Kinetics and secretion of placental growth hormone around parturition

Jens Fuglsang, Puk Sandager, Niels Møller¹, Sanne Fisker¹, Hans Ørskov¹ and Per Ovesen²

Gynecological/Obstetrical Research laboratory Y, Aarhus University Hospital, Skejby Sygehus, DK-8200 Aarhus N, Denmark, ¹Medical Research Laboratories, Aarhus University Hospital, Aarhus Kommunehospital, DK-8000 Aarhus C, Denmark and ²Gynecological/Obstetrical Department, Aarhus University Hospital, Skejby Sygehus, DK-8200 Aarhus N, Denmark

(Correspondence should be addressed to J Fuglsang; Email: Fuglsang@ki.au.dk)

Abstract

Objective: During pregnancy, placental growth hormone (PGH) is secreted into the maternal circulation, replacing pituitary GH. It is controversial whether PGH levels decline during vaginal birth. After placental expulsion, PGH is eliminated from the maternal blood. GH binding protein (GHBP) and body mass index (BMI) influence GH kinetics, but their impact on PGH kinetics is unknown. The present study was undertaken to define the kinetics of PGH during vaginal delivery and Caesarian section and to relate these kinetics to GHBP and BMI.


Methods: Twelve women had repeated blood samples drawn during vaginal delivery. From 26 women undergoing planned Caesarian delivery (CS) repeated blood samples were withdrawn before, during and after the CS, allowing PGH half-life determination.

Results: During vaginal delivery, median PGH values did not change before expulsion of the placenta, although individual fluctuations were seen. Clearance of PGH from the maternal circulation was best described by a two-compartment model. The initial half-life of serum PGH was (mean ± S.D.) 5.8 ± 2.4 min, and the late half-life was (median) 87.0 min (range: 25.1 – 679.6 min). The late half-life was correlated to the pre-gestational BMI (r = 0.39, P = 0.047), but not to the serum GHBP concentration.

Conclusions: Serum PGH did not decrease significantly during vaginal delivery. Elimination of PGH fitted a two-compartment model, with an estimated initial half-life of 5.8 min. The late phase serum half-life of PGH was related to BMI, suggesting a role for maternal fat mass in PGH metabolism.

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Introduction

Human placental growth hormone (PGH) is secreted from the placental syncytiotrophoblast into the maternal circulation, whereas no PGH is found in the fetal circulation (1, 2). Secretion is evident early in the first trimester (3), and PGH gradually replaces pituitary growth hormone (GH), which is stabilised at very low, but still detectable levels, in the last half of pregnancy (2, 4, 5). Maximum levels of PGH approach acromegalic levels of GH around gestational weeks 35–37 (3–6). Pulsatile secretion is not a feature of PGH (7), justifying single blood sample evaluation of PGH levels. It is controversial whether PGH levels decline in the last weeks of pregnancy. In recent studies, no significant decrease was observed (6, 8, 9); however, the most recent study reported decreasing values from week 37 onwards, which was most pronounced in the group of mothers carrying the smallest fetuses (3). In addition, serum PGH levels have been reported to decline during labour (4, 9).

Only 13 amino acid residues constitute the difference between PGH and GH (10), and both GH and PGH are transported by the high affinity GH-binding protein (GHBP), which, in turn, influences the half-life of GH (11–13). In addition, body mass index (BMI) influences both GHBP levels and GH turnover (14). The half-life of PGH has been estimated in a few small series with direct (9) or indirect (2, 15) measurements of serum PGH concentrations, but none of these studies has been large enough to take the impact of GHBP or body weight into account.

The physiology of PGH is largely undetermined, although it is surmised that it mimics GH physiology. Studies of PGH elimination may provide new insights into basal PGH metabolism and uncover potential
factors influencing PGH turnover. By comparison with GH physiology, such studies may unravel the discrepancies between the two growth hormones and lead to a better understanding of PGH function.

In this report, we describe the changes in PGH during and after vaginal delivery and Caesarian section. Special attention has been paid to define (i) whether serum PGH levels decline during labour, (ii) the kinetics of PGH and (iii) the interrelationship between PGH and factors of potential importance for its turnover.

Materials and methods

In the present study, forty-two healthy women with singleton pregnancies were included. 12 women delivering vaginally and 30 admitted for elective Caesarian section (CS). All participants gave informed consent. The protocol was approved by the Regional Ethical Committee for Aarhus County (journal no. 2002 0312). The Danish Data Protection Agency, Copenhagen, approved the collection of data (journal no. 2003-41-311).

In one woman delivering vaginally, betamethasone and indomethacin were administered in week 33 due to suspected preterm delivery; however, pregnancy continued to week 40. In all other women, pregnancies did not require intervention. Systemic medication was noted in two CS participants: one was using cetirizine due to allergy and one patient was using mesalazine sporadically, but not within the last 10 days before CS. One participant had had an (unremarkable) oral glucose tolerance test performed in pregnancy. No participant had glucosuria diagnosed at routine examination by their general practitioner and midwife during pregnancy. Characteristics of the participants are given in Table 1.

### Table 1 Characteristics for the two groups of participants and their newborns.

<table>
<thead>
<tr>
<th></th>
<th>Vaginal delivery (n = 12)</th>
<th>Caesarian section (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.8±6</td>
<td>33±4</td>
<td>NS</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (0–4)</td>
<td>1 (0–3)</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170±4</td>
<td>169±6</td>
<td>NS</td>
</tr>
<tr>
<td>Pre-gestational weight (kg)</td>
<td>70.1±13.1</td>
<td>66.5±11.5</td>
<td>NS</td>
</tr>
<tr>
<td>Pre-gestational BMI (kg/m²)</td>
<td>24.0 (19.3–36.8)</td>
<td>22.4 (18.4–33.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Weight gain in pregnancy (kg)</td>
<td>20.0 (11.6–26)</td>
<td>14.0 (4.0–29.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>40.3±1.1</td>
<td>39.0±0.4</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>- weeks</td>
<td>282±8</td>
<td>273±3</td>
<td></td>
</tr>
<tr>
<td>Pregnancies obtained after ART (no.)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Newborns female/male (no.)</td>
<td>6/6</td>
<td>20/10</td>
<td>NS</td>
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<tr>
<td>Birth weight (g)</td>
<td>3688±364</td>
<td>3528±399</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight z-score</td>
<td>0.2±0.9</td>
<td>0.3±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>52±2</td>
<td>52±2</td>
<td>NS</td>
</tr>
<tr>
<td>Ponderal index (kg/m³)</td>
<td>25.7±2.1 ¹</td>
<td>25.2±2.1</td>
<td>P = 0.05</td>
</tr>
<tr>
<td>Placenta weight (g)</td>
<td>670±85 ²</td>
<td>733±130</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are given as means±s.d. for normally distributed data, otherwise as median and range in parentheses.

¹ Data were available for 7 subjects only; ² n = 11; ³ n = 10. ART, assisted reproductive techniques; NS, not significant.

Vaginal delivery

Of the 12 participants, 9 had spontaneous labour and three underwent induction of labour by rupture of the membranes.

According to the protocol, participants had non-fasting venous blood samples withdrawn during labour at cervical dilatation of less than 3 cm, 3–6 cm, 6–9 cm, and at full dilatation. Finally, a blood sample was withdrawn one hour post partum.

Caesarian section

Thirty women with singleton pregnancies were enrolled in the study. Elective Caesarian section (CS) was performed due to breech presentation (n = 4), former CS (n = 6), former lesion of the anal sphincter (n = 8), or on maternal request (n = 12). Participants had non-fasting blood samples taken on the day of hospitalisation. On the day of CS, participants had fasting blood samples taken after placement of an intravenous cannula in the forearm. Blood samples were withdrawn just before removal of the placenta (t = 0 min), and then at 10, 20, 30, 40, 50, 60, 90, 120 and 240 min. Participants were fasted, but were not deprived of drink (free access to tap water), until t = 120 min. The i.v. cannula was kept patent with saline, and for each blood sample the first ml was discarded. According to the study protocol, blood sampling during CS was aborted if estimated bleeding > 500 ml occurred. This was the case for two participants. In two patients, difficulties with the intravenous catheter prompted cessation of blood sampling. These four patients were included in the analyses of non-fasting PGH levels, but they were excluded from half-life analyses. Thus, determinations of PGH half-life took place in 26 participants, who had a median estimated bleeding volume of 300 ml (range: 150–500 ml).

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All CSs were elective and were performed using spinal analgesia. Isotonic saline was administered during establishment of spinal analgesia and during the CS. Most participants received antacida on the morning before CS. After birth, intravenous syntocinon was administered, and rectal naproxen was given postoperatively. Antibiotics were administered according to routine guidelines.

Blood samples for the study of the growth hormone axis before and after parturition were drawn from participants undergoing CS (16).

Biochemical analyses
The blood was allowed to clot, then cooled in an ice bath until centrifugation, and serum was pipetted off and stored at –80°C. All hormone samples were determined in duplicate. Serum PGH was measured with a commercially available solid phase immuno-radiometric assay (PGH IRMA, BC1017, Biocode, Liege, Belgium). Cross-reactivity with pituitary GH was below 0.001%, according to the manufacturer. In our setting, both the intra- and interassay coefficients of variation were <6%. The minimum detectable concentration was calculated as the mean of 10 replicate determinations of the 0-standard + 3 × s.d., and was lower than 0.4 μg/l. Serum total GHBP was determined in an in-house immunofunctional assay as earlier described (17). In our laboratory, this assay has an intra-assay coefficient of variation of 3.4% and an interassay coefficient of variation of 12% at 0.563 nmol/l and 6.3% at 1.4 nmol/l. Serum total GH was measured by Delfia TRIFMA specific to the growth hormone binding to GHBP at serum levels of GHBP (21), a molecular weight of GH and non-glycosylated PGH of 22 000 Da (22, 23), and an affinity constant (K_a) of GHBP of 0.911 nmol/l (24).

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Data were log transformed where appropriate to obtain normality. Unless otherwise stated, normally distributed data are given as means±s.d., non-normally distributed data are given as median and range. Repeated measurements ANOVA with the Bonferroni t-test for multiple comparisons were used to compare hormone concentrations over time. The Pearson product moment correlation coefficient was used for testing correlations. A P value <0.05 was considered significant. All statistics were performed using the software SigmaStat 2.03 (SPSS Inc., Chicago, IL, USA) Curve fitting was performed in SigmaPlot 8.02 (SPSS Inc.).

Results
Table 1 gives the characteristics for the two groups of participants. Data for the two groups of participants and their newborns were similar, except for a higher gestational age at delivery in the vaginal delivery group, who also tended to give birth to babies with a slightly higher ponderal index (P = 0.05).

Vaginal delivery
Non-fasting serum PGH was (median) 10.1 (range: 7.7–35.3) μg/l upon admittance to the delivery ward (Fig. 1). This was significantly lower than the non-fasting serum PGH levels in women admitted for elective CS (19.9 μg/l, range: 8.2–92.4; P = 0.033), despite a higher gestational age (282 (269–297) days vs 274 (264–278) days; P < 0.001) and similar BMI. Unexpectedly, large fluctuations in serum PGH values were observed during the progression of labour; however no consistent changes in PGH values were observed, especially no significant decrease was
observed, regardless of stage of labour (Fig. 1A) or duration of labour (Fig. 1B). As expected, very low levels of PGH were detected 1 h after expulsion of the placenta \( (P, 0.001) \). Caesarian section

Serum GHBP

Before CS, fasting serum GHBP levels averaged 1.75 ± 0.55 nmol/l. Serum GHBP levels were stable during and after CS.

Growth hormones

Before CS, 49.5 ± 12.6% (range: 18.2–73.3%) of PGH was bound to GHBP at a PGH level of 24.0 ng/ml (range: 10.2–87.2).

Correlation studies

Correlation coefficients for variables related to PGH metabolism are given in Table 2. The two half-lives tended to correlate \( (r = 0.36, P = 0.069) \). Neither \( T_{1/2\text{init}} \) nor \( T_{1/2\text{late}} \) was associated to GHBP levels; however, the \( T_{1/2\text{late}} \) correlated to the pre-pregnant BMI \( (r = 0.39, P = 0.047) \) (Fig. 3).

Before birth, an inverse correlation was observed between PGH and GHBP levels \( (r = -0.55, P = 0.005) \).
The bound fraction of PGH at CS was not correlated to either of the two half-lives, to the pre-pregnant BMI, or to BMI at CS.

Discussion

The major findings of the present study are that PGH displays two-compartment elimination kinetics which are associated with BMI. An initial fast plasma elimination of PGH with half-lives of ~5–6 min and a second phase with half-lives of ~1.5 h were observed. No changes in PGH levels were observed during vaginal delivery, although baseline levels were low in the normal range.

PGH is secreted by the syncytiotrophoblast solely to the maternal circulation (1, 2). It is found in high concentrations during the last half of pregnancy, and at the same time pituitary GH is virtually absent in plasma (4, 5). The regulation and physiology of PGH is far from being understood, but the secretion of PGH is described as non-pulsatile (7). Pituitary GH has a well-known impact upon adipose tissue metabolism (25, 26), and PGH binds equally as well as GH to GHBP (11), which is the extracellular part of the GH receptor. Lipolytic effects of PGH have been demonstrated using rodent adipocytes (27).

The pattern of PGH levels during the last weeks and days of pregnancy is controversial. Cross-sectional and longitudinal studies have suggested a slight decrease in PGH (3–5), especially in women giving birth to children with lower birth weights (3). At birth, abruptly decreasing values at the onset of labour were noted by Mirlesse and colleagues (4, 28). In contrast, Coutant et al. could not demonstrate any difference in PGH levels in weeks 35–40 compared with early labour (8). We observed that in women delivering vaginally, serum PGH levels upon admittance to the delivery ward were somewhat lower than the PGH levels in women admitted for elective CS, despite a slightly higher gestational age in the former group. Birth weight z-scores and BMI were comparable; hence no obvious explanation for the lower PGH levels in early labour was found. In a recent paper, one woman delivering vaginally was observed to have decreasing values of serum PGH shortly before delivery (9), and Wu et al. found no explicit decline in PGH values up to 30 min before parturition in two patients (6). The present findings thus resemble these latter observations, as no significant changes in serum PGH values were observed during the course of vaginal delivery. In particular, none of the participants demonstrated markedly decreased PGH levels during labour as previously described (4), even at full cervical dilatation. These findings do not exclude the possibility that some reorganisation of placental physiology occurs during or shortly before vaginal delivery, but it appears that at least a basal secretory tone of PGH is maintained.

Figure 2 Serum PGH during Caesarian section. (A) An example of the disappearance of PGH from maternal serum after elective Caesarian section. The presented elimination curve followed a two-compartment elimination curve with a fit of $r^2 = 0.997$. For the entire group of participants, the fit was (median) 0.996 (range 0.972–0.999). In this example, the elimination curve was characterised by the formula: serum PGH = 12.91 × $e^{-0.1280 \times \text{time}} + 5.589 \times e^{-0.0109 \times \text{time}}$, giving a $T_1/2\text{init}$ of 5.5 min and a $T_1/2\text{late}$ of 63.4 min. In (B), absolute values for serum PGH concentrations are displayed ($n = 26$). The dotted line represents the minimum detectable PGH concentration of the assay. In (C), the relative decrease in serum PGH levels over time is shown. The PGH level at time = 0 min has been set to 100%. Note the log scale of the y-axis. Data in B and C are given as median with 10th and 90th centile.
even during advanced labour. Altogether, serum or placental samples obtained in relation to vaginal delivery may not be representative of third trimester hormone secretion, and this should be taken into account in future study designs.

Shortly before removal of the placenta at CS, a decrease in PGH was observed and this decrease was not explained by simple haemodilution. At least three mechanisms may contribute to the observed decrease in PGH levels. First, peripheral vasodilation may cause vascular 're-entry' influx of extravascular fluids with different PGH content. Secondly, sympathetic/adrenergic tone at CS may influence PGH liberation from the placenta. Thirdly, maternal blood flow may be redirected as a consequence of the preparatory procedures for CS. Regarding the latter, all newborns had normal umbilical cord pH, indicating that the blood supply to the placenta was sufficient for fetal aerobic metabolism.

Most studies of GH half-life describe elimination after GH infusion, finding a half-life of total GH of around 15–20 min following mono-exponential decay curves (13, 18, 25, 29). Alterations in GH levels induce minor changes in the half-life, suggesting saturation of elimination pathways at high concentrations (18, 29). Rather than being influenced by differing PGH levels, the present results suggest that the elimination of PGH is dissociated into two phases following a two-compartment model. Studies of GH metabolism sometimes assume one-compartment models to facilitate calculations; however two-compartment models following double-exponential decay curves have been recognised for both GH and GH–GHBP complexes (30–32), and also for the analogous human placental lactogen (hPL), which apparently has an early half-life of less than 13 min (33).

Previously, indirect estimates of PGH levels at parturition have indicated that the elimination of PGH was a rapid process (2, 15). We observed a mean half-life of PGH in the initial phase of PGH disappearance from the blood; $T_{1/2}$ in the second phase. $n = 24–28$.

A theoretical half-life of free GH of 2–12 min was found by Veldhuis et al. when no GHBP was present (13). Similarly, a half-life of 3.5 min was estimated for the free GH fraction in infusion studies at a total half-life of 13 min (34). In our setting, rapid plasma elimination independent of GHBP levels was observed at calculated levels of bound PGH averaging 50%. The bound value depends on the GHBP measurements, and different GHBP assays may provide differing GHBP values (17); however, regardless of assay, a substantial proportion of PGH will be bound, even in the third trimester. Also, the high levels of PGH justify the assumption of 1:1 complexing between PGH and GHBP (21, 35). Pregnancy is a long-lasting condition, allowing the maternal organism sufficient time to adapt to high PGH levels, and GH kinetics at birth may therefore be different from the elimination of high levels of GH after acute infusion. Adenomectomy in acromegalic subjects may be a comparable situation to CS with

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Correlation coefficients between variables in univariate analyses.</th>
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<tr>
<td>$T_{1/2}\text{init}$</td>
<td>$T_{1/2}\text{late}$</td>
</tr>
<tr>
<td>$T_{1/2}\text{init}$</td>
<td>—</td>
</tr>
<tr>
<td>$T_{1/2}\text{late}$</td>
<td>—</td>
</tr>
<tr>
<td>PGH*</td>
<td>0.18</td>
</tr>
<tr>
<td>GHBP</td>
<td>0.15</td>
</tr>
<tr>
<td>BMI pre-pregnant*</td>
<td>0.10</td>
</tr>
<tr>
<td>BMI at CS</td>
<td>—</td>
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</table>

* Data were log transformed before analysis. CS; caesarian section; $T_{1/2}\text{init}$, $T_{1/2}$ in the first phase of PGH disappearance from the blood; $T_{1/2}\text{late}$, $T_{1/2}$ in the second phase. $n = 24–28$.

$^{*}P < 0.05; **P < 0.01; ***P < 0.001; \dagger P = 0.069$. 

Figure 3 Late phase serum PGH half-life vs pre-pregnant BMI ($n = 26$). Note the logarithmic scales on the axes. Exclusion of the two outliers only strengthened the association ($r = 0.54$, $P = 0.006$).
regard to cessation of high growth hormone secretion of long duration. In such situations, however, intraoperative GH half-lives are slightly higher than half-lives found in acute studies (36, 37). Hence, among growth hormone analogues, plasma elimination of the placental variants PGH and hPL appears to be described most accurately by two-compartment kinetics, different from most observations on GH elimination from plasma. Furthermore, a rapid half-life of PGH predominates at physiological levels of GHBP and is independent hereof. The mechanism behind the rapid elimination of PGH is unclear. Intuitively, PGH clearance takes place through GH receptors (GHRs), although the importance of GHRs in plasmatic clearance of pituitary GH has been questioned (18). Tissue expression of GHRs during pregnancy is virtually unknown but could have an impact on PGH kinetics.

At a pituitary GH level of 25 μg/l, as much as 46% of the total metabolic clearance of GH may be renal in non-pregnant subjects (19), and renal GH clearance depends on BMI and fat mass (38). During pregnancy, renal blood flow and glomerular filtration rate increase as does the maternal fat mass, and, hence, renal clearance could be a substantial contributor to PGH elimination. Evidently, the observed rapid half-life was not caused by PGH clearance, even though the placenta could be a potential influence on plasmatic PGH elimination in pregnancy.

The initial phase of rapid PGH elimination is followed by a phase in which a larger proportion of PGH is placed extravascularly or is bound to GHBP, the latter also demonstrated here. We attempted to investigate whether GHBP had an influence on PGH half-lives, but we were unable to demonstrate such a relationship. Instead, the pre-gestational BMI was associated with the late phase half-life and with GHBP levels at birth. As for the BMI at birth, this parameter not only reflects the maternal fat mass but also the presence of the pregnancy product and no significant association to PGH metabolism was observed. Altogether, the positive associations between pre-gestational BMI and both PGH metabolism and GHBP levels suggest that adipose tissue may interact with PGH metabolism, possibly involving the GHRs (39).

Most studies, although not all (31), point to an effect of GHBP on GH kinetics (12, 13, 29, 30). GHBP is the extracellular domain of the GHR, but it has not been clarified whether circulating GHBP mirrors tissue GHR status (40). Notwithstanding, the majority of GHBP is presumed to derive from hepatic and adipose tissue. Local GHBP accumulation could tend to withhold PGH, decrease PGH exchange with serum, and form inert complexes when PGH cross-binds GHBP and GHRs.

Such situations are compatible with longer half-lives at high BMI and are expected to be most influential at low levels of circulating PGH. Thereby, effects of PGH, e.g., lipolysis (27), would be down-regulated and this could be expedient when the caloric supply to the placenta is assured otherwise, for example, by frequent or large caloric intakes. Supporting this, decreased PGH levels are observed with increasing BMI (3, 41, 42). Although the latter association was not demonstrated here, indirect evidence in terms of a negative association between PGH and GHBP levels was found, confirming the results of McIntyre et al. (43).

The PGH assay uses antibodies raised against the non-glycosylated hormone; however, a glycosylated variant has been recognised (23). It is not known whether glycosylation affects assay PGH detection or PGH kinetics. Finally, it should be stressed that the late half-life of PGH is not influenced by any sudden occurrence of pituitary GH in the maternal circulation, as the level of GH was constant at very low levels during the observation period.

In conclusion, PGH levels fluctuated during advanced labour and mean levels were unchanged. Serum PGH appears initially to be eliminated at a very rapid rate with a half-life of ~5–6 min. Later, at lower levels, a half-life of ~1.5 h was observed, showing some relation to the maternal BMI. Assuming steady-state or near steady-state concentrations of PGH in the last weeks of pregnancy, the short half-life suggests a high turnover rate of PGH. Elimination pathways for PGH remain to be clarified.

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