

Original Article

Full title: Reappearance of C-peptide during the third trimester in type 1 diabetes pregnancy: pancreatic regeneration or fetal hyperinsulinism?

Running title: C-peptide in type 1 diabetes pregnancy

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On behalf of the CONCEPTT collaborative group (See Appendix 1)

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Abstract:

Objective: We assessed longitudinal patterns of maternal C-peptide concentration to examine the hypothesis of beta-cell regeneration in type 1 diabetes pregnancy.

Research Design & Methods: C-peptide was measured on maternal serum samples from 127 participants (12, 24, 34 weeks) and cord blood during the continuous glucose monitoring in type 1 diabetes pregnancy trial (CONCEPTT). C-peptide was measured using a highly sensitive direct and solid-phase competitive electrochemiluminescent immunoassay.

Results: Three discrete patterns of maternal C-peptide trajectory were identified: Pattern 1 undetectable throughout pregnancy, n=74 (58%, maternal C-peptide <3 pmol/l); Pattern 2 detectable at baseline, n=22 (17%, maternal C-peptide 7-272 pmol/l at baseline); Pattern 3 undetectable maternal C-peptide at 12 and 24 weeks which first became detectable at 34 weeks, n=31 (24%; maternal C-peptide 4-26 pmol/l at 34 weeks). Baseline characteristics and third trimester glucose profiles of women with pattern 1 and pattern 3 C-peptide trajectories were similar but women in pattern 3 had suboptimal glycemia (50% time above range) at 24 weeks gestation. Offspring of women with pattern 3 C-peptide trajectories had elevated cord blood C-peptide (geometric mean 1319 vs 718 pmol/l; p=0.007), increased rates of large-for-gestational-age (90% vs 60%; p=0.002) neonatal hypoglycemia (42% vs 14%; p=0.001), and neonatal intensive care admission (45% vs 23%; p=0.023) compared to pattern 1 offspring.

Conclusion: First maternal C-peptide appearance at 34 weeks was associated with mid-trimester hyperglycemia, elevated cord blood C-peptide and high rates of neonatal complications. This suggests transfer of C-peptide from fetal to maternal serum and is inconsistent with pregnancy-related beta-cell regeneration.

Keywords:

C-peptide, beta cell regeneration, biomarker, pregnancy, type 1 diabetes, fetal hyperinsulinism, large-for-gestational age, neonatal hypoglycemia.

Abbreviations

BMI Body mass index

CGM Continuous glucose monitoring

CV Coefficient of variation

NICU Neonatal intensive care unit

Type 1 diabetes in pregnancy is associated with increased neonatal complications including large-for-gestational age, neonatal hypoglycemia and admission to the neonatal intensive care unit (NICU) (1). Despite recent advances in diabetes technology and improved maternal glycemia associated with the use of continuous glucose monitoring (CGM), neonatal outcomes remain suboptimal in this population (2-4). Fetal hyperinsulinism is a physiological response to maternal hyperglycemia and mediates many of these neonatal complications (5).

Pregnancy is considered to be a time when beta cell function and/or mass may increase in response to the rising gestational insulin resistance. Rodents expand beta cell numbers and one pancreatic autopsy study of pregnant women suggested a 1.4 times rise in beta cell area with increased numbers of small islets (6). The small number of human studies of serum C-peptide concentration during pregnancy have yielded conflicting results (7-10). We previously showed no rise in maternal C-peptide concentration in 10 pregnant women studied under strict experimental conditions during early (12-16 weeks) and late (28-32 weeks) gestation (7). Another study, performed in routine clinical care, showed a rise in maternal C-peptide, including in women with previously undetectable C-peptide (8). Newer highly sensitive ELISAs and electrochemiluminescent assays have improved the ability to study small changes in serum C-peptide, even in established type 1 diabetes, and allow more detailed analysis of gestational changes in beta cell function across pregnancy.

The aim of this study was to assess longitudinal patterns of maternal C-peptide concentration using a highly sensitive electrochemiluminescent assay to examine the hypothesis of pregnancy-induced beta cell regeneration in women with type 1 diabetes.

Methods

The Continuous Glucose Monitoring in Women with Type 1 Diabetes in Pregnancy Study (CONCEPTT) was a multinational randomised controlled trial to assess the effects of real-time CGM in comparison to standard care (capillary blood glucose monitoring) in pregnant women with type 1 diabetes (ClinicalTrials.gov NCT01788527; trial registered 11/2/2013.). All women gave written informed consent. Further details on study design, eligibility criteria, endpoints and results are given elsewhere (1). The CONCEPTT trial included 225 women recruited in early pregnancy or pre-pregnancy, who completed the study with a liveborn singleton infant. 127/225 women gave a voluntary additional non-fasting serum sample for the biorepository at 12, 24 and 34 weeks for C-peptide analysis and have been included in this analysis. The sample was rapidly processed and

stored frozen at -80°C prior to batch analysis at the end of the study. Outcome definitions used in the CONCEPTT study were adjudicated by the steering group and are provided in Appendix 2. Gestational age at delivery was based upon ultrasound measurements in early pregnancy (approximately 12 weeks).

Cord blood C-peptide was measured using a Dynacare test (Brampton, Ontario, Canada) on the Siemens Immulite 2000 platform (Siemens, Cambridge, UK). This is a solid-phase, competitive chemiluminescent immunoassay. Both intra-assay and inter-assay coefficients of variation (CV) were less than 6% throughout the concentration range. Maternal serum C-peptide was measured using a highly sensitive direct electrochemiluminescence immunoassay with a mouse monoclonal anti-C-peptide antibody (Roche Diagnostics, Mannheim, Germany) on an E170 analyser (Roche, Mannheim Germany) at the Academic Department of Blood Sciences, Royal Devon and Exeter Hospital. The limit of detection is 3.3 pmol/l with a CV of <4% across the reported range pmol/l. This assay is capable of measuring extremely low levels of C-peptide with superior analytical performance compared to many other highly-sensitive assays in common use. It is described more fully elsewhere (11).

Analysis

Our primary objective was to assess whether there was evidence for an increase in maternal C-peptide concentration during pregnancy. We also aimed to identify patterns of maternal C-peptide change during pregnancy by stratifying CONCEPTT participants into categories depending upon their baseline maternal serum C-peptide and the trajectory of gestational changes. We planned to use unadjusted logistic regression to compare maternal characteristics, antenatal glycaemic and pregnancy outcomes between women with and without detectable C-peptide in maternal serum, and considered undetectable C-peptide in maternal serum (pattern 1) to be the reference category.

We described continuous data using mean \pm standard deviation (SD) and categorical data as n (%) as appropriate. Data regarding birthweight were analysed as customised percentiles (adjusted for sex, gestational age, ethnicity and maternal BMI) (12; 13). C-peptide concentrations in maternal serum which were below the limit of detection (<3 pmol/l) were considered equal to the limit of detection for analysis and graphical representation. C-peptide concentrations in maternal and cord blood were converted logarithmically (base 10) prior to analysis. Although often cord C-peptide >90th centile is considered consistent with fetal hyperinsulinism in other populations (for example, obese pregnancy or in gestational diabetes) we considered this to be inappropriate for our population, as 61.8% of infants were large-for-gestational age at birth and therefore hyperinsulinism was likely to affect a

higher proportion of infants. There was a natural inflection point at the 75th centile (1415.5 pmol/l), which we took as our threshold. Student's t-tests were used to assess basic comparisons between groups classified according to maternal C-peptide patterns. Linear and logistic regression were used to assess the associations between maternal C-peptide pattern with continuous or categorical maternal and neonatal outcomes. Missing data were not imputed.

Results

Women included in this study (n=127) had type 1 diabetes with mean age of onset at 14.9 (SD 7.9) years and duration 16.9 (SD 7.7) years (Table 1). Included women were statistically similar to the whole CONCEPTT cohort (Table S1). The mean age and BMI at enrolment were 31.8 years (SD 4.4) and 25.7 kg/m² (SD 4.6) respectively. At baseline, HbA1c was 6.9% (SD 6.6) or 51.5 mmol/mol (SD 6.3), CGM time in range 51.3% (SD 13.0), time above range 39.9% (SD 14.4), time below range 8.8% (SD 6.5) and mean CGM glucose 129 mg/dl (SD19.8). Most women had large-for-gestational-age infants (83/127; 65.4%) and were delivered by caesarean section (86/127; 67.7%). Common neonatal complications included neonatal hypoglycemia requiring intravenous dextrose (27/127; 21.3%) and hyperbilirubinemia (31/127; 24.4%) with almost one third admitted for neonatal intensive care (37/127; 29.1%). Cord blood was only available from a proportion of the cohort (85/127; 66.9%).

Three longitudinal patterns of maternal C-peptide trajectory were identified (Table 1, Figure 1). Pattern 1, which included women with undetectable C-peptide in maternal serum at all time-points was the most common (74/127; 58.3%, maternal C-peptide <3 pmol/l). Pattern 2 included women with detectable C-peptide at 12 weeks in maternal serum and was less common (22/127; 17.3%, maternal C-peptide mean 68.8 ± 82.1 (range 7-272) pmol/l at 12 weeks, mean 59.0 ± 74.4 (range 3-308) pmol/l at 24 weeks and 47.0 ± 38.8 (range 3-134) pmol/l at 34 weeks).

Pattern 3 included women with undetectable C-peptide in maternal serum at 12 and 24 weeks, with appearance of detectable maternal C-peptide for the first time at 34 weeks gestation, (31/127; 24.4%, maternal C-peptide <3 pmol/l at 12 and 24 weeks, 4-26 pmol/l at 34 weeks). Their mean C-peptide concentrations were lower than women with detectable C-peptide throughout pregnancy (47.0 ± 38.8 vs 8.8 ± 5.3 at 34 weeks). Women with pattern 2 had marked inter-individual variability in C-peptide. No women with undetectable C-peptide in maternal serum at 12 weeks had detectable C-peptide in maternal serum at 24 weeks (Figure 1a-c).

We compared baseline maternal characteristics, antenatal glycemia and pregnancy outcomes between the three groups (Table 1, S2 & S3; figure 1-2). Compared to women with undetectable C-peptide in maternal serum throughout pregnancy (pattern 1), women with detectable C-peptide in maternal serum (pattern 2) had a lower pre-pregnancy BMI (mean ± SD: 23.6 ± 2.5 vs 26.6 ± 4.9 kg/m²; p=0.006), older age at diabetes diagnosis (22.5 ± 7.4 vs 13.2 ± 7.0 years; p<0.001) and a shorter duration of type 1 diabetes (10.6 ± 5.7 vs 18.6 ± 7.8 years; p<0.001). For women with detectable C-peptide in maternal serum during the first trimester, there was a trend for falling

maternal C-peptide throughout pregnancy (mean \pm SD: 68.8 ± 82.1 pmol/l at 12 weeks; 59.0 ± 74.3 pmol/l at 24 weeks; and 47.0 ± 38.9 pmol/l at 34 weeks; 12 vs 34 week C-peptide; $p=0.1$). Despite their favourable maternal characteristics and detectable C-peptide, glycemic control as assessed by HbA1c and CGM metrics, was comparable between women in pattern 1 and pattern 2 at all time-points (Table 1, S2). Their pregnancy outcomes were also similar, although obstetric and neonatal comparisons are limited by the small numbers of women (22) in pattern 2 (Table 1, S2 & S3). Total daily insulin doses were similar for women in patterns 1, 2 and 3 (Figure S1).

Women with undetectable maternal C-peptide throughout pregnancy (pattern 1) and detectable maternal C-peptide at 34 weeks' only (pattern 3) had comparable baseline characteristics, first and third trimester glycemic profiles (Table 1). However, for CGM metrics, maternal time in range 63-140 mg/dl (3.5-7.8 mmol/L) at 24 weeks gestation was significantly lower in women in pattern 3 ($45.3 \pm 16.8\%$) compared to women in pattern 1 ($51.9 \pm 13.9\%$; $p=0.050$) and pattern 2 ($55.0 \pm 14.8\%$, $p=0.037$). Women in pattern 3 also had a significantly higher time above range (49.8 ± 18.9 vs $38.0 \pm 14.8\%$; $p=0.021$) and a higher mean CGM glucose (146 ± 25.2 vs 132 ± 18.0 mg/dl; 8.1 ± 1.4 vs 7.3 ± 1.0 mmol/l; $p=0.027$) compared to women in pattern 2 at 24 weeks (figure 2).

Offspring of women with first appearance of C-peptide in maternal serum at 34 weeks had higher cord blood C-peptide concentration (available in a subset only; geometric mean 1319 vs 718 pmol/l; $p=0.007$). Logistic regression results are given in Table S3. Compared to pattern 1 offspring, infants of pattern 3 women had striking rates of large-for-gestational age (90.3% vs 59.5% pattern 1; $p=0.002$), neonatal hypoglycemia (41.9% vs 13.5%; $p=0.001$), respiratory distress (12.9 vs 2.7%; $p=0.040$), admission to NICU (45.2 vs 23.0%; $p=0.023$) (Table 1, S2 & S3, figure 2).

The new appearance of maternal serum C-peptide, when expressed as a categorical variable, was able to improve the prediction of suboptimal outcomes in women with type 1 diabetes in pregnancy, compared to the use of HbA1c at 24 weeks alone (Figure S2).

Discussion

We found three discrete patterns of C-peptide trajectories in maternal serum in pregnant women with type 1 diabetes. Most women (58%) had undetectable maternal serum C-peptide levels throughout pregnancy. A smaller second group included 15% of women with detectable maternal serum C-peptide levels throughout pregnancy. They were characterised by favourable maternal characteristics lower BMI, later onset, and shorter duration of type 1 diabetes, and improved glycemic control at 24 weeks suggesting that they may still have had some functioning beta cells. However, their serum C-peptide levels tended to fall during pregnancy, possibly due to changes in maternal vascular volume,

and their later gestation glycaemic outcomes were comparable to women with and without detectable C-peptide. A third group of women had the unexpected first appearance of C-peptide in maternal serum at 34 weeks gestation. This occurred in 25% of women and was associated with hyperglycemia at 24 weeks gestation, higher cord C-peptide, and striking rates of neonatal complications attributed to excess fetal pancreatic insulin secretion, including 90% large-for-gestational age neonates.

We previously found no longitudinal differences in fasting or meal-stimulated C-peptide production in maternal serum between early (12-16 weeks) and late pregnancy (28-32 weeks) in 10 women with type 1 diabetes using newer generation C-peptide assay methodology under strictly standardised laboratory conditions (7). However, Nielsen and colleagues measured C-peptide in a larger cohort of 90 Danish women with type 1 diabetes at six time-points (8, 14, 21, 27, 33 weeks and postpartum) (8). They found detectable C-peptide in 43% of women during early pregnancy, rising to 97% by 33 weeks gestation, with the median C-peptide concentration increasing from 6 to 11 pmol/l. The largest increase in the number of women with detectable C-peptide, and in median C-peptide concentration occurred in late pregnancy (27 and 33 weeks gestation). The proportion of women with detectable C-peptide, and the median C-peptide concentration were similar between early pregnancy and postpartum periods. The authors did not address the disappearance and/or postpartum decline in C-peptide concentration but commented that C-peptide “did not cross the placenta in either direction”. Another report in 10 women with type 1 diabetes also suggested an increase in insulin secretion before 10 weeks’ gestation (9). However, the precision and sensitivity of C-peptide assays has improved in recent years, so earlier studies may not have consistently measured C-peptide at low concentrations. The assay used for maternal serum C-peptide quantification in this study allows maternal serum C-peptide to be measured at very low concentrations, with robust analytical performance and good reproducibility (11). Relatively little data exist regarding cord C-peptide in type 1 diabetes pregnancy using modern assay technology.

Our study suggests that the first appearance of C-peptide in maternal serum at 34 weeks gestation is likely of fetal origin, due to its associations with higher cord blood C-peptide and striking rates of neonatal complications related to hyperinsulinism. This raises the possibility that previous reports of increased C-peptide in type 1 diabetes pregnancy may have also been caused by fetal transfer to the maternal circulation rather than maternal beta cell hyperplasia. Further information is needed about the elimination of C-peptide from the fetus, distribution in fetal body fluids and amniotic fluid, renal clearance rates, and the proportion of peptide which might cross the placenta for accurate assessment of fetal to maternal transfer. However, preliminary mathematical modelling (appendix 3) suggests

that transfer of C-peptide from the fetus could feasibly result in measurable maternal plasma C-peptide concentrations in mothers with type 1 diabetes.

Our study also highlights the limitations in our understanding of fetal-to-maternal transport in the placenta. Although it is generally believed that intact insulin and C-peptide do not cross the placenta, these data are based upon early studies. The report by Gerö in 1982 (14) used older C-peptide assays and does not exclude the possibility of fetal-maternal transfer of C-peptide or related fragments at low concentrations. A study in rhesus monkeys demonstrated that immunoreactive C-peptide fragments could cross the placenta from the maternal to fetal circulation (15). It is unclear if the first appearance of detectable C-peptide in maternal blood at 34 weeks gestation represents intact C-peptide or immunoreactive fragments only. Previous work demonstrating that C-peptide may have biological roles influencing insulin action and degradation suggests this may be a fruitful avenue for further study (16).

This study raises a number of other questions. Pregnancies with fetal hyperinsulinism were generally similar to those without hyperinsulinism, having comparable duration of diabetes and glycemic status at 12 and 34 weeks, but with higher mean glucose, higher time above range and a lower CGM time in range (but not HbA1c) at 24 weeks. It is therefore possible that maternal hyperglycemia at 24 weeks, or increasing hyperglycemia between 12 and 24 weeks gestation, might be important for the development of fetal hyperinsulinism. This limited improvement in maternal glycemia between 12 and 28-30 weeks is apparent from recent data showing that most women do not achieve the CGM time in range targets until the final weeks of pregnancy (17-19). Increased use of CGM continuously throughout pregnancy will facilitate more detailed longitudinal glycemic assessment. It is also possible that the fetal response to maternal hyperglycemia affects the degree of fetal hyperinsulinism (20; 21).

Alternative, unmeasured factor(s) stimulating both maternal and fetal beta cell function, causing simultaneous maternal and fetal C-peptide release or other non-pancreatic cells producing insulin during pregnancy cannot be excluded. Increased maternal beta cell function would be beneficial in type 1 diabetes pregnancy but there was no evidence of benefit in group 3. It is also possible that women in pattern 2 had enhanced beta cell function, but larger studies are required in women with detectable C-peptide to better understand the apparent decreasing maternal C-peptide concentration across gestation its impacts on glycemic and pregnancy outcomes. Glucose is considered the most important insulin secretagogue and very few nutrients are able to initiate insulin secretion in the absence of glucose. Several amino acids and fatty acids can amplify glucose-stimulated insulin

secretion (reviewed in (22)). Situations where beta cells are unable to demonstrate glucose-stimulated insulin secretion but demonstrate an insulin response to other nutrients have not, to our knowledge, been described in type 1 diabetes. The amino acid leucine can stimulate insulin release and could feasibly be responsible for this phenomenon. However, dietary information from a subset of CONCEPTT participants (n=94) suggests that maternal antenatal protein intake was not higher than expected in the general population (mean protein intake 69g or 17% of daily food energy, range 11-31%)(23) . Furthermore, no consistent associations were found between leucine intake and maternal or cord C-peptide concentration(24). It is also possible that the new appearance of C-peptide in maternal serum is caused by problems in the placenta which regulates crucial hormones. Further assessment of maternal C-peptide in type 1 diabetes pregnancy, with placental histology and postpartum C-peptide trajectory (25) for comparison would be useful. We cannot exclude the possibility that the C-peptide measured in this study is coming from an ectopic source, but this seems an unlikely explanation for one in four pregnant women.

We consider the first appearance of C-peptide in maternal serum at 34 weeks to be most likely due to fetal-to-maternal C-peptide transfer. It is also possible that this process occurred in some women with detectable C-peptide throughout pregnancy, e.g. those in group 2. These women have higher C-peptide concentrations in maternal serum, possibly reflecting some residual beta cell function as well as fetal-to-maternal C-peptide transfer. In embryonic development, pancreatic beta cells form at 7-8 weeks and begin to secrete insulin at around 12-14 weeks gestation (26). It is possible that the fetus of a mother with type 1 diabetes might have altered beta cell development, with capacity for insulin secretion in advance of this, but we consider it unlikely that fetal beta cell mass would be sufficiently large or functional at 12 weeks to provide measurable C-peptide in the maternal circulation.

Our study benefited from longitudinal measurements of maternal serum C-peptide in 127 pregnant women with type 1 diabetes with detailed CGM glycemic profiles and paired cord blood C-peptide for the majority of the cohort. Maternal C-peptide concentration was measured using an established and highly-sensitive assay with robust performance (11). There were some limitations; we did not have cord blood samples from all pregnancies, and detailed glycemic assessments using CGM were only available at 12, 24 and 34 weeks gestation with no post-partum C-peptide measurement and no data about longer-term consequences of offspring hyperinsulinism. There were no intrapartum maternal samples taken around the time of delivery (~37 weeks) for direct comparison with cord blood C-peptide. We also did not have pre-pregnancy samples for the majority of women, and cannot exclude an early first trimester rise in maternal beta cell function, as has been reported elsewhere (9; 27). We also lacked simultaneous plasma or serum glucose data and details regarding the time of day

for maternal samples and timing in relation to the last meal but did previously report substantial diurnal variability in maternal C-peptide concentration across the 24-hr day (7).

Although the Pedersen hypothesis explains the pathology of diabetes in pregnancy, it has not been possible to measure fetal hyperinsulinism at a time-point which could still influence clinical management. Previous attempts by Weiss and colleagues (28) and Carpenter and colleagues(29) using amniotic fluid sampling were effective but challenging to implement clinically on a large scale. Our findings suggest that increases in maternal C-peptide at 34 weeks could provide an opportunity for more precise monitoring of the hyperinsulinemic fetus. If confirmed by others, third trimester maternal serum C-peptide could be used to assess fetal metabolic function and predict neonatal complications in mothers with undetectable C-peptide in early pregnancy, especially those with mid-gestation hyperglycemia. As highly sensitive C-peptide assays become more widely available, this biomarker has potential for clinical use. Further improvements to C-peptide assay performance may also allow better characterisation of women with detectable and undetectable C-peptide.

Future work is needed to assess if maternal C-peptide has potential as a biomarker above and beyond CGM time-in-range metrics to facilitate early identification of fetal hyperinsulinism. The detection of fetal hyperinsulinism could facilitate targeted interventions for example, more stringent glycemic targets (17), automated insulin delivery, improved delivery planning (30), or specific perinatal protocols such as neonatal continuous glucose monitoring (31), or prophylaxis with buccal mucosal glucogel to prevent neonatal hypoglycemia (32). It is also plausible that a maternal C-peptide related biomarker could be used to identify hyperinsulinism in pregnancies affected by gestational diabetes or type 2 diabetes, or in pregnancies with evidence of accelerated fetal growth.

In conclusion, we found that C-peptide first detected in maternal serum at 34 weeks was associated with higher cord-blood C-peptide and clinical complications of fetal hyperinsulinism, including large-for-gestational age and neonatal hypoglycemia. We suggest that increasing maternal C-peptide in late gestation represents detectable fetal hyperinsulinism, rather than enhanced maternal beta cell function. Increasing focus on early biochemical identification of hyperinsulinemic offspring could provide new opportunities for personalised fetal monitoring in type 1 diabetes pregnancies.

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Contribution Statement

CLM designed the study, analysed and interpreted the data, wrote and revised the manuscript. RO designed the study, contributed to data analysis and interpretation, contributed to manuscript writing and revisions. TMcD measured C-peptide, contributed to data interpretation, reviewed and revised the manuscript and contributed to the discussion. DF and ATH all contributed to data interpretation, discussion and reviewed and revised the manuscript. HRM contributed to study design, data analysis, data interpretation, discussion, and reviewed and revised the manuscript. All authors gave approval of the final version of the manuscript prior to publication.

Statement of Assistance

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*Dr Lois Jovanovic died during the preparation of this manuscript

Guarantor

CLM is the guarantor of this work and, as such, has had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest/ Disclosures

CLM, RAO, TMcD, DSF and ATH have no conflicts of interest to declare. Helen R Murphy has received honoraria for speaking engagements from Medtronic, Roche, Novo Nordisk, Eli-Lilly and is a member of the Medtronic European Advisory Board. Medtronic supplied the CGM sensors and CGM systems for the CONCEPTT study at reduced cost.

Prior Publication & Data Availability

This study has not been previously published in abstract form. The data that support the findings of this study are available on request from the CONCEPTT trial steering committee via the senior author [HRM; Helen.Murphy@uea.ac.uk]. The data are not publicly available as they contain information that could compromise research participant privacy/consent.

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Tables & Figures

Table 1: Characteristics of study participants

	CONCEPTT Biorepository participants n=127	PATTERN 1 Undetectable maternal C- peptide n=74 (58.3%)	PATTERN 2 Detectable maternal C- peptide n=22 (17.3%)	PATTERN 3 Maternal C- peptide first detected at 34 weeks n=31 (24.4%)
BASELINE CHARACTERISTICS				
Maternal age years	31.8 ± 4.4	31.8 ± 4.4	33.1 ± 3.8	30.8 ± 4.9
Pre-pregnancy BMI kg/m ²	25.7 ± 4.6	26.6 ± 4.9	23.6 ± 2.5	24.9 ± 4.6
Duration T1D yrs	16.9 ± 7.7	18.6 ± 7.8	10.6 ± 5.7	17.3 ± 6.7
Age of onset of T1D yrs	14.9 ± 7.9	13.2 ± 7.0	22.5 ± 7.4	13.5 ± 7.0
Insulin Pump [#] %	60/127 (47.2%)	36/74 (48.7%)	9/22 (40.9%)	15/31 (48.4%)
Total daily insulin dose at 36 weeks (units per kg)	84.4 ± 36.2	87.4 ± 41.8	77.3 ± 22.9	82.4 ± 28.5
MATERNAL SERUM C-PEPTIDE				
At 12 weeks pmol/l	14.4 ± 41.8	<3.0 ± 0.0	68.8 ± 82.1	<3.0 ± 0.0
At 24 weeks pmol/l	12.7 ± 37.1	<3.0 ± 0.0	59.0 ± 74.4	<3.0 ± 0.0
At 34 weeks pmol/l	12.0 ± 22.8	<3.0 ± 0.0	47.0 ± 38.8	8.8 ± 5.3
GLYCEMIA AT 12 WKS				
HbA1c %	6.9 ± 0.6	6.9 ± 0.6	6.8 ± 0.5	6.8 ± 0.7
HbA1c mmol/mol	51.5 ± 6.3	51.9 ± 6.3	50.7 ± 5.3	51.1 ± 7.1
Mean CGM glucose mg/dl	135 ± 19.8	135 ± 21.6	133 ± 19.8	137 ± 21.6
Mean CGM glucose mmol/l	7.5 ± 1.2	7.5 ± 1.2	7.4 ± 1.1	7.6 ± 1.2
CGM Time in range %	51.3 ± 13.0	51.1 ± 13.1	54.3 ± 13.8	49.6 ± 12.3
CGM Time above range %	39.9 ± 14.4	39.7 ± 14.2	38.2 ± 14.7	41.6 ± 15.0
CGM Time below range %	8.8 ± 6.5	9.3 ± 6.5	7.3 ± 7.0	8.8 ± 6.4
GLYCEMIA AT 24 WKS				
HbA1c %	6.3 ± 0.6	6.3 ± 0.6	6.2 ± 0.6	6.4 ± 0.7
HbA1c mmol/mol	45.2 ± 6.8	44.8 ± 6.5	44.1 ± 6.7	46.8 ± 7.2
Mean CGM glucose mg/dl	130 ± 19.8	137 ± 19.8	132 ± 18.0	146 ± 25.2
Mean CGM glucose mmol/l	7.7 ± 1.2	7.6 ± 1.1	7.3 ± 1.0	8.1 ± 1.4
CGM Time in range %	50.9 ± 15.1	51.9 ± 13.9	55.0 ± 14.8	45.3 ± 16.8
CGM Time above range %	43.5 ± 16.5	42.7 ± 15.3	38.0 ± 14.8	49.8 ± 18.9
CGM Time below range %	5.5 ± 5.5	5.3 ± 4.3	6.8 ± 9.0	5.0 ± 5.3
GLYCEMIA AT 34 WKS				
HbA1c %	6.4 ± 0.6	6.4 ± 0.6	6.3 ± 0.6	6.5 ± 0.6
HbA1c mmol/mol	46.4 ± 6.6	46.1 ± 6.6	45.7 ± 6.2	47.7 ± 7.1
Mean CGM glucose mg/dl	131 ± 19.8	123 ± 14.4	121 ± 18.0	128 ± 19.8
Mean CGM glucose mmol/l	6.9 ± 0.9	6.8 ± 0.8	6.7 ± 1.0	7.1 ± 1.1
CGM Time in range %	64.7 ± 14.1	65.2 ± 13.7	66.6 ± 15.9	62.3 ± 13.9
CGM Time above range %	30.4 ± 14.0	29.1 ± 13.2	29.2 ± 15.6	34.0 ± 14.1
CGM Time below range %	4.9 ± 4.8	5.7 ± 5.2	4.2 ± 5.5	3.8 ± 3.1
PREGNANCY OUTCOMES				
Caesarean section	86/127 (67.7%)	48/74 (64.9%)	13/22 (59.1%)	25/31 (80.6%)
Vaginal delivery	31/127 (24.4%)	19/74 (25.7%)	8/22 (36.4%)	4/31 (12.9%)
Large for gestational age	83/127 (65.4%)	44/74 (59.5%)	11/22 (50.0%)	28/31 (90.3%)
Respiratory Distress	6/127 (4.7%)	2/74 (2.7%)	no events	4/31 (12.9%)
Neonatal hypoglycemia	27/127 (21.3%)	10/74 (13.5%)	4/22 (18.2%)	13/31 (41.9%)
NICU admission	37/127 (29.1%)	17/74 (23.0%)	6/22 (27.3%)	14/31 (45.2%)
Hyperbilirubinaemia	31/127 (24.4%)	16/74 (21.6%)	3/22 (13.6%)	12/31 (38.7%)

Cord blood available	85/127 (66.9%)	46/74 (62.2%)	17/22 (78.9%)	22/31 (71.0%)
Cord blood C-peptide >75 th centile	19/85 (22.4%)	6/46 (13.0%)	2/17 (11.8%)	11/22 (50.0%)
Cord blood C-peptide pmol/l *	802 (55-4965)	718 (172-4551)	570 (160-4518)	1319 (55-4965)

Data are shown for all women who gave a biorepository sample for C-peptide analysis (n=127) and subdivided into groups according to the pattern of maternal serum C-peptide. Pattern 1: undetectable maternal serum C-peptide throughout pregnancy. Pattern 2: detectable maternal serum C-peptide at 12 weeks' gestation. Pattern 3: Undetectable maternal serum C-peptide at 12 and 24 weeks which first became detectable at 34 weeks' gestation.

Data are shown as mean \pm SD or n (%); # Among women on insulin pumps, we have detailed insulin regimen information available for 90/110 women. 41/90 (45.6%) were taking Lispro and 49/90 (54.4%) were taking Aspart at 36 weeks. Among women who were using multiple daily injections (MDI), information is available for 108/115 women. For women on MDI, long acting insulin use included Glargine (50/108; 46.3%), Detemir (48/108; 44.4%), NPH insulin (4/108; 3.7%), human insulin (2/108; 1.9%) and Degludec (2/108; 1.9%). Short-acting insulin use for women on MDI included Lispro (41/108; 38.0%) and Aspart (67/108; 62.0%)

*geometric mean and range. The CGM time in range (TIR), time above range (TAR) and time below range (TBR) were defined according to international recommendations as TIR 3.5-7.8mmol/l (63-140mg/dl) and TBR <3.5 mmol/l (<63mg/dl)(17).

Figure Legends

Figure 1: Longitudinal patterns of maternal serum C-peptide change in pregnancy (a & b) with more detail of women in pattern 3 (c). Pattern 1: undetectable maternal serum C-peptide throughout pregnancy. Pattern 2: detectable maternal serum C-peptide at 12 weeks' gestation. Pattern 3: Undetectable maternal serum C-peptide at 12 and 24 weeks which became detectable at 34 weeks' gestation.

Figure 2: Associations between maternal serum C-peptide patterns 1-3 and cord blood C-peptide (a), maternal time-in-range (b; 3.5-7.8 mmol/l; 63-140 mg/dl), large-for-gestational age (c) and neonatal hypoglycemia (d). Pattern 1: undetectable maternal C-peptide throughout pregnancy. Pattern 2: detectable maternal serum C-peptide at 12 weeks' gestation. Pattern 3: Undetectable maternal serum C-peptide at 12 and 24 weeks which first became detectable at 34 weeks' gestation.