Maternal obesity associated with inflammation in their children

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Background: This study explored the association between maternal obesity during pregnancy and the inflammatory markers, tumor necrosis factor-α, interleukin-6 and high sensitivity C-reactive protein (hs-CRP), and the cytokine, adiponectin, in the offspring.

Methods: Weight, height, Tanner stage and biomarkers were measured in thirty-four 12-year-old children, from the Infant Growth Study, who were divided into high risk (HR) and low risk (LR) groups based on maternal pre-pregnancy body mass index (BMI).

Results: The two groups differed markedly in their hs-CRP levels, but no group difference was found for the other three biomarkers. The odds ratio (OR) of HR children having detectable hs-CRP levels was 16 times greater than that of LR children after adjusting for confounding variables, including BMI *z*-score, Tanner stages and gender (OR: 16; 95% CI: 2-123).

Conclusions: These results suggest that maternal obesity during pregnancy is associated with later development of elevated hs-CRP in the offspring, even after controlling for weight.

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Introduction

Maternal obesity is known to increase the risk of obesity in children.[1] Furthermore, exposure to gestational diabetes, excessive weight gain or nutritional restriction during pregnancy is associated with later development of obesity and its complications in the offspring.[1-4] While obesity is known to be a chronic, inflammatory state,[5] it is unclear whether obesity specifically during pregnancy affects the development of inflammation in the offspring before the onset of obesity. Whereas one study has shown that the inflammatory marker, C-reactive protein, is elevated in non-obese adult offspring of two obese parents,[6] the impact of maternal obesity during pregnancy on this or other inflammatory markers in non-obese children is unknown.

Therefore, we examined 12-year-old children who were classified into two risk groups for obesity, high risk (HR) and low risk (LR), based respectively on the high and low maternal pre-pregnancy body mass index (BMI) levels. Since the HR subjects, a majority of whom are not yet obese, are known to be prone to excess weight gain,[1] this unique cohort allows one to examine these children during early stages of the development of obesity as compared to the LR group. The aim of this study was to compare inflammatory markers and cytokines in children with (HR) and without (LR) exposure to maternal obesity during fetal life.

Methods

Design and participants

This is a cross-sectional study of the subjects from the Infant Growth Study,[7,8] an ongoing, longitudinal cohort study designed to assess anthropometric and

metabolic measures from infancy to adolescence. These Caucasian infants were initially enrolled by 3 months of age[8] based on their mothers' pre-pregnancy BMI, with the BMI cutoffs based on nationally representative data from the National Health and Nutrition Examination Survey (waves 1 and 2).[9] The mothers' BMI cutoffs were greater than 66th percentile for the HR group and less than 33rd percentile for the LR group.[9]

Enrollment criteria included: 1) infants born between 36 and 42 weeks gestational age; 2) infants with weight appropriate for gestational age; 3) mothers without history of gestational diabetes; and 4) maternal age greater than 18 years.

The present study reports on 34 subjects whose weight, height, and biomarkers were ascertained at 12 years of age. None of the 34 subjects reported any other causes of inflammation, such as smoking, acute illnesses, or chronic inflammatory conditions.

Procedures

All anthropometric assessments were performed at the Growth and Nutrition Laboratory and the Clinical and Translational Research Center at the Children's Hospital of Philadelphia, between July 2005 and February 2007. After an overnight stay at the Children's Hospital of Philadelphia, 12-hour fasting blood samples were obtained between 8 and 9 am and were stored at -70 degrees.

Written informed consent was obtained from the parents, and assent was obtained from the children. The protocol was approved by the institutional review boards of the University of Pennsylvania and the Children's Hospital of Philadelphia. All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

Anthropometric measures and Tanner stage

Height, using a stadiometer (Holtain, Crymych, United Kingdom), and weight, using a digital scale (model 6002; Scaletronix, Carol Strea, IL), were obtained in triplicate by trained research anthropometrists, using standardized techniques.[10] BMI (kg/m^2) was calculated from the mean height and weight, and the children's BMI z-scores were calculated based on the 2000 CDC growth charts.[11] The mothers' BMI was calculated based on self-report of their height and weight. Subjects completed the established Tanner stage self-assessment forms.[12] Even though female breast and male genitalia are better measures of puberty when assessed by experienced clinicians, pubic hair was used because it has been shown to be more reliable in self-assessment surveys when compared to a physician's examination.[13]

Biomarkers

Measurements were taken of high sensitivity C-reactive protein (hs-CRP) by immunonepholometry (Dade Behrin BNII instrument), of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) using high-sensitivity ELISAs (R and D in Minneapolis, MN), and of adiponectin by radioimmunoassay (Millipore, formerly Linco, St. Charles, MO).

Data analyses

Descriptive statistics are presented as means and standard deviations for continuous variables and as percentages for categorical variables. Student's t test was used to compare continuous variables between the HR and LR children. Risk status differences in gender, Tanner stage group, and hs-CRP levels were examined using the Chi-square test.

While TNF-α, IL-6 and adiponectin were normally distributed, hs-CRP was an outcome variable that was dichotomized as detectable (≥0.16 mg/L) versus undetectable (<0.16 mg/L), due to the non-normal distribution of this variable and to clinical significance of detectable cutoff levels.

Analysis of covariance regression models were fit to assess whether risk status (HR vs. LR) was associated with IL-6, TNF-α and adiponectin levels. Multivariable logistic regression was used to analyze group differences in levels of hs-CRP. The regression models were adjusted for the following a $priori$ confounders: BMI z-score, gender and Tanner stage. Residual diagnostic analyses were performed to assess model fit, violation of model assumptions, and any potential collinearity concerns. No violations were found. All analyses were conducted using the statistical software package SAS 9.1.3 with alpha level equal to 0.05 and 2-tail significance test.

Results

Table 1 shows the descriptive statistics of the study variables, for the total group and by risk status. As expected and previously reported,[7] subjects in the HR group had higher weight and BMI than those in the LR group. The adjusted associations between risk status and biomarkers are presented in Table 2. No group differences were found for IL-6, TNF-α and adiponectin levels. However, hs-CRP was more likely to be detectable among the HR than LR subjects. This difference remained significant after adjusting for confounding factors, including BMI z-score, gender and Tanner stages.

Brief report

Table 1. Descriptive statistics of anthropometric measures, inflammatory markers and gender differences in risk status groups at year 12 (mean ± SD or percentage)

Variables	Overall (n=34)	Risk status		
		Low risk (n=18)	High risk (n=16)	P value
Maternal pre-pregnancy BMI, kg/m^2	24.0±5.9	19.4±0.8	29.6±4.1	<0.0001
Child characteristics				
Age, y	12.2±0.2	12.2±0.3	12.2±0.2	0.99
Gender, % female	41%	39%	44%	0.77
Tanner stage				0.50
1	9%	12%	7%	
2	38%	47%	27%	
3	31%	18%	47%	
4	16%	18%	13%	
5	6%	6%	7%	
BMI, kg/m^2	20.0±5.4	17.9±2.5	22.3±6.8	0.02
BMI z-score, SD	0.20±1.19	-0.26±1.07	0.71±1.14	0.02
IL-6, pg/mL	0.49±0.32	0.46±0.34	0.52±0.29	0.61
TNF-α, pg/mL	2.05±0.66	1.96±0.50	2.15±0.82	0.43
Adiponectin, ng/mL	28.7±11.5	31.8±9.8	25.2±12.6	0.09
hs-CRP, mg/L detectable (≥0.16)	47%	17%	81%	0.0002

Mean ± SD and *t* test *P* value for continuous variables, such as weight, height, body mass index (BMI), BMI z-score, age, maternal BMI; ANCOVA mean ±SD and *P* value for inflammatory markers, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and the cytokine, adiponectin. Row % and Chi-square *P* value for categorical variables, such as gender, Tanner stages, and high sensitivity C-reactive protein (hs-CRP). Low risk and high risk groups were defined based on the mothers' pre-pregnancy BMI.

Table 2. Multivariable ANCOVA and logistic regression models with adjustment for confounders (BMI z-score, Tanner stage, and gender)

Variables	Low risk mean (SE)	High risk mean (SE)	Mean difference/odds ratio (95% CI)	P value
IL-6 (pg/mL)	0.46 (0.08)	0.53 (0.09)	0.08 (-0.20, 0.35)*	0.56
TNF-α (pg/mL)	1.94 (0.18)	2.25 (0.21)	0.31 (-0.29, 0.91)*	0.29
Adiponectin (ng/mL)	30.35 (2.74)	29.58 (3.13)	-0.78 (-9.82, 8.27)*	0.86
hs-CRP (mg/L, detectable *vs.* undetectable)	NA	NA	16.23 (2.14, 123.12)†	0.007

*: *P* value and mean difference (95% CI) from ANCOVA are presented (reference group: low risk); †: *P* value and odds ratio (95% CI) from logistic regression are presented (reference group: low risk). Low risk and high risk groups were defined based on the mothers' pre-pregnancy BMI. IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; hs-CRP: high sensitivity C-reactive protein; NA: not applicable.

Discussion

The present finding, revealing higher levels of hs-CRP in 12-year-old children exposed (HR) compared to not exposed (LR) to maternal obesity during pregnancy, is in agreement with two other studies examining the relationship of parental obesity to inflammatory markers in the offspring. In a study by Labayen, parental BMI was shown to be associated with various cardiovascular risk factors, including CRP, in the offspring, although this was not evident after controlling for their fatness.[1] Also, in non-obese adult offspring from the Framingham Heart Study, those with two obese parents (versus none or one parent) at any age had higher levels of CRP.[6] Together with the observations from the present study, these two reports support the hypothesis that parental obesity may increase susceptibility to an inflammatory state in the offspring that can be detected before onset of obesity, suggesting that inflammation may be a precursor to the development of obesity rather than just a consequence of it.

Whereas these two reports studied the effects of parental obesity on the offspring's CRP levels, the present study focused attention specifically on maternal obesity during pregnancy. It has been demonstrated that obesity during pregnancy, by itself or in conjunction with its co-morbidities such as gestational diabetes,[2] hypercholesterolemia[14] and inflammation[15,16] has great effect on the fetus and the future health of the offspring.[17] Providing further support for this, our present observation suggests that hs-CRP may be an early sign of the risk for excess weight gain in children exposed to obesity during pregnancy.

Since this study is a cross-sectional design with a small sample size, our results are primarily hypothesis-generating, requiring the proposed concepts to be tested using larger longitudinal studies. With the small sample size, the Tanner stages were divided into two groups, as opposed to controlling for each Tanner stage, which may have hidden a possible impact of puberty on our findings. Having only one ethnic group (Caucasians)

limits the generalizability of the study. Despite these limitations, the present study has important strengths. While most studies examine already obese children, this cohort enabled us to compare two groups, a majority of which were not yet obese, that differed as a function of exposure to maternal obesity during pregnancy. Also, none of the subjects reported any other possible causes of inflammation that could have affected these results.

In conclusion, this study showed that detectable hs-CRP levels were significantly more frequent in children who were exposed to maternal obesity during pregnancy. Whereas it is known that CRP is associated with the future development of cardiovascular disease,[5] this inflammatory marker may itself contribute to future weight gain, as suggested by previous adult studies,[18-20] rather than just be a consequence of it. Therefore, identifying risk factors for elevated CRP, such as maternal obesity during pregnancy or parental obesity,[1,6] may be helpful in developing methods for preventing future onset of obesity and its complications.

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