The association between TNF-α and insulin resistance in euglycemic women

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Abstract

Chronic low levels of inflammation have links to obesity, diabetes and insulin resistance. We sought to assess the relationship between cytokine tumor necrosis factor (TNF-α) and insulin resistance in a healthy, euglycemic population.

This is a prospective study of 574 non-diabetic mother and infant pairs. Maternal body mass index (BMI), TNF-α, glucose and insulin were measured in early pregnancy and at 28 weeks. Insulin resistance was calculated by HOMA index. At delivery birthweight was recorded and cord blood analysed for fetal C-peptide and TNF-α.

In a multivariate model, maternal TNF-α in early pregnancy was predicted by maternal insulin resistance at the same time-point (β = 0.54, p < 0.01), and maternal TNF-α at 28 weeks was predicted by maternal insulin resistance in early pregnancy (β = 0.24, p < 0.01) and at 28 weeks (β = 0.39, p < 0.01).

These results, in a large cohort of healthy, non-diabetic women have shown that insulin resistance, even at levels below those diagnostic of gestational diabetes, is associated with maternal and fetal inflammatory response. These findings have important implications for defining the pathways of fetal programming of later metabolic syndrome and childhood obesity.

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1. Introduction

Maternal weight exerts a significant influence on obstetric outcome [1,2]. In particular, an increased maternal body mass index (BMI) confers an elevated risk of delivering a heavier infant [3]. In the long term, infants that are at the highest end of the distribution for weight or body mass index (BMI) are more likely to be obese in childhood, adolescence, and early adulthood than other infants [4], and are at risk of cardiovascular and metabolic complications later in life [5,6]. The pathogenesis underlying the fetal programming of later metabolic health and obesity is complex and multifactorial [7]. Adipose tissue produces a number of inflammatory factors which regulate fat deposition and appetite [8]. In non-pregnant populations, evidence is accumulating for a role for these pro and anti-inflammatory mediators in the pathogenesis of obesity related consequences, and, in particular, the development of insulin resistance and overt diabetes mellitus [9,10]. During pregnancy, it has been shown that levels of inflammatory markers are higher in both women with a prior history of gestational diabetes, and in those who subsequently develop gestational diabetes, independent of maternal BMI [11,12]. Specifically, insulin resistance during late gestation has been shown to be significantly correlated with changes in circulating cytokine tumor necrosis factor (TNF-α), irrespective of fat mass [13]. More recently, an analysis of a cohort of women enrolled the large multi-centre HAPO trial [14], a trial designed to clarify the risk of adverse outcome associated with degrees of glucose intolerance during pregnancy, suggested that levels of certain inflammatory mediators were associated with maternal glucose in non-diabetic pregnancies [15]. These findings would suggest that fetal programming of later metabolic dysfunction and insulin resistance, and any potential role of inflammatory mediators, is likely to represent a continuous relationship not solely confined to diabetic pregnancies. If this is the case, then a far greater proportion of pregnancies are in fact, at risk, and any interventions to improve maternal metabolism in pregnancy should be applied to the general, rather than to a specific cohort of the pregnant population.

We recently performed a randomized control trial of low glycemic index diet in pregnancy to prevent the recurrence of macrosomia, the ROLO study [16]. As per the trial protocol, all women recruited were healthy and non-diabetic, with no previous history of gestational diabetes [17]. As well as assessments of maternal and fetal size and adiposity, women recruited to the ROLO study
had longitudinal analysis of insulin resistance in pregnancy with assessments of maternal insulin resistance in early pregnancy and again at 28 weeks, and the assessment of fetal insulin resistance with C-peptide in cord blood. We now report a secondary analysis of this study, in which we sought to clarify the relationship between cytokine tumor necrosis factor (TNF-α) and insulin resistance in a healthy, euglycemic population.

2. Materials and methods

2.1. Study sample

This is a prospective study of 574 mother and infant pairs recruited as part of the ROLO study, with institutional ethical approval and written informed maternal consent. The ROLO study was from January 2007 to January 2011, and the final women delivered in August 2011. During the study period, 909 women who met inclusion criteria were contacted by telephone and informed of the study. Of these, 851 agreed to meet with a researcher. A further 51 women met exclusion criteria or miscarried before randomisation, and 800 were recruited and randomised [16]. There were 20 women lost to follow up. As per the trial protocol, women were excluded if they had any underlying medical conditions, if they were less than 18 years of age, if they had previous gestational or pre-existing Type 1 or Type 2 diabetes or if they were unable to give full informed consent [17]. Patients were recruited at first antenatal consultation at 13.8 ± 2.4 weeks. For the purposes of this analysis, only women with TNF-α and insulin resistance data were included; those who developed gestational diabetes were excluded.

2.2. Assessments

At the initial visit and at 28 weeks all women had measurement of weight, height, and upper arm circumference; fasting serum glucose, fasting insulin and TNF-α concentrations were measured. Based on the BMI at the early pregnancy consultation women were categorised as either underweight (BMI < 18.5 kg/m²), normal weight (BMI 18.5–24.9 kg/m²), overweight (BMI 25.0–29.9 kg/m²) or obese (BMI ≥ 30.0 kg/m²) as per the Institute of Medicine (IOM) guidelines [18].

At delivery, infant birth weight, infant length and head circumference were recorded and a cord blood sample for fetal TNF-α and C-peptide concentrations taken.

2.3. Fetal ultrasound

Fetal biometry was assessed ultrasonographically at 34 weeks’ gestation using a Voluson 730 Expert (GE Medical Systems, Germany). Biparietal diameter, head circumference, abdominal circumference, femur length and anterior abdominal wall width (AAW), a marker of fetal adiposity were recorded. Fetal AAW was measured at the traditional abdominal circumference view, 2–3 cm lateral to the cord insertion, and included fetal skin and subcutaneous tissue [19,20]. Three measurements were obtained and the mean recorded.

2.4. Laboratory methods

Multianalyte profiling was performed on the Luminex Magpix system (Luminex Corporation, Austin, USA.). Plasma concentrations of TNF-α, insulin, and C-peptide were determined by the Human Endocrine Panel. Maternal insulin resistance was calculated using the HOMA index [21]: HOMA score = (fasting insulin μU/mL × fasting glucose mmol/l)/22.5. Fetal insulin resistance was assessed with cord blood C-peptide estimation.

2.5. Statistical analysis

Data were assessed for normality using Shapiro Wilk and P-P plot. Non-parametric data were log transformed prior to regression analysis and normal distribution confirmed with histograms. Positive and negative correlations were assessed using Pearson's correlation coefficient for normally distributed data and Spearman's rho for non-parametric data. Multiple linear regression analysis was used to produce a multivariate model with co-efficients adjusted for the following outcome measures; maternal BMI, fetal adiposity (AAW), estimated fetal weight at 34 weeks (EFW), birthweight, early pregnancy insulin resistance (HOMA), HOMA at 28 weeks gestation, and cord blood C-peptide. Results are expressed as beta-coefficients and corresponding p-values. Statistical significance was set at p < 0.05. Statistical analysis was performed using SPSS Windows version 18.0 (SPSS, Chicago, IL).

3. Results

The baseline maternal characteristics are contained in Table 1. The mean BMI at recruitment was 26.7 ± 4.9 kg/m². The mean fasting blood glucose was 4.4 ± 0.03 mmol/l. The low glycaemic index diet had no effect on maternal or fetal TNF-α (5.6 ± 3.8 vs. 5.7 ± 4.3 pg/ml in early pregnancy, 5.8 ± 4.3 vs. 5.8 ± 3.5 pg/ml at 28 weeks, and 6.1 ± 5.9 vs. 5.5 ± 5.1 pg/ml in cord blood, p > 0.05 for all)

The median TNF-α in early pregnancy was 4.79 pg/ml (interquartile range 3.05–7.55 pg/ml), at 28 weeks it was 5.17 pg/ml (interquartile range 3.18–7.85 pg/ml) ad in cord blood the median TNF-α was 5.23 pg/ml (interquartile range 0.81–8.8 pg/ml). The median HOMA index in early pregnancy was 2.14 (interquartile range 1.1–3.5), which rose to 2.82 at 28 weeks (interquartile range 1.6–4.6).

No relationship was noted between maternal TNF-α in early pregnancy and either maternal weight, BMI or arm circumference. Similarly, no significant relationship existed between maternal or fetal TNF-α and fetal biometry or neonatal biometry, including estimated fetal weight and fetal anterior abdominal wall width, as measured at 34 weeks gestation.

A significant relationship was identified between maternal TNF-α in early pregnancy and at 28 weeks gestation, and maternal insulin resistance, a relationship that was independent of the effect of maternal BMI. When the cohort was subdivided according to early pregnancy BMI as either normal weight (BMI 18.5–24.9 kg/m²), overweight (BMI 25.0–29.9 kg/m²) or obese (BMI ≥ 30.0 kg/m²) as measured at 28 weeks gestation.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline maternal characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at delivery (years)</td>
<td>31.92 ± 4.2</td>
</tr>
<tr>
<td>Early pregnancy BMI (kg/m²)</td>
<td>26.78 ± 4.9</td>
</tr>
<tr>
<td>Early pregnancy maternal arm circumference (cm)</td>
<td>29.51 ± 3.495</td>
</tr>
<tr>
<td>Early pregnancy fasting glucose (mmol/l)</td>
<td>4.45 ± 0.37</td>
</tr>
<tr>
<td>Early pregnancy HOMA index</td>
<td>2.14 ± 1.1–3.5</td>
</tr>
<tr>
<td>Early pregnancy TNF-α (pg/ml)</td>
<td>4.79 ± 3.05–7.55</td>
</tr>
</tbody>
</table>

Baseline maternal characteristics at recruitment. Results are expressed as mean and SD (standard deviation) for parametric and median and interquartile range for non-parametric data. Early pregnancy is gestation at first antenatal consultation, 13.8 ± 2.4 weeks. BMI is maternal body mass index. HOMA = (fasting insulin μU/mL × fasting glucose mmol/l)/22.5.
Relationship between maternal, fetal and infant size, insulin resistance and TNF-α according to the Institute of Medicine guidelines [18], a significant correlation was noted between maternal insulin resistance and maternal TNF-α existed in each weight category Table 2.

Multiple linear regression analysis confirmed the relationship between TNF-α and insulin resistance as assessed by the HOMA index. Maternal TNF-α in early pregnancy was predicted by maternal insulin resistance at the same time-point, (β = 0.54, p < 0.01), and maternal TNF-α at 28 weeks was predicted by maternal insulin resistance in early pregnancy (β = 0.24, p < 0.01) and at 28 weeks (β = 0.39, p < 0.01). The multivariate model produced for fetal TNF-α in cord blood failed to reach statistical significance Table 3.

4. Discussion

Our findings have confirmed a significant relationship between cytokine tumor necrosis factor (TNF-α) and insulin resistance in a healthy, euglycemic population. We found that TNF-α increased with advancing gestation, associated with a fall in maternal insulin sensitivity. Maternal TNF-α correlated with maternal insulin resistance both in early pregnancy and at 28 weeks gestation, and fetal TNF-α correlated with fetal C-peptide in cord blood.

Most literature to date investigating the relationship between inflammatory mediators and insulin resistance has focused on obese and/or diabetic pregnancies [9,12]. Importantly, all women recruited to this study were healthy and non-diabetic with a

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### Table 2

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>Early Pregnancy TNF-α</th>
<th>28 Week TNF-α</th>
<th>Cord Blood C-peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal BMI</td>
<td>Correlation coefficient</td>
<td>P-Value</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>18.5–24.9 kg/m²</td>
<td>0.58</td>
<td>0.37</td>
<td>−0.08</td>
</tr>
<tr>
<td>Overweight BMI 25–29.9 kg/m²</td>
<td>0.000</td>
<td>0.000</td>
<td>0.3</td>
</tr>
<tr>
<td>Obese BMI &gt; 30 kg/m²</td>
<td>0.02</td>
<td>0.3</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Early pregnancy is gestation at first antenatal consultation, 13.8 ± 2.4 weeks.

BMI is maternal body mass index. BMI classified as normal, overweight or obese according to the IOM guidelines (Ref).

HOMA = (fasting insulin μU/mL · fasting glucose mmol/l)/22.5.

Fetal TNF-α is TNF-α concentration in cord blood.

A p-value of < 0.05 was considered statistically significant.

### Table 3

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Beta-coefficient</th>
<th>P-Value</th>
<th>Beta-coefficient</th>
<th>P-Value</th>
<th>Beta-coefficient</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early pregnancy HOMA index</td>
<td>0.543</td>
<td>0.06</td>
<td>0.243</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 week HOMA index</td>
<td>0.392</td>
<td>0.06</td>
<td>2.075</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cord blood C-peptide</td>
<td>0.02</td>
<td>0.13</td>
<td>0.97</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal BMI</td>
<td>0.02</td>
<td>0.12</td>
<td>0.02</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAW at 34 weeks</td>
<td>0.03</td>
<td>0.12</td>
<td>0.02</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAW at 34 weeks</td>
<td>0.07</td>
<td>0.6</td>
<td>0.07</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Multivariate model with co-efficients adjusted for the following outcome measures: maternal BMI (body mass index), fetal anterior abdominal wall width (AAW), estimated fetal weight at 34 weeks (EFW), birthweight, early pregnancy insulin resistance (HOMA), HOMA at 28 weeks gestation, and cord blood C-peptide. Results are expressed as beta-coefficients and corresponding p-values. Statistical significance was set at p < 0.05.
distribution that included normal, overweight and obese subjects. We identified a clear relationship between TNF-α and insulin resistance in this non-diabetic cohort, and confirmed that this relationship existed for each BMI category. These findings would suggest that the relationship between insulin resistance and the up regulation of inflammatory pathways is a continuous relationship, with women at the highest end of the spectrum of weight distribution at highest risk of metabolic and inflammatory dysregulation. Interestingly, we found that maternal insulin resistance in early pregnancy was in fact a more significant predictor of TNF-α during pregnancy than maternal BMI. This would suggest that aberrations in maternal inflammatory response in pregnancy may be a more sensitive predictive of later metabolic dysfunction in pregnancy, and may explain why some women of normal weight develop pregnancy complications, such as gestational diabetes, more commonly attributed to overweight and obese women.

Changes in carbohydrate and lipid metabolism occur during pregnancy to ensure a continuous supply of nutrients to the fetus. These changes include alterations in insulin sensitivity and secretion. These metabolic changes are progressive and may be accentuated in women who develop gestational diabetes mellitus [22]. As such, these physiological changes in pregnancy may unmask an inherent predisposition to insulin resistance. Indeed, women who develop gestational diabetes have a significant risk of developing Type 2 diabetes mellitus in later life [23]. Our findings would suggest that the fall in insulin sensitivity in pregnancy is associated with a concurrent rise in inflammatory response, which may explain the association between gestational diabetes and later metabolic syndrome and cardiovascular disease.

Despite the association between TNF-α and insulin resistance, we did not identify a relationship with fetal or neonatal size, nor did we identify a relationship between maternal or fetal TNF-α and fetal adiposity. These findings are similar to a previous smaller study, which examined the correlation between maternal inflammatory markers and feto-maternal adiposity and did not identify an association between TNF-α and fetal adiposity [24]. Fetal growth and birthweight are subject to a variety of genetic and environmental influences. Further longitudinal studies of fetal, neonatal and childhood growth are needed to elucidate this further.

Our study does have some limitations worthy of consideration. Our study cohort were selected to investigate the potential for a low glycemic index diet in pregnancy to reduce macrosomia, and as per that protocol were all secundigravid, having previously given birth to an infant weighing greater than 4 kg. They may not, therefore, be a representative sample of all pregnant women. Also, there is significant heterogeneity between countries in rates of maternal obesity in pregnancy; we can only draw conclusions with this specific Irish cohort. There was however, a representative mix of women from all BMI categories, and the strict criteria of the randomised control trial allowed for exclusion of diabetes, and for uniform and standardised measurements of maternal and fetal size.

Our findings have implications for clinical practice. There is an established association between obesity, chronic low levels of inflammation and later metabolic and vascular dysfunction. Our findings would suggest that in pregnancy at least, the relationship between insulin resistance and inflammation is a continuous association evident in normal weight as well as overweight and obese women. Pregnancy may be considered as a screening test for later adult health, as the physiological changes designed to enhance fetal growth unmask a predisposition to later insulin resistance and inflammatory dysregulation. This may have important metabolic and cardiovascular implications not only for the mother, but also for the fetus. Future studies should now be directed toward the interrogation of the relationship between inflammatory pathways, insulin resistance and later cardiovascular risk, and, importantly, to assess if any pregnancy interventions, such as lifestyle and exercise, may ameliorate this risk.

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References


