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RESEARCH: PREGNANCY

Pregnancy induces longitudinal changes in urinary C-peptide creatinine ratio in women with type 1 diabetes

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Abstract

Aims: Changes in maternal serum C-peptide have been described during pregnancy in women with Type 1 diabetes. We aimed to determine whether in these women, C-peptide, as measured by the urinary C-peptide creatinine ratio (UCPCR), display changes during the course of pregnancy and in the postpartum period.

Methods: In this longitudinal study including 26 women, UCPCR was measured in the first, second, and third trimester of pregnancy, and postpartum, using a high sensitivity two-step chemiluminescent microparticle immunoassay.

Results: UCPCR was detectable in 7/26 (26.9%) participants in the first trimester, 10/26 (38.4%) in the second trimester, and 18/26 (69.2%) in the third trimester. Changes in UCPCR concentrations were observed throughout pregnancy, significantly increasing from first to third trimester. UCPCR concentration in the three trimesters was associated with a shorter duration of diabetes and in the third trimester also with first trimester UCPCR.

Conclusion: UCPCR detects longitudinal changes during pregnancy in women with type 1 diabetes mellitus, more marked in those with shorter diabetes duration.

KEYWORDS

C-peptide, pregnancy, type 1 diabetes, urinary C-peptide creatinine ratio

1 | INTRODUCTION

In women with type 1 diabetes mellitus (T1DM), some studies have shown an increase in serum C-peptide during pregnancy[1–3] although not all studies concur.[4] It has been speculated that pregnancy might be associated with β-cell regeneration/neogenesis in these women with T1DM.[1,2] In 2000, Ilic reported the presence of C-peptide at 10 weeks' gestation in 10 pregnant women with T1DM and previously undetectable concentrations.[1] Nielsen also described a gradual increase in serum C-peptide from 8 to 33 weeks' gestation in a group of 90 women,[2] while a smaller study showed no significant differences from early to late pregnancy.[4] A recent report, involving 127 women, observed three different serum C-peptide patterns: ~60% of the participants had undetectable C-peptide throughout pregnancy, 17% had detectable C-peptide since baseline, and ~24% displayed C-peptide appearance at 34 weeks.[3] Serum C-peptide increase in pregnancy is transient as for the few reports that have addressed it. In Nielsen's report,[2] serum C-peptide concentrations decreased rapidly postpartum.

Abovementioned studies have used blood C-peptide to evaluate changes in β-cell function during pregnancy since due to a longer half-life, it is preferable to measure C-peptide than insulin.[5] Remarkably, the concentration of C-peptide in urine is 10–20 times higher than in blood, collection is not invasive, and the sample is stable for up to three days.[6,7]

Previous studies have shown that 24-h urinary C-peptide strongly correlate with fasting and stimulated serum insulin and C-peptide.[8,9] Studies in non-pregnant population have shown that C-peptide measured in a second-void fasting urine sample expressed as Urinary C-peptide to Creatinine Ratio (UCPCR) correlates with 24-h urinary C-peptide.[7] Stimulated UCPCR, both after a mixed meal test or a home evening meal, has also shown to have a strong correlation with stimulated serum C-peptide in subjects without diabetes,[10] and patients with T2DM[11] and T1DM.[12]

We have previously evaluated the feasibility of UCPCR to measure changes in β-cells function during pregnancy in women with and without diabetes. Our results showed a good correlation between both fasted and stimulated serum C-peptide and UCPCR during an OGTT in women without diabetes.[13] Furthermore, our data suggests that UCPCR can be used to assess insulin sensitivity and β-cell function in pregnancy.[13,14] In a small group of 7 pregnant women with T1DM, UCPCR was measured in 2 samples obtained over 10 weeks apart. UCPCR was detectable at first assessment in all 7 women and rose in 6 women during follow-up.[13]

We aimed to determine if in women with T1DM, dynamic changes in maternal serum C-peptide during pregnancy and postpartum can be appreciated using UCPCR.

What's new?

- An increase in serum C-peptide concentrations during pregnancy has been reported in most, but not all studies addressing this matter.
- We observed changes in C-peptide concentrations throughout pregnancy using the urinary C-peptide creatinine ratio. We also observed an association between a shorter duration of diabetes and UCPCR in the three trimesters.
- Current results suggest that longitudinal modifications of UCPCR in pregnancy could be of maternal origin, while other studies support a foetal origin. Both would be clinically relevant, either indicating maternal β-cell regeneration, or suggesting UCPCR as a biomarker of foetal hyperinsulinemia.

2 | PARTICIPANTS AND METHODS

In this observational longitudinal study performed in two tertiary care centres (Imperial College NHS Trust, London, UK; and Hospital de la Santa Creu i Sant Pau, Barcelona, Spain), pregnant women with T1DM > 18 years were eligible for the study and invited to participate. Women with impaired renal function (eGFR < 60 mL/min/m^2) were excluded. The study was approved by the corresponding Ethics Committees.

After giving written consent, second-void urine samples were collected in the first, second and third trimesters, and at 48 h and 3–6 months postpartum. Women were advised to collect the urine samples 1–3 h after a main meal. Urine samples were transferred into boric acid, then aliquoted into 1 mL cryotubes and frozen at −80°C for storage. Serum C-peptide was collected in the baseline assessment. All laboratory analyses were performed at Charing Cross North West London pathology laboratory. Urinary and serum C-peptide concentrations were measured using a two-step chemiluminescent microparticle immunoassay, on the Abbott Diagnostics Architect platform (Abbot Laboratories, Abbott Park, IL, USA), with a coefficient of variation of <10% and detection range of 3.33 to 10,000 pmol/L. Creatinine values were determined using the kinetic alkaline picrate method, Abbott Architect ci16200 system (Abbot Laboratories, Abbott Park, IL, USA) with a coefficient of variation of <6%.

Statistical analysis was performed using SPSS version 26. Descriptive data is presented as mean and SD or median and range when appropriate. Maternal and newborn clinical and anthropometric data were obtained from medical records. Gestational age was based upon last menstrual period (or early pregnancy ultrasound when discordant). Grow centiles were calculated using version 8 of the GROW calculator.[15,16] Fisher's and Cochran's Q tests were used to address differences in UCPCR positivity between study periods. To assess differences between UCPCR concentrations, Friedman's and Wilcoxon signed-rank were used. Bivariate linear regressions were performed to evaluate associations between UCPCR at different periods and baseline maternal characteristics (age, duration of diabetes, HbA1c at booking), gestational age at each pregnancy visit, and, for UCPCR in subsequent measurements, UCPCR at baseline. Variables with a p value < 0.100 were used in the multivariate analysis.

3 | RESULTS

We recruited 34 participants (18 at the UK site and 16 at the Spanish site) from 01/05/2019 to 30/03/2021.

Some visits were lost due to COVID clinic restrictions enforced during most of the study period. One participant was excluded due to diagnostic concerns (anti-glutamine decarboxylase antibodies positivity but, C-peptide > method upper limit). Four participants did not have longitudinal data due to early miscarriages.

Twenty-six participants had longitudinal data throughout pregnancy. Baseline and follow-up characteristics from these women are displayed in Table 1.

Serum C-peptide data was available in 14 of the 26 women included in this analysis. In this subset of women evaluated at baseline, serum and urinary C-peptide showed a good correlation ($r = 0.726$, $p < 0.003$).

TABLE 1 Maternal baseline characteristics, follow-up variables and neonatal outcomes.

Variable	Mean ± SD, median (P_{25-75}), or *n*, %	*n*
Baseline		
Maternal age (years)	34.2 ± 5.5	26
Ethnicity, Caucasian, *n* (%)	19 (76)	25
Weight at booking (kg)	67.1 ± 12.5	26
BMI at booking (kg/m^2)	23.6 (21.2–28.3)	26
Diabetes duration (years)	19.1 ± 8.6	26
Insulin regime (MDI, *n* (%))	16 (61.5)	26
Microvascular complications, *n* (%)	9 (36)	25
Retinopathy, *n* (%)	9 (36)	25
Nephropathy, *n* (%)	2 (8)	25
HbA_{1c} at booking		26
mmol/mol	46 (41–54)	
%	6.4 (5.9–7.1)	
Previous pregnancies, *n* (%)	24 (92.3)	26
Follow-up	Gestational age (weeks)	
1st trimester	9.8 (8.3–12.4)	26
2nd trimester	24.3 (23.0–25.6)	26
3rd trimester	35.0 (33.4–36.0)	26
Delivery	37.3 (36.4–38.9)	19
3–6 months postpartum	–	12
Neonatal outcomes		
Gestational age at delivery (weeks)	37.4 (36.6–38.8)	25
Preterm birth, *n* (%)	3 (12)	25
Birth weight (gr)	3410 (3125–3675)	25
Birth weight centile (%)	92 (54–98)	25
Large for gestational age, *n* (%)	13 (52)	25
Macrosomia, *n* (%)	2 (8)	25

Abbreviation: MDI, multiple daily injections.

UCPCR was detectable in 7 (26.9%) women in the first trimester, 10 (38.4%) in the second trimester and 18 (69.2%) in the third trimester (Figure 1-a1, $p < 0.001$). Overall, UCPCR appeared or displayed increased concentrations during pregnancy in 16/26 (61.5%) women. Significant changes in UCPCR concentrations were observed throughout pregnancy (median 0 pmol/mmol; P25-75 0-88.8 in first trimester, 0 pmol/mmol; P25-75 0-51.7, in second trimester, 8.3 pmol/mmol; P25-75 0-63.1 in third trimester, $p < 0.001$, Figure 1-b1).

All women with detectable UCPCR in the first trimester had detectable UCPCR throughout pregnancy (Figure 1-a2). In this group of women, the apparent increase in UCPCR concentrations during pregnancy was not significant (median 115 pmol/mmol, P_{25-75} 80-237 in first trimester; 142 pmol/mmol, P_{25-75} 78-583 in second trimester; 218 pmol/mmol, P_{25-75} 161-263 in third trimester, $p = 0.406$, Figure 1-b2).

In women with undetectable UCPCR in the first trimester, UCPCR was detectable in the third in 11/19 (57.9%) ($p < 0.001$, Figure 1-a3). UCPCR concentration changes in this group of women are displayed in Figure 1-b3.

UCPCR showed an inverse association with the duration of diabetes in all three pregnancy periods evaluated ($\beta = -0.46$, $p < 0.021$; $\beta = -0.42$, $p < 0.034$ and $\beta = -0.53$, $p < 0.005$ respectively). Third trimester UCPCR was also associated with first trimester UCPCR ($\beta = 0.96$, $p < 0.001$). In the multivariate regression, both duration of diabetes and first trimester UCPCR, were independently associated with third trimester UCPCR ($\beta = -0.12$, $p < 0.045$; $\beta = 0.91$, $p < 0.001$ respectively; overall R2 = 0.95). Multivariate regression full data is shown in Table S1.

Nineteen participants had full data from first trimester to 48 h postpartum. In this subset of women, UCPCR was detectable in 4 (21%) in the first trimester, 7 (36.8%) in the second trimester and 13 (68.4%) in the third trimester. At 48 h postpartum, UCPCR was detectable in the 4 women with initially detectable UCPCR in first trimester and undetectable in the rest ($p < 0.001$). No changes were observed between 48 h to 3–6 months postpartum in 10 women with long term follow-up.

Post-hoc, we explored clinical differences between women according to their UCPCR status (undetectable throughout pregnancy, detectable throughout pregnancy and initially undetectable that became detectable later in pregnancy). There were no significant differences at baseline regarding HbA1c, maternal age, duration of diabetes or BMI. We also analyzed neonatal outcomes, especially birth weight centile as a surrogate for hyperinsulinism, and found no differences between groups with the exception of a lower gestational age at delivery in the third group. Results from this analysis are displayed in Table S2.

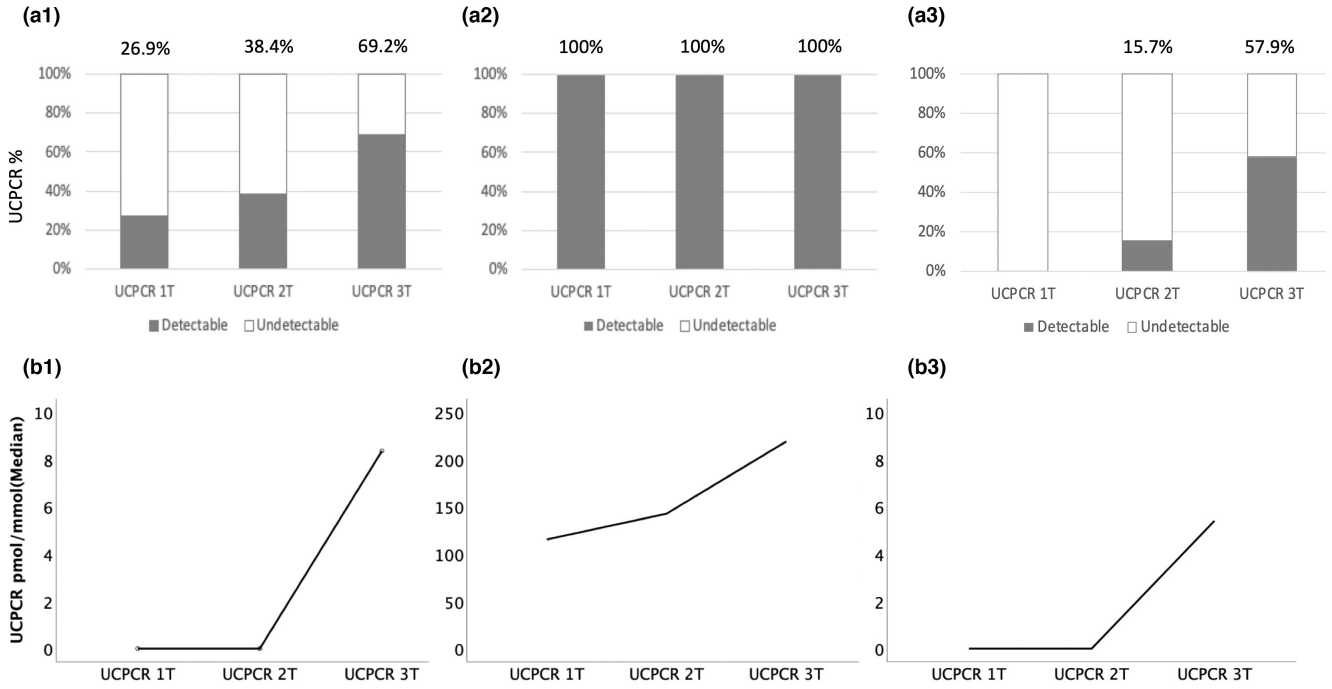

FIGURE 1 Longitudinal changes in UCPCR throughout pregnancy and postpartum, (a) Rate of women with detectable UCPCR at different pregnancy and postpartum periods. (a1) all women, (a2) women with detectable UCPCR in the 1st trimester (a3) women with undetectable UCPCR in the 1st trimester. (b) Concentrations of UCPCR at different pregnancy and postpartum periods. (b1) all women, (b2) women with detectable UCPCR in the 1st trimester, (b3) women with undetectable UCPCR in the 1st trimester. UCPCR, urinary C-peptide creatinine ratio *Y-axis scale is different in Figure 1-b2. See text for significance.

4 | DISCUSSION

In this study, we observed significant changes in UCPCR during pregnancy.

Outside pregnancy, UCPCR has been used to assess β-cell function in patients with T1DM, showing a strong correlation with serum C-peptide.[12,17] A UCPCR threshold >=0.53 nmol/mmol for significant endogenous insulin secretion has been proposed (94% sensitivity/100% specificity).[17] In a report involving 74 patients with long-duration T1DM, stimulated (after mix-meal test) serum C-peptide and UCPCR were detectable in 66.2% and 67.5% of the participants, respectively. In pregnancy, the correlation observed between UCPCR and serum C-peptide in 90 glucose-tolerant women at 0 and 120 min during a 75 g oral glucose tolerance test at 28 weeks of gestation,[13] supports the use of the UCPCR to assess insulin secretion during pregnancy.

In this cohort of women with T1DM, detectable UCPCR significantly increased from 7/26 (26.9%) in first to 18/26 (69.2%) in third trimester. New detectable UCPCR was transient as per women assessed after delivery. Higher rates of detectable C-peptide in late pregnancy have been previously described.[2,3] We confirm these prior observations using urinary C-peptide.

This is the second time that postpartum follow-up has been included in a report assessing C-peptide changes during pregnancy in women with T1DM. As in the previous report,[2] we observed that the rate of detectable C-peptide decreased postpartum, confirming the transient nature of the pregnancy-induced increase. We observed this decrease as early as 48 h postpartum with no changes in women with longer follow-up.

The rate of 26.9% participants with detectable UCPCR in the first assessment is in line with the previous studies, were detectable serum C-peptide in early pregnancy ranged from 17 to 43%.[2,3] The 69.2% rate of detectable UCPCR in the third trimester is also in agreement with reports of serum C-peptide ranging from 40 to 97% at 28 to 35 weeks in the same studies. Remarkably, in the report of Ilic, the conversion took place at 10 weeks in all participant women.[1] We observed that in some participants, UCPCR became detectable in the second trimester which would be in agreement with the report of Nielsen,[2] but not with that of Meek where all C-peptide new appearances were observed at 34 weeks. In the current report, an association between duration of diabetes and UCPCR concentrations was observed in all three pregnancy periods evaluated. This would be in line with reports addressing persistence of C-peptide secretion subjects with T1DM, in[1,2,18] and outside pregnancy.[10]

The early C-peptide appearance in women with previously undetectable concentrations observed by Ilic,[1] and

the association of C-peptide increase with improvement in HbA1c from 8 to 33 weeks reported by Nielsen,[2] suggest a maternal origin of C-peptide. Conversely, undetectable C-peptide becoming detectable at 34 weeks in Meek's report, was associated with mid-pregnancy hyperglycaemia, foetal hyperinsulinemia and adverse pregnancy outcomes, suggesting a foetal origin of C-peptide.[3] The latter would be possible since even when it is generally assumed that C-peptide, does not cross the placenta, a study conducted in Rhesus monkeys using 125I-tyrosylated-C-peptide showed that the placental barrier is not absolute.[11] Recently, Ivanisevic also described an increase in C-peptide during pregnancy, but no correlation between maternal and cord blood C-peptide was observed.[18] Instead, they observed an inverse association between maternal C-peptide and duration of diabetes that would be analogous to the inverse association between UCPCR and duration of diabetes in the current study.

The associations observed in this study would favour a maternal origin of UCPCR. First, the consistent negative association of UCPCR in all pregnancy periods with diabetes duration; where a shorter duration of diabetes would imply a higher preservation of endogenous β-cell function and a lower likelihood of foetal hyperinsulinism. Second, the independent association of UCPCR in the third trimester with that in the first trimester.

It would be interesting to elucidate the maternal vs. foetal origin of "maternal" C-peptide increase in pregnancy. Both would be clinically relevant, either indicating maternal β-cell regeneration, or as a biomarker of foetal hyperinsulinemia.

Our study had several limitations, essentially the limited sample size, which does not allow to explore associations between UCPCR concentrations and perinatal outcomes, or perform subgroup analysis. We do not have serum C-peptide data at follow-up to correlate with UCPCR. HbA1c was measured at baseline, but not during follow-up, which does not allow us to correlate UCPCR and glycaemic control at different periods. The loss of some follow-up samples after delivery is another limitation.

We confirm a transient increase in C-peptide during pregnancy using UCPCR, in line with previous studies using serum C-peptide. The rapid decrease in UCPCR after delivery supports that changes observed are related to pregnancy factors. However, whether these changes are explained by maternal β-cell regeneration, or, as recently suggested, by foetal hyperinsulinemia is yet to be clarified.

AUTHOR CONTRIBUTIONS

Lilian C. Mendoza, Anne Dornhorst and Rosa Corcoy designed the study, analysed, and interpreted the data, wrote, and revised the manuscript. Martina Tashkova contributed to data analysis and interpretation and to manuscript writing and revisions. All authors gave approval of the final version of the manuscript prior to publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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