CLINICAL STUDY

Alterations in circulating adiponectin levels occur rapidly after parturition

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Abstract

Objective: To determine the levels of adiponectin and its subforms before and immediately after delivery to estimate the effect of cessating advanced pregnancy on circulating adiponectin levels.

Design and methods: In a cohort of 37 women with uncomplicated singleton pregnancies and 6 women with twin pregnancies, serum adiponectin was measured before caesarean section (CS) in the fasting state, and 24 and 48 h after CS.

Results: Serum adiponectin levels declined within 24 h of delivery from median 8.34 mg/l (range 5.57–20.47) to 6.81 mg/l (4.16–17.39) after 24 h and 6.84 mg/l (3.83–17.42) after 48 h. This corresponded to a relative decrease to 83% and 81% of pregnant values after 24 and 48 h respectively (P<0.001, ANOVA). In twin pregnancies, maternal adiponectin levels displayed a decrease that was the same as that displayed by them after birth (P<0.001).

High-molecular weight adiponectin constituted 50% (range 34–68%) of total adiponectin. Absolute changes in adiponectin levels after delivery were most pronounced in this subfraction. The percentage medium-molecular weight adiponectin decreased slightly, but significantly (from 37% to 35%, P<0.001), and a similar statistically significant rise was observed in the low-molecular weight fraction (from 13% to 15%; P<0.001) within 48 h of delivery.

Conclusions: Decreases in adiponectin levels occur shortly after delivery, and adiponectin subforms initiate the changes towards the non-pregnant state.

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Introduction

Adiponectin is a recently discovered plasma protein. It is synthesised in and secreted from adipose tissue, and it is present in large amounts in the circulation. Intriguingly, adiponectin is inversely related to the amount of body fat in adults. Low levels of adiponectin are observed in obesity and type 2 diabetes, and low levels of adiponectin correlate with markers of type 2 diabetes, i.e. increased levels of fasting insulin, fasting glucose and triglyceride. Accordingly, a growing body of evidence indicates a role for adiponectin in the development of insulin resistance.

In pregnancy, adiponectin may be involved in the development of insulin resistance during the second and third trimesters (1, 2). However, only limited data exist for the physiological course of adiponectin levels during pregnancy, and the role of adiponectin for maternal insulin resistance and diabetes during pregnancy clearly needs further elucidation.

Foetal growth may be influenced by the maternal energy metabolism and by the energy metabolism of the placenta and the foetus itself. Proteins and hormones derived from adipose tissue are found in the foetal circulation as well, and both leptin and adiponectin are present in cord blood. The placenta is a potential source of these proteins; however, for leptin, it has been demonstrated that, by far, the majority of placentally produced leptin is secreted towards the maternal circulation (3). It has not been investigated whether placenta is able to secrete adiponectin. However, reports on the presence of adiponectin protein and its mRNA in the placenta indicate that placenta is capable of synthesising adiponectin, though contrasting reports exist (4–9).

In the maternal blood, adiponectin levels decline during late pregnancy (1, 2, 10, 11). The present study was undertaken to investigate alterations in circulating adiponectin levels at parturition to estimate the impact of advanced pregnancy on maternal adiponectin levels. Given the potential impact of placenta on maternal adiponectin levels, twin pregnancies, having a larger placental mass than singleton pregnancies, were investigated in the present study.
Materials and methods

The present study was conducted in a group of individuals, from whom data on the changes in the GH axis have been reported previously (12, 13). In short, blood was drawn from 37 singleton pregnant and 6 twin pregnant women undergoing caesarean section (CS). Samples were taken first thing in the morning on the day of CS, and fasting 24 and 48 h after CS. Blood samples were allowed to clot and centrifuged, and serum was pipetted off and stored at −80 °C until analysis.

The study took place in a university hospital setting, all CSs were elective, and all mothers were healthy and had uncomplicated pregnancies. The study protocol was approved by the regional ethical committee for Aarhus County (reg. no. 2002 0312), and written consent was obtained from all participants.

**Adiponectin and adiponectin subfractions**

Serum total adiponectin was measured using a validated in-house time-resolved immunofluorometric assay (TR-IFMA) as described previously (14), having an intra-assay coefficient of variance (CV) <5% and inter-assay CV <10%. The three major subforms of circulating adiponectin (high-molecular weight (HMW), medium-molecular weight (MMW) and low-molecular weight (LMW)) were isolated by fast protein liquid chromatography (FPLC), followed by immunoassay of the obtained fractions corresponding to the three major subforms. The FPLC method has been described previously in detail elsewhere (15). The intra-assay CV values for the relative concentrations of the HMW, MMW and LMW isoforms were <4, 6 and 3% respectively. The inter-assay CV values were 6, 12 and 7% respectively (15).

**Insulin sensitivity**

Blood was collected in NaF-coated vials for the determination of plasma glucose (Beckman Instruments, Palo Alto, CA, USA). Insulin was determined using a time-resolved fluorimmunoassay (TR-IFMA; AutoDELFA, PerkinElmer, Turku, Finland). Insulin sensitivity indices (ISIs) were calculated using the QUICKI and the HOMA equations as described previously (13). In the calculations, the plasma insulin concentration was replaced by serum insulin. ISIQUICKI was calculated as ISIQUICKI = 1/log(fasting insulin) + log(fasting glucose)), and the ISIHOMA was calculated as ISIHOMA = (fasting glucose × fasting insulin)/22.5.

**Statistical analysis**

Non-parametric data were normalised by log transformation whenever appropriate to obtain normalisation of data. One-way repeated-measures ANOVA followed by the Tukey test for multiple comparisons was used for the comparison of changes over time. The Pearson product moment correlation coefficient was obtained when studying associations. \( P < 0.05 \) were considered significant. Data were analysed using Sigmastat (Vers. 2.03, SPSS Inc., Chicago, Illinois, USA).

For construction of normalised data on birth weights (z-score), reference data were obtained from the Perinatal Epidemiology Research Unit, Aarhus University Hospital, Skejby. A total of 32 573 live births in non-diabetic singleton mothers and 1405 live births among twin mothers constituted the reference cohort. Details are given in (12, 13).

**Results**

Clinical characteristics of participants and their newborns are given in Table 1.

**Singletons**

Median basal adiponectin levels in the fasting state were 8.34 mg/l (range 5.57–20.47). After delivery, levels fell to 6.81 mg/l (4.16–17.39) after 24 h and to 6.84 mg/l (3.83–17.42) after 48 h (Fig. 1a). This corresponded to a decrease to 83±6 and 81±7% of adiponectin levels before CS at 24 and 48 h respectively (\( P < 0.001 \) versus fasting levels before CS), Fig. 1b.

Before delivery, fasting total adiponectin levels correlated inversely to fasting serum insulin (\( r = -0.35, P = 0.035 \)) and positively to the QUICKI ISI (ISIQUICKI: \( r = 0.34, P = 0.041 \); and ISIHOMA: \( r = -0.31, P = 0.064 \)).

No statistically significant associations were observed between fasting maternal total adiponectin levels before delivery and measures of foetal growth, i.e. birth weight, placental weight, the ratio of placental weight to birth weight, or to any of the z-scores of these variables. Neither any significant associations between fasting total adiponectin levels and the maternal weight gain in pregnancy, the body mass index (BMI) before pregnancy or the BMI at delivery were observed.

**Adiponectin subfractions**

Before CS, the primary adiponectin fraction was HMW adiponectin, constituting 50±8% (range 34–68%) of the total adiponectin fraction (equal to HMW to total adiponectin ratio). This ratio did not change significantly after delivery, whereas the actual concentrations of HMW as expected declined significantly from median 3.35 mg/l (range: 1.66–10.59) to 2.76 mg/l (1.33–7.41) and 2.76 mg/l (1.33–7.50) after 24 and 48 h respectively (\( P < 0.001 \)).
The percentage MMW adiponectin decreased slightly, but significantly (from $37 \pm 6$ to $35 \pm 5$%, $P < 0.001$) and a similar minimal, yet statistically significant rise was observed in the LMW fraction (from $13 \pm 2$ to $15 \pm 3$%; $P < 0.001$) $48$ h after delivery. The corresponding MMW adiponectin concentrations were median $2.42 \text{ mg/l}$ (range: $1.72$–$4.39$) before CS and $2.09 \text{ mg/l}$ (range: $1.36$–$3.78$) and $2.05 \text{ mg/l}$ (range: $1.17$–$3.95$) at $24$ and $48$ h respectively ($P < 0.001$), and the LMW concentrations were $0.88 \text{ mg/l}$ (range: $0.52$–$1.51$) before CS and $0.85 \text{ mg/l}$ (range: $0.50$–$1.53$) and $0.93 \text{ mg/l}$ (range: $0.48$–$1.31$) at $24$ and $48$ h respectively. The changes in actual concentrations of LMW adiponectin did not reach a statistically significant difference.

Using the adiponectin sensitivity index (ratio of HMW to total adiponectin), a stronger correlation was found to the ISIs ($\text{ISI}_{\text{QUICKI}}$: $r = 0.67$; $P = 0.002$; and $\text{ISI}_{\text{HOMA}}$: $r = -0.71$; $P < 0.001$).

**Twin pregnancies**

Twin pregnant women had statistically insignificant higher third trimester adiponectin levels than the singleton pregnant women (fasting: median $12.29 \text{ mg/l}$; range: $7.98$–$16.11$ vs $8.34 \text{ mg/l}$; range: $5.57$–$20.47$). After delivery, total adiponectin decreased to a stable level of $9.03 \text{ mg/l}$ (range: $6.72$–$12.83$) and $9.46 \text{ mg/l}$ (range: $6.32$–$14.20$) $24$ and $48$ h after delivery respectively corresponding to $80 \pm 3$ and $78 \pm 9$% of fasting levels before delivery ($P < 0.001$).

Adiponectin subfractions were not analysed in this small subgroup.

**Discussion**

The major findings in this longitudinal study are the observations of decreasing adiponectin levels within $24$ h of delivery. In addition, we observed minor, but significant, changes in the subfractions of adiponectin after delivery, favouring the LMW fractions as in the non-pregnant state.

These observations demonstrate that circulating adiponectin levels are affected rapidly after birth and removal of the placenta, and they further indicate that the composition of adiponectin multimers changes towards the non-pregnant state with the termination of pregnancy.

In our study, adiponectin levels decreased to $\sim 80\%$ of preganant levels in singleton and twin pregnancies respectively after the expulsion of the placenta, with a rearrangement of adiponectin fractions favouring the LMW form. Before and after delivery, the HMW adiponectin subforms constitute approximately half of the circulating adiponectin; thus, the major decrease in adiponectin subforms is observed in HMW adiponectin. Longitudinal changes in adiponectins around delivery have not been described before, and even though we report only discrete changes in adiponectin levels around parturition, our findings are in agreement with previous cross-sectional observations (2, 16, 17), and they indicate i) a rapid adaptation of adiponectin levels after delivery and ii) alterations in the subforms towards the state known in non-pregnant women of reproductive age.

In pregnancy, low adiponectin levels are associated with high insulin levels and low insulin sensitivity.

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**Table 1 Clinical characteristics of participants and their offspring.** Data are given as mean ± S.D. Modified from (13). Fasting glucose, insulin and insulin sensitivity index (ISI) are given for singleton pregnancies only. The same applies to the z-scores for placental weight and the ratio of placental to foetal weight.

<table>
<thead>
<tr>
<th></th>
<th>Singletons for subform analysis ($n = 19$)</th>
<th>Twin pregnancies ($n = 6$)</th>
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<tbody>
<tr>
<td><strong>Mothers</strong></td>
<td></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>$33.2 \pm 3.6$</td>
<td>$33.2 \pm 3.4$</td>
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<tr>
<td>Pre-gestational BMI (kg/m$^2$)</td>
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<td>$22.6 \pm 3.3$</td>
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<td>Gestational age at delivery (weeks)</td>
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<td>$39 \pm 0 \pm 3$ days</td>
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<td>BMI at delivery (kg/m$^2$)</td>
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<td>$28.1 \pm 4.2$</td>
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<td>Weight gain in pregnancy (kg)</td>
<td>$15.7 \pm 5.3$</td>
<td>$15.5 \pm 5.1$</td>
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<td>Fasting insulin (pmol/l)</td>
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<td>$58.3 \pm 26.1$</td>
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<tr>
<td>Fasting glucose (mmol/l)</td>
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<td>$0.19 \pm 0.02$</td>
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<tr>
<td>$\text{ISI}_{\text{HOMA}}$</td>
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<td>$11.36 \pm 5.47$</td>
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<td><strong>Newborns</strong></td>
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<td>Weight (g)</td>
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<td>$3465 \pm 377$</td>
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<td>Placental weight (g)</td>
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<td>Placental weight $Z$-score</td>
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<td>Ratio of placental to foetal weight</td>
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<tr>
<td>$Z$-score, ratio</td>
<td>$0.76 \pm 1.02$</td>
<td>$0.98 \pm 0.96$</td>
</tr>
</tbody>
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* $P < 0.001$ versus singleton pregnancies.

* $n = 6$.
level/serum adiponectin level before CS calculated according to the following formula: serum adiponectin levels after CS in singleton mothers. Serum adiponectin levels were levels on the day of CS. (b) Relative changes in serum adiponectin (adiponectin levels declined significantly within 24 h of delivery www.eje-online.org

Figure 1 (a) Serum adiponectin versus time in singleton mothers. Boxes indicate median, and 25 and 75 percentiles with 5 and 95 percentiles are indicated. Individual data sets are represented by grey dots and lines in the background. ***P<0.001 versus pregnant levels on the day of CS. (b) Relative changes in serum adiponectin levels after CS in singleton mothers. Serum adiponectin levels were calculated according to the following formula: serum adiponectin level/serum adiponectin level before CS×100%. Serum adiponectin levels declined significantly within 24 h of delivery (P<0.001). CS, caesarean section.

Supporting previous data, a statistically significant association was found between circulating third trimester adiponectin levels and estimates of insulin sensitivity, and this relationship was further strengthened using the ratio of HMW to total adiponectin rather than total concentrations (2, 16, 18). Late pregnancy is a state of insulin resistance, and these observations further add to the evidence of a role for especially HMW adiponectin in determining insulin resistance (19).

In adult non-pregnant women, adiponectin is secreted by adipose tissue. In pregnancy, placental secretion of adiponectin is in contrast controversial, despite the presence of adiponectin mRNA and immunoreactive adiponectin in the placenta (4–9). Any placental adiponectin may then be secreted into either the foetal or the maternal compartment.

Immediately after delivery, profound changes in the maternal metabolism occur. Insulin sensitivity is rapidly reversed, and fasting insulin levels are reduced by 50–60% within 24 h of removal of the placenta (13). It appears, though, that within 24 h of delivery, only minor changes in the amount of maternal adipose tissue can be expected, as opposed to the irreversible removal of the foetus and the placenta. In our study, significantly decreasing adiponectin levels were observed uniformly shortly after the removal of the placenta. A similar decrease in adiponectin levels was implied in a study that compared adiponectin levels around gestational week 30 with postpartum levels within 5 days of delivery, though the decrease was significant only among women who were diagnosed with gestational diabetes, whereas the decrease in the non-diabetic control group was insignificant (18). Taken together, a placental contribution to the circulating pool of adiponectin could be speculated, though only indirectly suggested by the present results. Given the discrete, but significant, changes in the subfractions of adiponectin after delivery, this placental contribution could be skewed towards the HMW and MMW adiponectin fractions, a notion that is in agreement with cross-sectional data (20).

In twin pregnancies, the total size of the pregnancy product exceeds the size of the pregnancy product in singleton pregnancies at the same gestational age, and twin gestations call for increased energy supplies to and across the placenta. The increased placental mass leads to an increased capacity for hormone synthesis. Increased hormone levels influence the maternal metabolism, and twin gestations are associated with an increased risk of gestational diabetes and hypertensive disorders of pregnancy, which are conditions that have been associated with alterations in maternal adiponectin levels. Compared with singleton pregnant women, twin pregnant women had numerically higher levels of circulating adiponectin, but this difference was not statistically significant. Still, the same relative postpartum decline in circulating adiponectin as that observed in singleton pregnant mothers was observed among twin mothers. Thus, the small sample size here calls for larger studies on multiple gestations.

In extension of and in context with the immediate changes in maternal adiponectin levels reported here, previous works on adiponectin levels in the puerperium have reported levels lower than or comparable to third trimester levels from a few days to 6 months after delivery, even in women who had stopped breastfeeding (10, 17, 18, 21–23). Shortly after delivery, fasting insulin levels decline dramatically as demonstrated for this cohort also (13, 23). Similarly, maternal weight loss usually takes place over weeks or months, facilitated by the energy loss accompanying lactation. Intriguingly, it appears that maternal adiponectin levels reach their pre-pregnant levels months after delivery at a much slower pace than expected from changes in maternal insulin sensitivity or weight.

In summary, we have demonstrated here that circulating adiponectin levels decrease shortly after
delivery, and that the composition of adiponectin subfractions subsequently displays changes towards the composition of subfractions in the non-pregnant state. These rapid changes after parturition clearly indicate a role for circulating adiponectins during advanced pregnancy. The present study does not clarify whether the placenta contributes to the circulating pool of adiponectins.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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