected in serum clotting tubes from mothers at approximately 28 weeks gestation. Umbilical cord blood (≤ 5ml) was collected before delivery of the placenta from placental vessels into a serum clotting tube. Both maternal and newborn blood samples were centrifuged within 2 hours of collection and the serum aliquoted and stored at -80^{0}C before assaying.

High-sensitivity CRP was measured on a COBAS Integra 400 *plus* Analyser (Roche Diagnostics Australia, Castle Hill, NSW, Australia); maximum recorded value was 20mg/dL. Soluble CD14 serum levels were assayed using the Human sCD14 Quantikine ELISA (R&D Systems, China).

Statistical Analysis

All serum markers were log-transformed prior to analysis because of right-skewed distributions. Mean differences between binary categories (eg BMI >25kg/m^2) were assessed using student t-tests. Associations between continuous variables were first visualized as scatter plots, and then analyzed by

multiple linear regression. Potential confounding variables were identified from substantive consideration of likely causal pathways, and then the effect of adjustment for these potential confounders on the magnitude of associations was assessed. Primary results are presented as estimated mean difference (MD) change per unit of exposure, with associated 95% confidence intervals. Statistical analysis was performed using Stata 13.1 (Stata Corp, College Station, TX).

Firstly, we investigated the association between the independent variable, maternal pre-pregnancy BMI, and the dependent variables infant birth weight and adiposity (skinfold thickness). To assess the extent to which the association between maternal BMI and birth weight was independent of infant adiposity, regression analysis was repeated with skinfold thickness as a mediator variable using a simple causal mediation approach[22, 23]. We then stratified our sample into overweight and obese (pre-pregnancy BMI>25kg/m^2) women and those of normal weight[17], and repeated the regression analysis for each group. Secondly, we investigated the association between maternal pre-pregnancy BMI and both maternal and infant inflammation. Finally, we evaluated whether associations between maternal pre-pregnancy BMI and infant outcomes were partially mediated by including maternal inflammation in linear regression models for these outcomes and using the same mediation approach[22].

RESULTS

Baseline characteristics of the BMI cohort:

The study population was similar to the larger cohort; baseline characteristics of the subjects in the study population compared to those in the rest of the cohort are displayed in Table 1. As expected, there was a moderate correlation between

birth weight and birth adiposity measured by skin fold thickness (R=0.49, p<0.001).

Table 1. Characteristics of the mothers and newborn

Higher maternal pre-pregnancy BMI is associated with increased newborn adiposity

Infants whose mothers were overweight or obese pre-pregnancy (BMI>25 kg/m^2) had increased birth weight (3588g versus 3481g, mean difference 106g, 95%CI -31 to 243g, p=0.13), skinfold thickness (5.16mm versus 4.73mm, mean difference 0.4mm, 95%CI 0.1 to 0.8mm, p=0.02) and z-score of birth weight for gestational age (71.6 centile versus 63.5 centile, mean difference 8.1, 95%CI 0.5 to 15.6 centiles, p=0.04) compared with mothers who were not (BMI <25kg/m^2).

There was evidence of positive associations between maternal pre-pregnancy BMI and birth weight (MD=17.3g per kg/m^2, 95%CI 6.3 to 28.4; p=0.002), between maternal pre-pregnancy BMI and birth skinfold thickness (MD=0.06 mm per kg/m^2, 95%CI 0.03 to 0.09; p<0.001), and between maternal pre-pregnancy BMI and weight z-score adjusted for sex and gestational age (MD=1.0 centile per kg/m^2, 95%CI 0.4 to 1.6; p=0.001). When newborn skinfold thickness was included as a covariate, the association between maternal pre-pregnancy BMI and birth weight attenuated (MD 6.9g per kg/m^2, 95%CI -3.7 to 17.4g, p=0.20). Indeed over half the magnitude of the association between higher maternal pre-pregnancy BMI and higher birth weight appeared to be accounted for by higher newborn skinfold thickness (61.5%, 95%CI 36.3% to 181.5%). In contrast, the associations between maternal pre-pregnancy BMI and offspring skinfold thickness persisted following adjustment for birth weight (MD = 0.04mm per kg/m^2, 95%CI 0.01 to 0.06, p=0.03)."

The magnitude of positive associations between maternal pre-pregnancy BMI and each of birth weight and newborn skinfold thickness were stronger when the cohort was restricted to mothers who were overweight or obese (BMI >25kg/m^2 n=71) than when it was restricted to mothers who were normal or underweight (BMI <25 kg/m^2 n=90). However the statistical evidence that these relationships were modified by the presence maternal overweight/obesity was relatively weak (Table 2).

Table 2: The association between maternal pre-pregnancy BMI and infant adiposity, stratified by maternal overweight and obesity.

Higher maternal pre-pregnancy BMI is associated with increased inflammation during pregnancy

Higher maternal pre-pregnancy BMI was associated with increased maternal inflammation in pregnancy. For every 1kg/m^2 increase in pre-pregnancy BMI, maternal hsCRP levels increased by 8.0%, 95%CI 5.6% to 10.4%; p<0.001 (Figure 1), and maternal sCD14 increased by 0.7%, 95%CI 0.3% to 1.1%; p=0.003. The magnitude of associations were minimally changed following adjustment for maternal age, smoking in pregnancy, and gestational diabetes (hsCRP 8.6% per mg/kg, 95%CI 5.9% to 11.3%, p<0.001; sCD14 0.6%, 95% CI 0.1% to 1.1%; p=0.019).

Figure 1: The association between maternal pre-pregnancy BMI and hsCRP protein at 28 weeks gestation (n=161) (logged). For every 1kg/m^2 increase in pre-pregnancy BMI, maternal hsCRP levels increased by 8.0%, 95%CI 5.6 to 10.4%; p<0.001

Higher maternal pre-pregnancy BMI is associated with increased cord blood inflammation

Following adjustment for maternal smoking, mode of delivery, gestational age and newborn sex, maternal pre-pregnancy BMI was correlated with newborn hsCRP (for each 1mg/kg change in BMI, hsCRP increased by 4.2%, 95%CI 0.6%

to 7.7%; p=0.02), but not with newborn sCD14 (for every 1mg/kg change in BMI, sCD14 increased by 0.5%, 95%CI -0.1% to 1.1%, p=0.10).

Increased maternal inflammation is associated with cord blood inflammation

Following adjustment for maternal smoking, mode of delivery, gestational age, and sex, maternal hsCRP was associated with cord blood hsCRP (for every 10% increase in maternal hsCRP there was a 2.7% increase in infant hsCRP, 95%CI 0.6% to 4.8%, p=0.01). Maternal hsCRP was not associated with cord sCD14, nor was maternal sCD14 associated with cord blood hsCRP or cord sCD14. The exclusion of infants with hsCRP>10mg/dl (suggestive of acute inflammation) did not substantially alter the results.

Maternal pre-pregnancy BMI, maternal inflammation, birth weight, and cord blood inflammation

The magnitude of the association between maternal pre-pregnancy BMI and birth weight reduced by approximately one quarter when maternal hsCRP was included as a covariate (mediation percentage 26.5%, 95%CI 16.0% to 80.8%). The reduction in the magnitude of association was less than 5% when maternal sCD14 was included (mediation percentage 3.2%, 95%CI 2.0% to 9.3%). The associations between maternal pre-pregnancy BMI and newborn skinfold thickness, and between maternal pre-pregnancy BMI and birth weight z-score, had a similar pattern.

The magnitude of association between maternal pre-pregnancy BMI and cord blood hsCRP reduced by 40.0% when maternal hsCRP was included as a covariate, however with extremely wide confidence intervals (mediation percentage 40.0%, 95%CI -140.0 to 213.6%). However in infants born without exposure to the acute stress of labour (ie. via elective caesarian section, n=29), the magnitude of the association between maternal pre-pregnancy BMI and cord

blood hsCRP was approximately halved when maternal hsCRP was included as a covariate (mediation percentage 50.5%, 95%CI 25.4% to 263.0%).

DISCUSSION

In this population-derived sample of mother-newborn dyads, higher pre-pregnancy BMI was positively associated with offspring birth weight and this primarily related to increased newborn adiposity. In addition, higher maternal pre-pregnancy BMI was associated with cord blood inflammation; and approximately half of this association could be accounted for by maternal inflammation (as measured by hsCRP) at 28 weeks gestation. Given the potential importance of early life adiposity and inflammation to long-term cardiometabolic risk, our findings suggest that strategies to reduce pre-conception overweight and obesity may be of benefit in reducing the risk of cardiometabolic disease in offspring.

Cardiometabolic risk trajectories are initiated in early life, but the causal pathways are poorly understood. Maternal obesity is associated with increased offspring birth weight[24], and specifically with increased adiposity and newborn total fat mass[25]. Whilst the association between maternal obesity and maternal inflammation has been described in pregnancy[26], our study extends these findings by showing that maternal pre-pregnancy BMI is also associated with cord blood inflammation, thought to be representative of endogenous fetal production. In adults, chronic inflammation is increased in metabolic syndrome and is strongly predictive of cardiovascular disease risk. The associations between the maternal pre-pregnancy BMI and each of infant adiposity and infant inflammation are of relatively small magnitude. Although these modest associations may not be of immediate clinical significance, the

implications for long-term cardiovascular health are as yet unknown. Given the latency of cardiovascular disease onset, there are currently no studies of sufficient duration to evaluate the relationship between infant adiposity and inflammation to cardiovascular events in later life. Nonetheless, it is concerning that in the current study maternal BMI was positively associated with multiple different measures of offspring adiposity as well as maternal and infant inflammation. These findings are consistent with mother-to-infant transmission of cardiometabolic risk, which may be in part mediated by maternal inflammation during pregnancy[27].

In mice, maternal high fat diet is associated with increased offspring adiposity and inflammatory gene expression[9]. Furthermore, in murine models, pathogenic features of obesity seen in the mother (such as hepatic steatosis) are observed in the newborn offspring, suggesting that the cardiometabolic risk can somehow be conferred from mother to fetus[27]. Our study confirms the likely relevance of these findings to humans by demonstrating an association between maternal pre-pregnancy BMI and both infant adiposity and cord blood inflammation, implying that both the metabolic and inflammatory consequences of maternal overweight and obesity may be transmitted to the offspring *in utero*.

Increased maternal adiposity promotes a chronic inflammatory state in the mother and this inflammatory milieu may contribute to the development of offspring adiposity. In the current study maternal pre-pregnancy BMI was associated with both maternal hsCRP and maternal sCD14, and hsCRP was associated with newborn adiposity, the association between maternal sCD14 and infant adiposity did not persist following adjustment for maternal age, gestational diabetes, smoking and sex. A mediation analysis suggested that log maternal hsCRP accounted for approximately one fifth of the association

between maternal pre-pregnancy BMI and birth weight, and up to half of the association between maternal pre-pregnancy BMI and cord blood hsCRP. Furthermore, there was some indication that the association between maternal hsCRP and birth weight was stronger in magnitude in overweight and obese women, although the statistical evidence for an interaction effect was weak, possibly due to the small sample size. Comparable cohort studies have been unable to demonstrate an association between maternal hsCRP and birth weight that is independent of pre-pregnancy BMI. However, given the emerging evidence of interplay between obesity and inflammation[5], investigating the independence of these associations would seem less informative than treating maternal inflammation as a mediating variable.

The strengths of this study include the use of a prospectively studied population-based cohort and inclusion of measures of newborn adiposity in addition to birth weight. The main limitation is the relatively small number of mother-infant dyads with paired inflammatory measures, which has reduced the precision of our estimates. Although these dyads seem to be similar to that of the overall cohort, there may be some residual confounding due to selection bias in this group that we have been unable to control for. It was not possible to determine whether elevated hsCRP and sCD14 represent a chronic or acute inflammatory response, however sensitivity analysis excluding those with CRP >10mg/dl (suggestive of acute inflammation) made minimal difference to results. Similarly, we only have one marker of maternal inflammation during pregnancy to reflect inflammation across pregnancy. Reassuringly, it was taken at a consistent time-point in well women in the third trimester.

Both hsCRP and sCD14 may be elevated due to chronic inflammatory stimuli[5], and also following acute stresses such as labor or infection[28]. However the

correlation between maternal and infant hsCRP, which were measured approximately three months apart, is compatible with a chronic inflammatory response. Another limitation was the use of self-reported pre-pregnancy weight, although the pre-pregnancy BMI of the women in this cohort are consistent with Australian population data[29]. In addition, we did not capture weight gain during pregnancy. Finally, the mediation analysis performed relies on the sequential ignorability assumption[23]; it assumes that there is no confounding in the relationship between the putative mediator and the outcome measure, and thus may overestimate the degree of mediation[30].

The findings of this study may have important clinical implications. Higher maternal pre-pregnancy weight is associated with higher birth weight, and this study suggests that this association is primarily relates to increased birth adiposity rather than increased overall infant size. Furthermore, a novel link has been made between maternal pre-pregnancy BMI and newborn inflammation, which appears to be partially mediated by maternal inflammation during pregnancy.

CONCLUSION

In addition to replicating the established association between maternal pre-pregnancy BMI and offspring birth weight, we found that higher maternal pre-pregnancy BMI was also associated with increased offspring adiposity and inflammation. Furthermore, these associations were in part mediated by maternal inflammation during pregnancy. Of clinical relevance, the relationships between maternal pre-pregnancy BMI and offspring adiposity were substantially stronger among mothers who were overweight or obese. These findings may have important public health implications given that in many countries the majority of women of child-bearing age are now overweight or obese.

CONFLICT OF INTEREST

The authors of this study have no conflict of interest to declare.

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Table 1. Characteristics of the mothers and newborn

	Study population n=161	Rest of cohort n=913
Sex of child: Male	84/161 (62%)	472/913
Socio-Economic Indexes for Areas (SEIFA):		
Low (most deprived)	45/158 (28%)	223/896 (25%)*
Mid	26/158 (16%)	178/896 (20%)*
High (least deprived)	87/158 (54%)	495/896 (55%)*
Unknown		17/913 (2%)
Maternal age, years (mean and standard deviation)	32.7 (4.0)	32.0 (4.9)
Maternal cigarette smoking (any during pregnancy):	6/155 (4%)	101/913 (11%)
Maternal pre-natal BMI kg/m^2 (median IQR)	24.1/161 (21.6-27.7)	24.1 (2.6 to 27.9)
Overweight (>25 kg/m^2)	71/161 (44%)	320/913 (35%)
Maternal gestational diabetes	8/146 (5%)	35/913 (4%)
Maternal Group B Streptococcal colonisation*	24/148 (15%)	181/913 (20%)
Delivery via Caesarean section	52/161 (32%)	283/913 (31%)
Gestational age at birth:		
32 to 36 completed weeks	5/161 (4%)	40/913 (4%)
37 to 42 completed weeks	156/161 (96%)	873/913 (96%)
Caucasian	129/138 (80%)	665/913 (73%)
Birth weight in grams (mean and standard deviation)	3528 (439)	3526 (532)
Mean newborn skinfold thickness - triceps and subscapular mm (median IQR)	4.7 (4.2-5.6)	4.8 (4.1-5.5)
Maternal hsCRP (median IQR) mg/dL	3.40 (1.63-5.66)	
Maternal sCD14 (median IQR) ng/mL	1244 (1130-1392)	
Cord blood hsCRP (median IQR) mg/dL	0.05 (0.03- 0.11)	
Cord sCD14 (median IQR) ng/mL	508 (442-563)	

*Variation in denominator reflects missing data

Table 2: The association between maternal pre-pregnancy BMI and infant adiposity, stratified by maternal overweight and obesity.

	Overall (n=161)	**BMI <25 kg/m^2 (n=90)**	**BMI >25 kg/m^2 (n=71)**	**p**
	MD per kg/m^2, (95%CI); p	**MD per kg/m^2, (95%CI); p**	**MD per kg/m^2, (95%CI); p**	**interaction**
Birth weight (g)	17.8, (6.6 to 28.9); p=0.002	7.8, (-35.0 to 50.6); p=0.72	26.6, (6.0 to 47.2); p=0.01	0.477
Skinfold thickness (mm)	0.06, (0.03 to 0.09); p<0.001	0.01, (-0.10 to 0.12); p=0.90	0.08, (0.03 to 0.13); p=0.002	0.267
Z-score birth weight (centile)	1.1, (0.4 to 1.7); p=0.001	1.2, (-1.5 to 3.9); p=0.38	1.2, (0.2 to 2.2); p=0.02	0.995

*MD = mean difference, **overweight and obesity = BMI >25 kg/m^2