

**Inflammation and endothelial activation is evident at birth in offspring of mothers with type 1 diabetes**

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Running Title: Inflammation in Offspring of Type 1 DM Mothers

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**Abstract**

Objective: Offspring of mothers with diabetes are at risk of obesity and glucose intolerance in later life. In adults markers of subclinical inflammation (CRP, IL-6) and endothelial activation (ICAM-1) are associated with obesity and with higher risk for incident type 2 diabetes. We examined whether these biomarkers were elevated at birth in offspring of mothers with type 1 diabetes (OT1DM).

Research Methods: Umbilical cord plasma CRP, IL-6 and ICAM-1 measured in 139 OT1DM and 48 control offspring, with analysis relative to fetal lipids and hormonal axes.

Results: OT1DM had higher CRP (OT1DM 0.17 [0.13-0.22]mg/l; control 0.14 [0.12-0.17]mg/l;  $P<0.001$ : median [interquartile range]) and ICAM-1 (OT1DM 180 [151-202]ng/ml; control 166 [145-187]ng/ml,  $P=0.047$ ). IL-6 was not different after necessary adjustment for mode of delivery. Birthweight was unrelated to inflammatory indices, however, leptin was correlated with CRP (controls  $r=0.33$ ,  $p=0.02$ ; OT1DM  $r=0.41$ ,  $p<0.001$ ) and with IL-6 ( $r=0.23$ ,  $p<0.01$ ) and ICAM-1 ( $r=0.29$ ,  $p<0.001$ ) in OT1DM. In OT1DM CRP correlated with maternal glycaemic control (HbA1c at 35-40 weeks;  $r=0.28$ ,  $P=0.01$ ). In multivariate analysis leptin was a determinant of CRP ( $p<0.001$ ), ICAM-1 ( $p=0.003$ ) and IL-6 ( $p=0.02$ ) in OT1DM. Inflammatory measures demonstrated positive relationships with triglycerides in OT1DM (CRP, IL-6 and ICAM-1  $P<0.05$ ) and controls (ICAM-1  $P=0.001$ ).

Conclusions: Inflammatory markers are increased in OT1DM and are related to measures of fetal adiposity- particularly leptin- and to maternal glycaemia. Sub-clinical inflammation is a novel component of the diabetic intra-uterine environment and should be considered as a potential etiological mechanism for *in-utero* programming of disease.

## **Introduction**

Maternal diabetes is associated with adverse consequences to mother and baby. Rates of macrosomia and fetal adiposity are higher resulting in substantive increases in intrapartum complications, independent of their increased background risk of perinatal morbidity and mortality. In the longer term offspring have increased risk of obesity and type 2 diabetes (1; 2) in high risk populations – reflecting potential *in-utero* programming of disease. In adults, inflammatory markers, in particular C-reactive protein (CRP), intracellular adhesion molecule-1 (ICAM-1) and interleukin-6 (IL-6), are associated with adiposity, altered glucose tolerance and prospectively with later risk of development of type 2 diabetes and vascular disease (3-8). In animal models intra-uterine exposure to cytokines, including IL-6, have been associated with increases in fat mass and insulin resistance in later life (9). Changes in inflammatory markers have not been extensively studied in offspring of type 1 pregnancies at birth, however, gestational diabetes has been associated with an alteration in the placental transcriptome with a dominance of genes regulating inflammatory responses and endothelial function (10). This suggests the hypotheses that short term complications and more speculatively the longer term metabolic sequelae of maternal diabetes for offspring of type 1 diabetes mothers (OT1DM) may be partially attributable to the intra-uterine environment.

The objective of the present study was to evaluate whether baseline plasma levels of the inflammatory markers; CRP, ICAM-1 and IL-6, were increased in OT1DM at birth and whether they were related to birth weight, measures of fetal adiposity, fetal insulinaemia and maternal metabolic control. Finally we have also investigated the relationship of inflammatory measures to fetal lipids.

## **Research Design and Methods**

### **Recruitment and collections of cord bloods:**

Recruitment, which began in January 1999 and ended in May 2001, took place in eight hospital-based antenatal centers in Scotland. A total of 250 women with type 1 diabetes consented to participate in the study (a 94% participation rate of those enrolled in and planning to deliver in the centers), and cord blood samples were obtained from 200 (80%). No differences in gestation at delivery, maternal age at delivery, years of diabetes, fetal sex or maternal HbA1c (where available) were found between those with and without cord samples. Because sample hemolysis results in increased degradation of insulin and pilot studies indicated significant (>10%) degradation of insulin in which samples were either not collected from cord for more than 20 min or there was delay between sample collection and freezing for more than 60 min, samples not fulfilling these criteria were excluded from the main data analysis. Thus, the 200 samples were further restricted to those in whom 1) there was no evidence of hemolysis of cord blood (17 excluded); 2) cord blood had been collected within 20 min [12 exclusions:

(median interquartile range) collection time for remaining samples, 2 min (1-7)]; 3) cord blood had been centrifuged and plasma frozen within 60 min [17 exclusions: time from collection to freezing for remaining samples, 17 min (11-26)]; 4) antenatal glucocorticoids had not been administered in the 24 h before birth (15 excluded); and finally 4) children delivered before 33 weeks gestation (five excluded).

A convenience sample of control mothers, who had no history of obstetric or metabolic disease, and in which routine screening for gestational diabetes (using national guidelines:

<http://www.sign.ac.uk/guidelines/fulltext/55/section8.html>) was negative were recruited from routine obstetric follow-up clinics after the 34th week of pregnancy in the same centers. Of the 145 women who gave initial consent, cord samples were attempted in 75 and obtained in 70. Forty-eight collections met the above restriction criteria, and samples were available for fetal lipids in all 48.

Data on clinical outcome including caesarean section, intercurrent medical conditions, and hypertensive conditions of pregnancy were obtained by case note review. Gestational ages were calculated from estimated dates of delivery from chart review. This date was derived from dates of last menstrual period (LMP), where available, or by ultrasound if there was either conflict with dates as assessed by LMP (>6 d) or LMP was unavailable.

Weight was measured at birth and, for offspring born between 33 and 42 weeks of gestation, further expressed as an SD score, as previously described (11). Skinfold thickness at subscapular and triceps was measured using Holtain calipers by pediatricians at each site using a centrally agreed protocol, available in writing at the time of measurement. Skinfolds were not measured in all subjects. However, there were no significant differences in baseline demographic or biochemical measures between those with and without skinfold measurements in either control subjects or OT1DM (data not shown). All mothers gave informed consent and the local ethical committees approved the protocols.

**Cord blood assays:** Plasma insulin, 32-33 split proinsulin, proinsulin, leptin, IGF-1, adiponectin, plasma total cholesterol, triglyceride, non-esterified fatty acid, VLDL-C, LDL-C and HDL-C were assayed as previously described (12-15). All lipid assays were carried out at the Biochemistry Department of Glasgow Royal Infirmary, which is a Centers for Disease Control and Prevention (Atlanta) reference laboratory and accredited by Clinical Pathology Accreditation U.K. Maternal HbA1c was measured centrally by one laboratory. Plasma IL-6 and ICAM-1 were measured in plasma stored at -80°C using high-sensitivity commercial ELISA kits (R&D Systems). CRP was measured using a high-sensitivity, 2-site enzyme-linked immunoassay (16).

### **Statistical Analysis**

Data were analysed using standard software (Minitab 14, Pennsylvania, USA and Stata 7, Texas, USA). In several

cases (insulin, leptin, triglyceride, VLDL, NEFA, TC:HDL-C ratio, CRP, ICAM-1 and IL-6) measures were not normally distributed and unadjusted values are presented as median (interquartile range) and for normally distributed variables mean  $\pm$  standard deviation. Variables were logarithmically transformed to obtain normal distributions. Inter-group differences were assessed by unpaired *t*-test, analysis of variance or, where further predictor variables were included, by general linear models. Spearman correlation coefficients are reported. Pearson partial correlation co-efficients on log transformed data allowed adjustment of analytes for gestational age. Stepwise logistic regression was performed using an alpha of  $p \leq 0.15$  for adding or removing predictors from the model. Quartiles for CRP, ICAM-1 and leptin were derived for controls and OT1DM separately. Assessment of direction across quartiles was performed by Chi squared test for trend. Statistical significance was determined at  $p < 0.05$ .

## Results

### Fetal Hormonal, Inflammatory and Lipid Analytes in OT1DM vs Controls

The maternal and fetal characteristics for this cohort have previously been described and are included in Table 1. Maternal type 1 diabetes was associated with marked increases in absolute values of cord insulin (12) and leptin (13) with reductions in adiponectin (14) HDL-C and NEFA (Table 1). All these differences remained significant after adjustment for sex and mode of delivery.

In absolute terms CRP and ICAM-1 were *higher* and IL-6 was *lower* in OT1DM (Table 1). IL-6 concentrations were related to mode of delivery (Table 2); being lower in babies delivered by elective caesarean section, in both OT1DM ( $p < 0.001$ ) and controls ( $p = 0.028$ ). After adjustment for mode of delivery there was no difference in IL-6 between OT1DM and controls ( $p = 0.24$ ), because more OT1DM were delivered by elective caesarean section. CRP was related to mode of delivery in controls being significantly higher in the 6 babies delivered by emergency caesarean section ( $p = 0.027$ ; Table 2), but no difference dependent on mode of delivery was observed in OT1DM. CRP remained higher in OT1DM compared to controls even after adjustment for mode of delivery ( $p = 0.018$ ). ICAM-1 was not affected by mode of delivery in either group, with overall significantly higher levels in OT1DM ( $p = 0.047$ ). Fetal sex, gestational age and birth weight were not significant determinants of IL-6, CRP or ICAM-1 in controls or OT1DM (in a model also including mode of delivery).

### Fetal Inflammatory Markers, Relationship with Fetal Adiposity and leptin

Initial analysis of the inter-relation of each of the inflammatory markers demonstrated that IL-6 and ICAM-1 expression were positively correlated (controls  $r = 0.47$ ,  $p = 0.002$ ; OT1DM  $r = 0.2$ ,  $p = 0.02$ ), and CRP was correlated to IL-6 only in cases (control  $r = 0.10$ ,  $p = 0.48$ ; OT1DM  $r = 0.22$ ,  $p = 0.01$ ) and ICAM-1 also only in cases (control

$r = 0.04$ ,  $p = 0.80$ ; OT1DM  $r = 0.37$ ,  $p < 0.001$ ). Given the higher levels of CRP and ICAM-1 in OT1DM we further explored the relation of these inflammatory markers to fetal anthropometry and cord hormonal profiles, in particular to measures of fetal adiposity, maternal glycaemia and fetal insulinaemia. Inflammation was correlated with various measures of fetal fat mass in controls and OT1DM (Table 3), but most consistently with leptin which was associated with CRP ( $r = 0.41$ ,  $p < 0.0001$ ), ICAM-1 ( $r = 0.29$ ,  $p < 0.001$ ) and IL-6 ( $r = 0.23$ ,  $p = 0.007$ ) in OT1DM (Table 3 and Figure 1). In addition to these relationships with fetal fat mass, CRP correlated maternal HbA1c at 35-40 weeks in OT1DM ( $r = 0.28$ ,  $p = 0.01$ ). Despite this association CRP did not correlate with insulin in controls or OT1DM (Table 3). Indeed insulin demonstrated a negative relationship with IL-6 ( $r = -0.32$ ,  $p = 0.01$ ) and ICAM-1 ( $r = -0.32$ ,  $p = 0.01$ ) in controls. With respect to other hormonal measures, adiponectin was negatively associated with ICAM-1 in controls ( $r = -0.31$ ,  $p < 0.01$ ), but unrelated to any of the fetal inflammatory indices in OT1DM. IGF-1 showed generally negative correlations with the inflammatory measures however these relationships were significant only for the association of IGF-1 and IL-6 in controls ( $r = -0.35$ ,  $p < 0.001$ ), and with CRP ( $r = -0.23$ ,  $p < 0.001$ ) in OT1DM. Analysis of the correlations presented in Table 3 after correction of analytes for gestational age produced similar results (data not shown).

To identify the principal associates of inflammation, analysis of the effect of insulin, leptin, adiponectin, IGF-1, mode of delivery and sex on cord inflammatory markers was performed using a stepwise regression model (Table 4). Most notably, leptin was a positively associated with CRP and ICAM-1 and IL-6, in OT1DM. Relationships of cord leptin to CRP and IL-6 were of marginal significance in controls (Table 4) but there was a significant association of leptin and CRP in controls in similar models with exclusion of babies delivered by emergency section (contribution to variance 11.8%,  $P = 0.03$ ). Insulin showed a negative relationship with IL-6 and ICAM-1 in controls but was not related to any of the inflammatory markers in OT1DM. Finally there was a generally negative relationship of IGF-1 to the markers in OT1DM with the largest effect (contribution to variance 12.8%,  $P < 0.0001$ ) for CRP in OT1DM. Inclusion of maternal HbA1c at 35-40 gestation as a determinant in these models attenuated the associations of leptin with CRP, IL-6 and ICAM-1 in OT1DM (data not shown).

### Relationship of inflammatory measures to fetal lipids

An association between inflammation and lipid metabolism was evident in cord blood. Triglyceride was most consistently associated with the inflammatory measures and particularly with ICAM-1 (controls  $r = 0.42$ ,  $p < 0.001$ ; OT1DM  $r = 0.35$ ,  $p = 0.001$ ; Table 3 and Figure 1). NEFA was also generally positively correlated with the inflammatory measures but significantly so only in the case of IL-6 (controls  $r = 0.39$ ,  $p = 0.009$ ; OT1DM  $r = 0.43$ ,  $p < 0.001$ ). Finally relationships of the inflammatory measures with HDL-C were generally, but inconsistently, negative, with

significant correlations between CRP and HDL-C ( $r=-0.23$ ,  $p=0.02$ ) in OT1DM and IL-6 and HDL-C in control ( $r=-0.33$ ,  $P=0.026$ , Table 3).

In a multivariate stepwise regression model incorporating log IL-6, log CRP, log ICAM-1, log insulin, log leptin, adiponectin, IGF-1, mode of delivery and sex, all of the inflammatory markers were independent predictors of triglyceride in OT1DM (Table 5). Although there was a small contribution from IL-6 in prediction of NEFA in OT1DM, mode of delivery was the principal predictor of NEFA (Table 5). IL-6 was also associated with HDL-C in OT1DM, however, IGF-1 was the dominant predictor of HDL-C in controls and OT1DM (Table 5).

## Discussion

Maternal type 1 diabetes is associated with significant alteration in cord inflammatory markers *in-utero*. Differences in metabolic, cardiovascular and inflammatory variables between OT1DM and controls have previously been observed in a single study in children as young as 5 to 11 years old (17). In the current study we establish that an inflammatory phenotype is present at birth, as CRP and ICAM-1, accepted markers of systemic inflammation and endothelial dysfunction with previous known associations to obesity, insulin resistance, and type 2 diabetes in children (18-22) and adults (3-7) are elevated in cord blood. Furthermore we demonstrate that the known positive inter-relationships between the plasma biomarkers CRP, IL-6 and ICAM-1 levels are established at birth.

IL-6 is one of the most studied cytokines and is considered for the most part to exhibit proinflammatory and proatherogenic activity. It is the main stimulant for hepatic production of CRP and other reactant proteins, but also has other important roles leading to increased endothelial cell adhesiveness by up-regulating E-selectin, ICAM-1, and VCAM-1 and releasing inflammatory mediators, including IL-6 itself (23). IL-6 expression correlates with plasma ICAM-1 expression in controls and OT1DM at birth, consistent with *in-vitro* IL-6 up-regulation of ICAM-1 expression (24) and the relationships observed in studies in children (25) and adults (26). The positive relationship that we observe between ICAM-1 and CRP may reflect the direct abilities of CRP to induce release of inflammatory cytokines, increase endothelial cell adhesion molecule expression facilitating endothelial monocyte binding (27; 28). The direct effects of CRP on endothelial function have recently been questioned (29; 30) and it remains highly likely that elevations in CRP and ICAM-1 simply reflect a more general response to an abnormal environment (31). Although all these proteins form part of the acute phase response, IL-6 is the most readily inducible, as reflected by the 300% increase in response to vaginal delivery in controls and OT1DM. In contrast ICAM-1 and CRP are much slower to rise, with the IL-6 induction of CRP production by liver cells taking at least 24 hours (32) and CRP and ICAM-1 levels peaking four days after an acute cardiac event (33). Therefore although IL-6 may be responsible for important mechanistic links with respect to inflammation and endothelial dysfunction,

cord blood levels may be reflecting short term changes - particularly relating to mode of delivery - in line with our findings. In contrast cord CRP and ICAM-1 are likely to reflect the longer term changes in the intrauterine environment, and thus their levels are likely to be more informative.

The association of low grade inflammation with both obesity and type 2 diabetes is well described. CRP and ICAM-1 are increased in adults with obesity (34-38) and type 2 diabetes (39; 40) and similar relationships also appear to be present in childhood (19). At birth OT1DM have increased fat mass and an associated increased circulating leptin concentration (41; 42). It is of considerable interest then that CRP and ICAM-1 are not only increased in OT1DM but are associated with cord leptin and skin fold thickness. Furthermore leptin is also associated with IL-6 at birth in OT1DM, raising the possibility that fetal adipose tissue is not only responsible for endothelial activation but induction of a pro-inflammatory phenotype which is already evident by the time of birth. In general relationships of leptin with the cord measures of subclinical inflammation were more consistent than relationships of skinfolds to the inflammatory measures. This may simply reflect that skinfolds were available for a smaller number of the cohort and is a less precise measurement than cord leptin, nevertheless we cannot exclude a particular role for leptin. Maternal HbA1c was also associated with CRP supporting a role of maternal glycaemic control in the inflammatory phenotype at birth.

The mechanistic link between adiposity, endothelial activation and CRP secretion *in-utero* may be cytokine mediated, including IL-6 (43), as leptin was associated with IL-6 in OT1DM. Although IL-6 levels were not increased in OT1DM, this may be as a consequence of the dynamic nature of IL-6 at the time of birth, potentially explaining why we and others have not shown a relationship between cord levels of IL-6 and leptin or anthropometric neonatal measures of adiposity in controls (42), despite a relationship being observed in adults (44).

We were also interested in the direct effects of insulin on inflammatory measures at birth. Insulin is capable of stimulating adipocyte IL-6 production *in-vitro* (45), however, there were no significant relationships of insulin to CRP, IL-6 and ICAM-1 in OT1DM. We are well placed to examine the effects of often very high cord insulin concentrations ( $>300\text{pmol/l}$  in 14% of our cohort) and our data would not support a substantial stimulatory effect of fetal insulin on IL-6 *in-utero*. Indeed given the negative relationship of insulin and IL-6 and ICAM-1 in the controls one might argue the opposite case- that low grade inflammation might suppress insulin secretion as suggested by recent investigation in adults. (46) or conversely that insulin is in part anti-inflammatory (47).

Lipid metabolism and inflammation are also linked via hepatic lipid metabolism with elevations of plasma triglycerides occurring during acute adult inflammatory responses (48), consequently, CRP and triglyceride are positively related in children (35) and adults (5). We

demonstrate that this relationship is present at birth in OT1DM, with markers; ICAM-1, CRP and IL-6 potentially all acting as independent determinants of triglyceride. Underlying this relationship may be lipoprotein lipase (LPL), a pivotal enzyme in lipid metabolism. LPL activity is known to be modulated by various stimuli including inflammation and adiponectin. In adults lower adiponectin and higher CRP are associated with lower lipoprotein lipase activity - in turn associated with the characteristic diabetic dyslipidaemia of low HDL and raised triglycerides (49). One might speculate that our observation of a pro-inflammatory state in OT1DM and association of CRP with HDL-C and triglycerides in cord blood in OT1DM reflect a similar role of lipoprotein lipase. We have previously demonstrated for this cohort a significant reduction in HDL-C and an increase in cholesterol:HDL-C ratio in male offspring, established risk factors for coronary heart disease (15).

Clearly further investigations are needed to examine the longer term implications of these findings. Inflammatory

markers are known to track in childhood (50) and to predict later metabolic (39; 40) and vascular disease (51). A single study has found raised inflammatory markers in offspring of mothers with type 1 diabetes in childhood (17). Our data demonstrates that this inflammatory phenotype is present at birth in OT1DM and that it is particularly related to fetal leptin. Future studies investigating potential long term effects of this change - including potential programming of inflammatory, metabolic and even vascular disease phenotypes in offspring of T1DM - are warranted.

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**Table 1:** Characteristics of mothers with type 1 diabetes and their singleton offspring *vs.* control mothers and children. Values are given as mean  $\pm$  standard deviation or median [interquartile range]. <sup>†</sup> Mode of delivery: vaginal delivery (vaginal), emergency caesarean section (Em LUSCS) and elective caesarean section (El LUSCS). <sup>‡</sup> Birth weights given as unadjusted, P value for difference dependent on maternal diabetes status adjusted for gestational age at delivery. <sup>§</sup> Z weight is standard deviation score compared to standard values for gestational age, gender and maternal parity. A subset of offspring had detailed anthropometry performed. Cord hormonal profiles of singleton offspring of mothers with type 1 diabetes *vs.* control offspring. Unadjusted values are given as mean  $\pm$  standard deviation or median [interquartile range]. \* P= value of significance in unpaired *t*-test,  $\chi^2$  or Mann-Whitney test as appropriate.

	Control Mothers	Mothers type 1 Diabetes	with P*
N	48	139	
Age (years)	28.8 ± 6.0	29.6 ± 5.7	0.36
Duration of diabetes (years)	-	13.2 ± 7.4	-
Parity			
0	20 (42%)	64 (46%)	
1	21 (44%)	59 (42%)	0.85
>1	7 (14%)	17 (12%)	
Children (male/ female)	21 M / 27 F	69 M / 70F	0.44
Gestational age at delivery (weeks)	40.2 ± 1.1	37.8 ± 1.3	<0.001
Mode of Delivery <sup>†</sup>			
Vaginal	33 (69%)	45 (32%)	
EI LUSCS	9 (19%)	48 (35%)	<0.001
Em LUSCS	6 (12%)	46 (33%)	
Birth weight (kg) <sup>‡</sup>			
Males	3.75 ± 0.51	3.84 ± 0.74	0.007 <sup>‡</sup>
females	3.41 ± 0.49	3.76 ± 0.64	<0.001 <sup>‡</sup>
Z weight <sup>§</sup>	0.32 ± 1.1	1.96 ± 1.5	<0.001
N	48	139	
Cord CRP (mg/l)	0.14 [0.12-0.17]	0.17 [0.13-0.22]	0.001
Cord ICAM-1 (ng/ml)	165.8 [144.5-187.0]	180.4 [150.6-201.76]	0.047
Cord IL-6 (pg/ml)	7.38 [3.0-13.1]	4.3 [2.5-9.6]	0.04
Cord Insulin (pmol/l)	22.4 [15.0 - 38]	111 [61 - 218]	<0.001
Leptin (ng/ml)	9.0 [4.3 - 18.0]	32.0 [15.0 - 60.0]	<0.001
IGF-1 (mmol/l)	8.11 ± 0.50	8.12 ± 0.27	0.98
Adiponectin (µg/ml)	22.1 ± 5.3	19.8 ± 6.1	0.03
HDL cholesterol (mmol/l)	0.69 ± 0.18	0.62 ± 0.25	0.05
NEFA (mmol/l)	0.24 [0.18 - 0.33]	0.18 [0.13 - 0.24]	<0.001
Triglyceride (mmol/l)	0.44 [0.36 - 0.57]	0.41 [0.34 - 0.54]	0.23
N	19	56	
Crown-rump length	33.8 ± 2.3	34.9 ± 2.4	0.11
Crown-heel length	50.6 ± 3.0	50.9 ± 2.5	0.60
Triceps skinfold thickness	6.03 ± 2.4	7.98 ± 3.1	0.014
Subscapular skinfold thickness	5.57 ± 2.0	7.43 ± 2.1	0.001

**Table 2:** Influence of maternal diabetes on CRP, ICAM-1 and IL-6 with stratification for mode of delivery. Values are medians [inter-quartile range]. Units for Y axes: CRP (mg/l), ICAM-1 (ng/ml) and IL-6 (pg/ml).

	<b>Offspring of Control Mothers</b>	<b>Offspring of Mothers with Type 1 Diabetes</b>	<b>P</b>
<b>CRP</b>			
Elective LUSCS	0.13 [0.11 - 0.14]	0.18 [0.13 – 0.29]	0.027
Emergency LUSCS	0.18 [0.15 – 0.55]	0.17 [0.14 – 0.21]	0.50
Vaginal delivery	0.13 [0.12 -0.16]	0.16 [0.13 – 0.21]	0.001
<b>ICAM-1</b>			
Elective LUSCS	165.6 [135.2 – 171.8]	183.1 [150.9 – 203.0]	0.19
Emergency LUSCS	161.4 [140.5 – 176.7]	182.0 [146.9 – 200.6]	0.16
Vaginal delivery	166.0 [147.0 – 192.6]	167.8 [ 150.0 – 210.8]	0.38
<b>IL-6</b>			
Elective LUSCS	3.0 [1.9 - 6.3]	2.6 [1.8 - 3.6]	0.28
Emergency LUSCS	10.4 [2.4 - 14.6]	5.2 [2.7 - 10.9]	0.57
Vaginal delivery	8.3 [3.8 - 14.1]	8.7 [5.2 - 13.1]	0.99

**Table 3:** Spearman correlation coefficients (r) of inflammatory markers vs. hormonal and lipid measures in cord blood at birth. Correlation coefficients in normal font denote  $P \geq 0.05$  and bold font  $P < 0.05$ .

	Offspring of Control Mothers			Offspring of Mothers with Type 1 Diabetes		
	CRP	IL-6	ICAM-1	CRP	IL-6	ICAM-1
<b>Indices of Fetal Fat Mass</b>						
Leptin	<b>0.33</b>	0.14	-0.04	<b>0.41</b>	<b>0.23</b>	<b>0.29</b>
Subscapular Skin Thickness	0.06	0.34	-0.07	-0.28	0.07	<b>0.31</b>
Triceps Skin Thickness	0.10	0.46	0.16	0.03	0.07	<b>0.28</b>
<b>Indices of maternal glycaemic control, fetal insulinaemia and cord hormonal measures</b>						
Maternal HBA1c				<b>0.28</b>	0.06	0.06
Insulin	0.18	<b>-0.32</b>	<b>-0.32</b>	0.04	-0.12	0.04
IGF-1	-0.22	<b>-0.35</b>	-0.22	<b>-0.23</b>	-0.04	-0.09
Adiponectin	-0.28	-0.12	<b>-0.31</b>	-0.07	0.01	-0.07
<b>Fetal Lipids</b>						
HDL	-0.14	<b>-0.33</b>	-0.17	<b>-0.23</b>	0.10	-0.13
Trig	0.12	<b>0.58</b>	<b>0.42</b>	<b>0.36</b>	<b>0.33</b>	<b>0.35</b>
NEFA	0.14	<b>0.39</b>	0.11	0.16	<b>0.43</b>	0.16

**Table 4:** Multivariate analysis of predictors of cord inflammatory measures in Controls and OT1DM.

Stepwise regression with log insulin, log leptin, adiponectin, IGF-1, mode of delivery and sex was performed using an alpha of  $p \leq 0.15$  for adding or removing predictors from the model. B (SE) =  $\beta$  co-efficient (Standard error of  $\beta$  co-efficient), % variance explained by predictor.

	Offspring of Control Mothers				Offspring of Mothers with type 1 Diabetes			
	Predictor	B (SE)	% variance	P	Predictor	$\beta$	% variance	P
<b>CRP</b>	Leptin	0.10 (0.06)	5.3%	0.13	Leptin	0.20 (0.04)	9.3%	<0.001
					IGF-1	-0.07 (0.02)	12.8%	<0.001
<b>ICAM-1</b>	Insulin	-0.08 (0.03)	20%	0.003	Leptin	0.05 (0.02)	6.4%	0.003
	adiponectin	-0.01 (0.004)	4.6%	0.12	IGF-1	-0.01 (0.006)	2.0%	0.10
<b>IL-6</b>	Mode of delivery	-0.19 (0.09)	15.5	0.009	Mode of delivery	-0.33 (0.05)	29.5	<0.0001
	Insulin	-0.32(0.12)	9.7	0.03	Leptin	0.11 (0.04)	3.0	0.02
	leptin	0.22 (0.12)	5.7	0.08	IGF-1	-0.03 (0.02)	1.3	0.12
					Sex		1.8	0.06

**Table 5:** Multivariate analysis of independent correlates of fetal cord lipids in OT1DM and controls. Stepwise regression with log CRP, log ICAM-1, log IL-6, log insulin, log leptin, adiponectin, IGF-1, mode of delivery and sex was performed using an alpha of  $p \leq 0.15$  for adding or removing predictors from the model. B (SE) =  $\beta$  co-efficient (Standard error of  $\beta$  co-efficient), % variance explained by predictor.

Offspring of Control Mothers					Offspring of Mothers with type 1 Diabetes			
	Predictor	B (SE)	% variance	P	Predictor	B (SE)	% variance	P
<b>Cholesterol</b>	nil				Sex	Male lower	8.8%	0.001
					Leptin	-0.13 (0.04)	3.6%	0.03
					Insulin	0.10 (0.04)	4.0%	0.02
					IL-6	0.19 (0.07)	3.4%	0.03
					Mode of delivery	0.07 (0.04)	1.9%	0.10
<b>HDL-C</b>	IGF-1	0.02 (0.01)	22.0%	0.002	IGF-1	0.03 (0.006)	12.2%	<0.001
	adiponectin	0.008 (0.004)	6.2%	0.08	Leptin	-0.07 (0.02)	8.8%	<0.001
					Sex	Male lower	7.0%	0.001
					IL-6	0.05 (0.03)	2.4%	0.049
<b>LDL</b>	Insulin	0.15 (0.05)	20.4%	0.003	Sex	Male lower	3.6%	0.04
					Mode of delivery	0.07 (0.03)	3.0%	0.05
					IL-6	0.10 (0.05)	3.5%	0.04
<b>Triglyceride</b>	IGF-1	-0.07 (0.02)	32.6%	<0.001	CRP	0.14 (0.04)	18.1%	<0.001
	ICAM-1	1.21 (0.34)	16.9%	0.001	Insulin	-0.08 (0.02)	4.9%	0.008
					ICAM-1	0.32 (0.14)	4.1%	0.01
					IL-6	0.12 (0.05)	3.4%	0.02
					Mode of Delivery	0.05 (0.03)	1.7%	0.09
					sex	0.09 (0.06)	1.5%	0.12

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<b>NEFA</b>	Mode of delivery	-0.14 (0.04)	21.3%	0.003	Mode of delivery	-0.09 (0.03)	19.3%	<0.001
					Sex	Male lower	7.5%	0.001
					IL-6	0.11 (0.05)	2.8%	0.04

**Figure 1:** Unadjusted CRP and ICAM-1 levels relative to group specific leptin quartiles in Controls and OT1DM and unadjusted Triglyceride levels relative to CRP and ICAM-1 group specific quartiles in Controls and OT1DM. Leptin, CRP and ICAM-1 quartiles calculated separately for controls and OT1DM. Values are geometric means and standard error for the means. Units for Y axes: Triglyceride (mmol/l), Leptin (mmol/l). A) CRP across group specific leptin quartiles for Controls; ANOVA p=0.29, and for OT1DM p=0.0001. \*, \*\*, \*\*\*, \*\*\*\* The test for trend across the CRP quartiles in OT1DM p<0.001. B) ICAM-1 across group specific leptin quartiles for Controls; ANOVA p=0.83, and for OT1DM p=0.004. \*, \*\*, \*\*\*, \*\*\*\* The test for trend across the ICAM-1 quartiles in OT1DM p<0.001. C) Triglyceride across group specific CRP quartiles for Controls; ANOVA p=0.58, and for OT1DM p=0.002. \*, \*\*, \*\*\*, \*\*\*\* The test for trend across the CRP quartiles in OT1DM p<0.001. D) Triglyceride across group specific ICAM-1 quartiles for Controls; ANOVA p=0.047, and for OT1DM p=0.001. \*, \*\*, \*\*\*, \*\*\*\* The test for trend across the ICAM-1 quartiles in controls and OT1DM p<0.001.

