

**PREGNANCY-INDUCED RISE IN SERUM C-PEPTIDE CONCENTRATIONS IN WOMEN
WITH TYPE 1 DIABETES**

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Objective: To investigate whether pregnancy induces increased insulin production as a marker of improved beta cell function in women with long-term type 1 diabetes.

Research, Design and Methods: Prospective study of 90 consecutive pregnant women with type 1 diabetes. At 8, 14, 21, 27 and 33 weeks blood samples were drawn for measurements of HbA1c, C-peptide and serum glucose. C-peptide (detection limit: 6 pmol/l) was considered stimulated at a corresponding serum glucose concentration ≥ 5.0 mmol/l. GAD antibody concentration was determined at 8 and 33 weeks in 35 women.

Results: C-peptide concentrations gradually increased throughout pregnancy regardless of serum glucose concentrations in the 90 women with a median duration of diabetes of 17 years (range 1-36). Among 35 women with paired recordings of stimulated C-peptide, C-peptide production was detectable in 15 (43%) at 8 weeks and in 34 (97%) at 33 weeks ($p < 0.0001$) and median C-peptide gradually increased from 6 to 11 pmol/l ($p = 0.0004$) with a median change of 50% (range -50% to 3271%) during pregnancy. GAD antibodies were present in 77% with no change from 8 to 33 weeks ($p = 0.85$). Multivariate regression analysis revealed a positive association between absolute increase in C-peptide concentrations during pregnancy and decreased HbA1c from 8 to 33 weeks ($p = 0.003$).

Conclusions: Pregnancy-induced increase in C-peptide concentrations in women with long-term type 1 diabetes was demonstrated, even in women with undetectable C-peptide concentrations in early pregnancy. This is suggestive of improved beta cell function and was associated with improvement of glycemic control during pregnancy.

Previously it has been shown that individuals with long-term type 1 diabetes have scattered beta cells in the pancreas (1) that retain the ability to develop new beta cells, probably by neogenesis from exocrine duct cells (2,3).

Pregnancy is a unique event in which the fetus usually survives to full term without rejection by the maternal immune system, which apparently accepts the foreign fetus (4). During normal pregnancy, mild suppression of the maternal immune system occurs in order to tolerate the allogenic fetus (5). This requires substantial changes in the maternal immune system over a programmed period. Pregnancy is associated with partial suppression of the immune inflammatory system so that autoimmune diseases often go into remission during pregnancy (5). During normal pregnancy, growth promoting factors are expressed in increased amounts, and the pancreas responds to the additional demand for insulin by an enlargement of existing islets of Langerhans and hyperplasia of the insulin producing beta cells (6,7), up-regulation of insulin synthesis and secretion (6), and increased sensitivity of beta cells to glucose stimulation (6). Increased mitotic activity has been seen both in vivo and in vitro in islets exposed to growth hormone. Receptors for growth hormone are expressed in islet cells and are upregulated during pregnancy (8).

In small series of women with long-term type 1 diabetes, even with previously undetectable C-peptide secretion, occurrence of maternal insulin production in pregnancy has been observed (9-11). This phenomenon may be a consequence of rejuvenated beta cell function due to pregnancy-induced growth promoting factors and suppression of the immune system (9) but improved metabolic control may also play a role in newly diagnosed type 1 diabetes (12) as well as type

1 diabetes beyond the initial remission phase (10,13).

The aim of this study was to investigate whether pregnancy induces increased C-peptide concentration as a marker of possible beta cell regeneration in a large unselected cohort of women with long-term type 1 diabetes. In addition, possible factors related to increased C-peptide concentration were investigated.

RESEARCH, DESIGN AND METHODS

As a part of another study a total of 108 consecutive Danish-speaking Caucasian women with clinical type 1 diabetes for more than one year and with non-fasting C-peptide concentrations <600 pmol/l were investigated prospectively during pregnancy. Details on the material and methods have been published previously (14). A total of 90 women with available measurements of C-peptide and serum glucose in both early and late pregnancy were included in the current study.

At inclusion at 8 (5-13) weeks (median (range)), at the visits at 14 (12-16), 21 (20-23), 27 (25-29) and 33 (31-35) weeks as well as within five days postpartum blood samples were drawn from an antecubital vein in the non-fasting state between breakfast and lunch and centrifuged at 3000 g. Serum for C-peptide, placental growth hormone (GH) and Insulin-like Growth factor-I (IGF-I) measurements obtained during pregnancy was stored at -80° C for later analysis within one run of an assay. Postpartum blood samples for C-peptide and serum glucose were analyzed separately six months later. The differences in C-peptide concentrations and serum glucose levels between the two runs of assays were 0% for C-peptide and 2% for serum glucose, respectively, tested in 20 representative samples.

Serum glucose concentrations were measured by the glucose-hexokinase method (Roche Diagnostics GmbH, Mannheim,

Germany) with intra-assay CV 1.0% and inter-assay CV 1.7%. In 67% of the samples obtained during pregnancy, glucose concentrations were measured immediately; in the remaining samples measurement of serum glucose concentrations was obtained from thawed serum samples.

C-peptide was measured by the electrochemiluminescence immunoassay ECLIA (Modular 170, Roche Diagnostics GmbH, Mannheim, Germany). The concentrations in healthy fasting subjects were >180 pmol/l. Intra-assay CV 1.9%, inter-assay CV 2.3%. C-peptide production was defined as concentrations above the detection limit of 6 pmol/l whereas C-peptide concentrations ≤ 6 pmol/l were set to 6 pmol/l.

In order to secure measurements of C-peptide at serum glucose concentrations known to stimulate C-peptide production, the C-peptide data were post hoc divided into two groups, i.e. with corresponding plasma glucose concentrations above or below 5.0 mmol/l (15). C-peptide concentrations with corresponding serum glucose concentrations ≥ 5.0 mmol/l are arbitrarily stated as “stimulated C-peptide” in the following. Thirty-five women had paired stimulated C-peptide concentrations at 8 and 33 weeks.

Glutamic acid decarboxylase (GAD) antibody concentrations were determined in early and late pregnancy in the 35 women with paired stimulated C-peptide concentrations using a quantitative radioligand assay as previously described (16). Upper detection limit was 250 U/ml.

Placental GH and IGF-I were analyzed as previously described (17). Serum creatinine was assayed on routine basis, and upper normal limit is 88 μ mol/l (18).

All women visited our and/or their local diabetes clinic at 1 or 2 weeks intervals throughout pregnancy where weight, HbA1c, insulin dose and blood pressure were recorded. The majority of women were treated with basal bolus insulin regimen. All

women registered their self-monitored plasma glucose (SMPG) readings in diabetes diaries, which were evaluated at each clinical visit. Routine SMPG was recommended at least seven times daily in order to obtain pre-prandial SMPG of 4.0-6.0 mmol/l, 90 minutes post-prandial SMPG of 4.0-8.0 mmol/l and pre-bedtime SMPG of 6.0-8.0 mmol/l (14).

HbA1c was measured by a latex immunoagglutination inhibition method at the same analyzer (DCA 2000[®], Bayer plc, England). Normal HbA1c range outside pregnancy was 4.7-6.3%, in early pregnancy 4.5-5.7% and in late pregnancy 4.4-5.6% (19). Selective serotonin reuptake inhibitors were given in three women and antihypertensive therapy, mainly with methyldopa, was given in 22 women during pregnancy. Thyroid dysfunction was treated with levothyroxine in 15 and with thiamazole in two resulting in normal thyroid function in all 17 women during pregnancy.

All participants gave written informed consent. The research protocol was approved by the regional committees for ethics and science and by the Danish Data Protection Agency.

Statistics: Descriptive statistics are given as median (range) or numbers (%). Categorical variables were compared by Chi-square test or Fisher’s exact test as appropriate. Continuous variables were analyzed by Kruskal-Wallis or Mann-Whitney tests when appropriate.

Changes during pregnancy were tested assessing the within-subject differences between continuous values at week 33 and week 8 using Kruskal-Wallis tests or, for within-subject differences between frequencies, McNemars test.

After logarithmic transformation to improve approximation of the normal distribution, correlation analyses were performed using Pearson’s coefficient, denoted r .

To determine factors associated with absolute change (after logarithmic transformation) in C-peptide concentrations from 8 to 33 weeks, univariate linear regression analyses were conducted with the following explanatory variables: Maternal age, duration of diabetes, pre-pregnancy BMI, weight gain during pregnancy and decrease in HbA1c from 8 to 33 weeks, as well as insulin dose (IU/kg), HbA1c (%), placental GH, IGF-I and GAD antibody concentrations at 8 and 33 weeks. Significant variables were further tested in backward stepwise multivariate linear regression analysis. A variable remaining significantly associated with change in C-peptide concentration during pregnancy in this analysis was regarded as an independent factor.

Differences were considered to be statistically significant at a two-sided p-value <0.05. All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

The 90 women were median 31 years old (range 21-42) with a duration of diabetes of 17 years (1-36) and a pre-pregnancy BMI of 24.3 kg/m² (18.4-34.4). At inclusion, HbA1c was 6.6% (4.9-8.8) and insulin dose was 0.75 IU/kg (0.33-1.17). At each blood sampling time, concentrations of both C-peptide and corresponding serum glucose were measured in 87-100% of women (Table 1).

At 8 weeks, C-peptide concentration was detectable in serum in 44% of women with a corresponding serum glucose concentration <5.0 mmol/l and in 41% of women with stimulated C-peptide (corresponding serum glucose concentration ≥5.0 mmol/l). Throughout pregnancy, these proportions gradually increased (Table 1), and at 33 weeks, the C-peptide production was detectable in 97% of women with a corresponding serum glucose concentration

<5.0 mmol/l and in 98% of women with stimulated C-peptide. Median serum glucose concentrations in the groups with concentrations below and above 5.0 mmol/l, respectively, were comparable throughout pregnancy (Table 1), but the individual women's glucose concentrations varied above and below 5.0 mmol/l at the different sampling times. C-peptide and serum glucose concentrations did not correlate significantly at any sampling time.

Serum creatinine concentrations were within normal range in all but two women with concentrations of 89 and 95 µmol/l at inclusion, increasing to 101 and 125 µmol/l at 33 weeks. C-peptide increased from undetectable concentrations at inclusion to 10 pmol/l at 33 weeks in both women.

Table 2 describes 35 women with paired recordings of stimulated C-peptide concentrations at 8 and 33 weeks. The number of women with detectable C-peptide concentrations increased from 15 (43%) at 8 weeks to 23/34 (97%) at 33 weeks (p<0.0001) at comparable serum glucose concentrations. From 8 to 33 weeks, the median C-peptide concentration increased from 6 to 11 pmol/l (p=0.0004) with a median absolute change in C-peptide concentrations of 50% (range -50% to 3271%).

Twenty women had undetectable C-peptide concentrations in early pregnancy and were older (p=0.04) with a tendency towards longer diabetes duration (p=0.06) compared with 15 women with detectable C-peptide concentrations in early pregnancy (Table 2). The C-peptide concentrations in these women measured in late pregnancy did not reach the values of women with detectable values at inclusion (Figure 1), but the percentage of absolute change in C-peptide concentrations was highest in the women with undetectable concentrations at inclusion. No differences were detected regarding prevalence of diabetic retinopathy, hypoglycaemia awareness or severe

hypoglycemia (requiring assistance from another person) during pregnancy (data not shown).

GAD antibodies were present in 27 (77%) of the 35 women. There was a tendency towards higher concentrations among women with undetectable C-peptide concentrations at baseline (Table 2), but this did not reach statistical significance ($p=0.14$). A decrease in GAD antibody concentrations during pregnancy could not be detected ($p=0.85$).

In univariate linear regression analyses, absolute change in C-peptide concentrations from 8 to 33 weeks was positively associated with HbA1c at inclusion ($r=0.34$; $p=0.047$) and with decrease in HbA1c from 8 to 33 weeks ($r=0.49$; $p=0.003$). No other significant associations were found. In multivariate linear regression analysis, decrease in HbA1c from 8 to 33 weeks was the only variable associated with absolute change in C-peptide concentrations (parameter estimate: $\beta=50$, 95% confidence interval 20-80, $p=0.003$). This means that C-peptide concentration increases 50 pmol/l per percent decrease in HbA1c.

CONCLUSIONS

In this prospective study of 90 pregnant women with long-term type 1 diabetes we demonstrated gradually increasing C-peptide concentration in serum throughout pregnancy. Even in women with undetectable stimulated C-peptide concentrations in early pregnancy a significant increase in stimulated C-peptide concentrations was obtained in late pregnancy. This increase was associated with improvement of glycemic control during pregnancy.

These findings are in extension of preliminary observations of rejuvenation of beta cell function in early pregnancy among 10 women with long-term type 1 diabetes (9) and in 23 type 1 diabetic women during the

entire pregnancy (11). In the current study, detectable C-peptide production was present in 42% of women in early pregnancy, which is comparable with recent findings in non-pregnant type 1 diabetic subjects with comparable duration of diabetes and good metabolic control (20).

C-peptide does not cross the placenta in either direction and, consequently, the C-peptide concentrations detected in the current study originate from the mother and not from the developing fetus. Likewise it is not likely that the demonstrated increase in C-peptide concentrations is due to cross-reactivity with other pregnancy-related hormones (9). In order to avoid overestimation of the increase in C-peptide concentrations in the subset of women with C-peptide concentrations below the detection limit in early pregnancy, these concentrations were all set to 6 pmol/l (detection limit).

Serum glucose concentrations were measured immediately in the majority of samples. However, for practical reasons, in a minority of samples, serum glucose concentrations were assayed on stored samples, which might reveal slightly lower concentration due to intermediate glucose metabolism.

For practical reasons standardized sampling either in the fasting state or after a standardized meal was not performed. To compensate for this weakness of the study, a post hoc analysis of women with paired C-peptide measurements at serum glucose concentrations known to stimulate C-peptide production (15) was performed and a significant increase in C-peptide concentration during pregnancy was demonstrated in this subgroup. Surprisingly, the C-peptide concentrations measured at serum glucose concentrations below 5 mmol/l were comparable with the concentrations measured at stimulated serum glucose concentrations. The explanation for this remains speculative. The duration of the low

serum glucose concentrations is unknown, and it is not known whether the relatively immature and stressed beta cells of these pregnant women are able to produce significant differences in C-peptide concentrations with the relatively small difference in serum glucose concentrations detected in this study.

In normal rodents, the number of insulin-producing beta cells increases during pregnancy and the sensitivity to secretagogues is increased in the islet beta cells (7). Another recent study of pregnant, non-diabetic mice found a two-fold increase in beta cell mass during pregnancy (21). In human studies, the area of the pancreatic endocrine tissue doubles during normal pregnancy and hyperplasia of the beta cells is demonstrated (7). The demonstrated increase in C-peptide production in the current study may thus reflect pregnancy-induced hyperplasia of the existing beta cells. Such a mechanism is supported by the rapid decline in circulating C-peptide concentration postpartum. However, beta cell neoplasia may also be present, which is supported by the autopsy finding of insulin positive beta cells in 88% of patients with long-standing type 1 diabetes (2). In that study, the histological investigations suggested that new beta cells are formed through differentiation of progenitor cells as duct cells or putative stem cells (2). In healthy pregnant women a similar increase in C-peptide concentration during pregnancy has been reported (7,10,11).

The mechanisms underlying the increased C-peptide production demonstrated in the present study remain unknown. Menin, a protein previously characterized as an endocrine tumor suppressor and transcriptional regulator, controls islet growth in pregnant, non-diabetic mice (21). Further studies of possible growth promoters during human pregnancy are warranted.

In the current study, the increased C-peptide concentrations were associated with improved

metabolic control during pregnancy. This is in accordance with previous findings of an association between better metabolic control and presence of C-peptide production in pregnant (11) and non-pregnant patients with type 1 diabetes (10,12,13,22). It is not known whether improved metabolic control results in C-peptide production or, vice versa, that the increased C-peptide production results in improved metabolic control. However, a prospective randomized trial demonstrated that improved metabolic control leads to increased C-peptide concentrations in patients with short-term type 1 diabetes (13) and in the current study the increased C-peptide concentrations were not associated with a reduced insulin dose in late pregnancy leading us to suggest that the improved metabolic control facilitated C-peptide production. In post-mortum analysis of pancreatic tissue from patients with type 1 diabetes, the number of beta cells was independent of duration of diabetes, but was higher in patients with lower blood glucose concentrations (2). This may indicate that obtaining and maintaining good metabolic control in pregnancy plays a pivotal role in the regeneration of insulin-producing beta cells during pregnancy.

C-peptide concentrations may be elevated in renal disease (23). It is unlikely that this confounds the present results as the renal clearance is generally increased during pregnancy (24), which may indeed underestimate the C-peptide concentrations. Notably, serum creatinine remained within normal range throughout pregnancy in the vast majority of our patients (18).

During normal pregnancy mild maternal immunosuppression occurs in order to tolerate the allogenic fetus (5). This may result in metabolically important regeneration of beta cell function. We could not demonstrate a relation between changes in GAD antibody concentration and regeneration of beta cell function during pregnancy in

agreement with Novak et. al. (10) who did not detect any association between C-peptide positivity during pregnancy and presence of antibodies against GAD, insulinoma antigen 2 or insulin (10). A possible role of pregnancy-induced changes of other aspect of the immune system cannot be ruled out.

Increased mitotic activity has been seen both in vivo and in vitro in islets exposed to GH (8) suggesting that this hormone may have played a role in the increasing C-peptide concentrations during pregnancy. Combination therapy with epidermal growth factor and gastrin induces neogenesis of human islet beta cells from pancreatic duct cells and an increase in functional beta cell mass (3). In the current study we demonstrated a significant increase in placental GH and IGF-I during pregnancy (17) but associations between these growth promoters and increased C-peptide production during pregnancy could not be demonstrated. However, due to the relative small number of pregnant women with paired recordings of stimulated C-peptide and the small number of examined growth promoters, this does not exclude an association between pregnancy-related growth promoters and increased c-peptid production.

In the current study, C-peptide concentrations decreased rapidly postpartum. This may imply that the increasing C-peptide concentrations demonstrated during pregnancy may be generated by placenta-derived growth promoters or hormonal factors that are not lasting after pregnancy. In favor of this hypothesis is the finding in mice of a pregnancy-stimulated proliferation of maternal rodent pancreatic islet beta cells with a prompt decline in the proliferation within four days postpartum (21). Alternatively, the increasing C-peptide concentrations during pregnancy may be ascribed interference with other substances. However, the C-peptide assay used in our

study measures C-peptide accurately and specifically (25).

In conclusion, pregnancy-induced increase in C-peptide concentrations in women with long-term type 1 diabetes was demonstrated, even in women with undetectable C-peptide concentrations in early pregnancy.

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Figure 1a. Paired recordings of stimulated C-peptide concentration in 15 pregnant women with type 1 diabetes and C-peptide production in early pregnancy.

Figure 1b. Paired recordings of stimulated C-peptide concentration in 20 pregnant women with type 1 diabetes without C-peptide production in early pregnancy.

Detection limit 6 pmol/l. C-peptide concentration is indicated on a logarithmic scale.

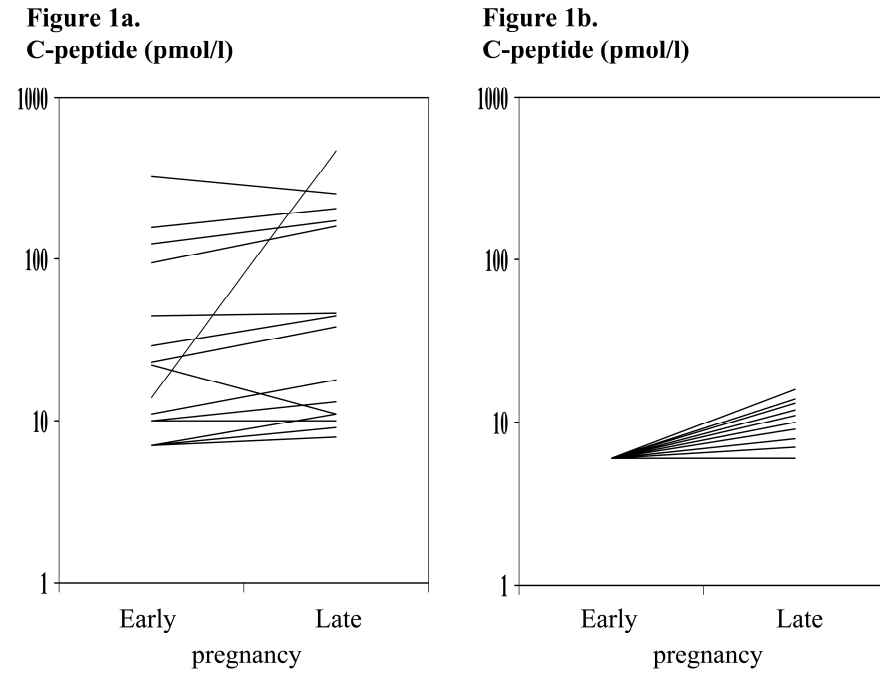


Table 1. C-peptide concentrations during pregnancy in 90 women with type 1 diabetes divided according to serum glucose values ≥ 5.0 mmol/l or < 5.0 mmol/l at the time of sampling.

	8 weeks	14 weeks	21 weeks	27 weeks	33 weeks	Postpartum
Number of women with blood sampling	90 (100%)	85 (94%)	87 (97%)	85 (94%)	90 (100%)	78 (87%)
Number of samples < 5.0 mmol/l	32	43	39	28	31	13
Number of samples ≥ 5.0 mmol/l	58	42	48	57	59	65
C-peptide concentration (pmol/l)	6 (6-325)	7 (6-598)	7 (6-434)	9 (6-527)	11 (6-472)	6 (6-1250)
C-peptide concentration (pmol/l) at serum glucose < 5.0 mmol/l	6 (6-194)	7 (6-248)	7 (6-120)	8 (6-308)	9 (6-164)	6 (6-350)
C-peptide concentration (pmol/l) at serum glucose ≥ 5.0 mmol/l	6 (6-325)	7 (6-598)	7 (6-434)	9 (6-527)	13 (6-472)	6 (6-1250)
Women with C-peptide production	38 (42%)	47 (49%)	54 (62%)	79 (93%)	88 (98%)	28 (36%)
Women with C-peptide production at serum glucose < 5.0 mmol/l	14 (44%)	25 (58%)	22 (56%)	23 (82%)	30 (97%)	4 (31%)
Women with C-peptide production at serum glucose ≥ 5.0 mmol/l	24 (41%)	22 (52%)	32 (67%)	56 (98%)	58 (98%)	24 (37%)
Non-fasting serum glucose concentration < 5.0 mmol/l	3.7 (0.8-4.9)	3.6 (1.8-4.9)	3.4 (1.4-4.9)	3.7 (1.8-4.9)	3.5 (2.1-4.9)	4.1 (2.0-4.8)
Non-fasting serum glucose concentration ≥ 5.0 mmol/l	7.2 (5.0-14.5)	8.1 (5.1-15.5)	7.0 (5.1-15.5)	6.6 (5.0-14.4)	6.9 (5.0-18.3)	8.4 (5.0-20.9)

Data are given as median (range)

C-peptide production was defined as concentrations above the detection limit of 6 pmol/l. Concentrations at or below 6 were set to 6 pmol/l

Table 2. Clinical data in 35 type 1 diabetic women who had paired values of stimulated C-peptide concentrations in both early and late pregnancy

	Women without C-peptide production in early pregnancy	Women with C-peptide production in early pregnancy
Number	20 (57%)	15 (43%)
Age (years)	32 (27-40) *	30 (26-34)
Duration of diabetes (years)	20 (6-28)	16 (2-31)
Last HbA _{1c} before pregnancy (%)	7.5 (6.1-10.0)	7.6 (6.0-9.3)
HbA _{1c} at 8 weeks (%)	7.1 (5.9-8.7)	6.8 (5.6-8.8)
HbA _{1c} at 33 weeks (%)	6.0 (5.4-7.2)	6.0 (5.5-7.1)
Median change in HbA _{1c} from 8 to 33 weeks (%)	0.8 (-0.2-2.1)	0.6 (-0.5-2.9)
Insulin dose before pregnancy (IU/kg)	0.75 (0.49-1.05)	0.56 (0.32-1.17)
Insulin dose at 8 weeks (IU/kg)	0.74 (0.45-1.04)	0.77 (0.33-1.14)
Insulin dose at 33 weeks (IU/kg)	1.04 (0.72-1.60)	1.05 (0.52-1.46)
BMI before pregnancy (kg/m ²)	24.2 (18.4-34.4)	23.5 (19.7-33.7)
Weight gain during pregnancy (kg)	14.3 (5.6-26.8)	15.0 (8.0-26.0)
C-peptide concentrations at 8 weeks (pmol/l)	6	22 (7-325)
C-peptide concentrations at 33 weeks (pmol/l)	10 (6-16)	38 (8-472)
Median change in C-peptide concentrations from 8 to 33 weeks (%)	67 (0-167)*	30 (-50-3271)
Serum glucose concentrations at 8 weeks (mmol/l)	6.8 (5.5-13.3)	7.7 (5.1-14.5)
Serum glucose concentrations at 33 weeks (mmol/l)	6.7 (5.4-18.3)	7.6 (5.1-12.8)
Placental growth hormone concentrations at 8 weeks (ng/ml)	1.0 (0.1-6.9)	1.3 (0.2-5.2)
Placental growth hormone concentrations at 33 weeks (ng/ml)	36.6 (3.5-157)	41 (8.2-87)
Insulin-like growth factor I concentrations at 8 weeks (ng/ml)	158 (26-234)	169 (100-268)
Insulin-like growth factor I concentrations at 33 weeks (ng/ml)	235 (115-483)	280 (144-331)
GAD antibody concentrations at 8 weeks (U/ml)	20.5 (0-250)	7 (0-250)
GAD antibody concentrations at 33 weeks (U/ml)	15 (0-250)	6 (0-129)

Median (range), number (%) * p<0.05