Placental growth hormone is increased in the maternal and fetal serum of patients with preeclampsia

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Abstract

Objectives—Placental growth hormone (PGH) is a pregnancy-specific protein produced by syncytiotrophoblast and extravillous cytotrophoblast. No other cells have been reported to synthesize PGH. Maternal PGH Serum concentration increases with advancing gestational age, while quickly decreasing after delivery of the placenta. The biological properties of PGH include somatogenic, lactogenic, and lipolytic functions. The purpose of this study was to determine whether the maternal serum concentrations of PGH change in women with preeclampsia (PE), women with PE who deliver a small-for-gestational age neonate (PE+SGA), and those with SGA alone.

Study Design—This cross-sectional study included maternal serum from normal pregnant women (n=61), patients with severe PE (n=48), PE+SGA (n=30), and SGA alone (n=41). Fetal cord blood from uncomplicated pregnancies (n=16) and PE (n=16) was also analyzed. PGH concentrations were measured by ELISA. Non-parametric statistics were used for analysis.

Results—(1) Women with severe PE had a median serum concentration of PGH higher than normal pregnant women (PE: median: 23,076 pg/mL (3473-94 256) vs. normal pregnancy: median: 12 157 pg/mL (2617-34 016); p < 0.05), pregnant women who delivered an SGA neonate (SGA: median 10 206 pg/mL (1816-34 705); p < 0.05], as well as pregnant patients with PE and SGA (PE + SGA: median: 11 027 pg/mL (1232-61 702); p <0.05); (2) No significant differences were observed in the median maternal serum concentration of PGH among pregnant women with PE and SGA, SGA alone, and normal pregnancy (p > 0.05); (3) Compared to those of the control group, the median umbilical serum concentration of PGH was significantly higher in newborns of preeclamptic women (PE: median 356.1 pg/mL (72.6-20 946), normal pregnancy: median 128.5 pg/mL (21.6-255.9); p < 0.01]; (4) PGH was detected in all samples of cord blood.

Conclusion—(1) PE is associated with higher median concentrations of PGH in both the maternal and fetal circulation compared to normal pregnancy. (2) Patients with PE+SGA had lower maternal serum concentrations of PGH than preeclamptic patients without SGA. (3) Contrary to previous findings, PGH was detectable in the fetal circulation. The observations reported herein are novel and suggest that PGH may play a role in the mechanisms of disease in preeclampsia and fetal growth restriction.

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Keywords
Pregnancy; placental growth hormone; preeclampsia; small for gestational age; fetal growth

Introduction
Placental growth hormone (PGH) is a pregnancy-specific hormone that has been proposed to play a role in trophoblast invasion [1] and fetal growth [2-6], as well as maternal adaptation to pregnancy [7-9]. PGH demonstrates somatotrophic, lactogenic and lipolytic properties similar to pituitary growth hormone (GH), although its growth-promoting activity surpasses its other functions [10-13]. Syncytiotrophoblast and extravillous cytotrophoblast (EVCT) express PGH mRNA and protein [14-16]. This hormone is secreted in a non-pulsatile fashion [17], in contrast to the pulsatile secretion of GH. PGH can be detected in maternal blood at as early as 5 weeks of gestation [4], and increases throughout pregnancy until term [3], at which time PGH concentration has been observed to either plateau [2,18,20-22] or slightly decrease [4].

Given the effect of PGH upon fetal growth and trophoblast invasion, several studies have investigated the relationship between the maternal serum concentration of PGH and fetal growth restriction [2,6,21,24]. Consistently lower concentrations of PGH were detected in small for gestational age neonates (SGA). There is, however, only minimal information about PGH in preeclampsia.

The aims of this study were to: (1) determine the maternal serum concentrations of PGH in the following groups: patients with severe preeclampsia, women with preeclampsia who delivered SGA neonates, patients with SGA alone, and normal pregnancies; and (2) Evaluate the serum concentrations of PGH in umbilical cord blood in both normal pregnancies and those complicated by preeclampsia.

Materials and methods
Study Population
A cross-sectional study was conducted through a search of our clinical database and biological sample bank. This study included normal pregnant women (n=61), patients with severe preeclampsia (n=48), those with preeclampsia who delivered a SGA neonate (n=30), and patients who delivered SGA neonates without preeclampsia (n=41). Blood samples were drawn between 20 and 42 weeks of gestation. Patients with multiple gestations, fetal anomalies or fetal demise, chronic hypertension, diabetes mellitus, systemic lupus erythematosus, or chronic renal diseases were excluded. In addition, cord blood was collected from patients with preeclampsia (n=16) and uncomplicated pregnancies (n=16). Eligible patients were enrolled at the Detroit Medical Center/Hutzel Women's Hospital in Detroit, Michigan. Preeclampsia was defined as hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on at least two occasions, 4 hours to 1 week apart) and proteinuria (≥ 300 mg in a 24 hour urine collection or one dipstick measurement ≥ 2+). Severe preeclampsia was defined as either severe hypertension (diastolic blood pressure ≥ 110 mmHg) plus mild proteinuria or mild hypertension plus severe proteinuria (a 24 hour urine sample containing 3.5 grams of protein or a urine specimen with ≥ 3+ protein by dipstick measurement). Patients with abnormal liver function tests (aspartate aminotransferase > 70 IU/L) plus thrombocytopenia (platelet count < 100 000/cm³), as well as those with eclampsia, were also classified in the severe preeclampsia category [25]. SGA was diagnosed if the neonatal birth weight was below the 10th percentile for gestational age [26]. The control group consisted of pregnant women with no medical or obstetrical complications, who delivered at term.
Sample collection and placental growth hormone assay

Samples of maternal peripheral blood were obtained by venipuncture and collected directly into tubes containing EDTA. The samples were centrifuged for 10 minutes at 4° C and stored at -70° C until assayed. Umbilical cord blood was collected at the time of delivery by umbilical vein venipuncture. Samples were collected directly into tubes containing EDTA, processed, and stored as described above.

Maternal and fetal serum PGH concentrations were determined using specific and sensitive immunoassays obtained from Diagnostics Systems Laboratories, Inc. (Webster, TX, USA). Briefly, standards and unknown maternal serum samples were incubated in duplicate wells of the micro titer plates, which had been pre-coated with PGH antibodies. During this incubation, PGH present in the standards or maternal and fetal serum samples were immobilized by the pre-coated antibodies (forming antigen-antibody complexes). Repeated washing and aspiration removed all other unbound materials from the assay plate. This step was followed by incubation with another anti-PGH antibody labeled with biotin. Following incubation, the wells were washed and incubated with a streptavidin-horseradish peroxidase (HRP) conjugate solution. After incubation and washing to remove excess and unbound materials, a substrate solution (containing TMB, tetramethylbenzidine) was added to the wells of the microtiter plate and color developed in proportion to the amount of antigen bound in the initial step of the assay. The color development was stopped with the addition of an acid solution, and the intensity of color was read using a programmable microtiter plate spectrophotometer (SpectraMax M2 micro plate workstation, Molecular Devices, Sunnyvale, CA, USA). The concentrations of PGH in maternal and fetal serum samples were determined by interpolation from individual standard curves composed of purified human PGH. The calculated inter- and intra-assay coefficients of variation (CV) for PGH immunoassays in our laboratory were 9.3% and 9.3%, respectively. The sensitivity of the PGH immunoassay was calculated to be 16.1 pg/mL.

All patients provided written informed consent prior to the collection of maternal and umbilical cord blood samples. The collection and utilization of the samples for research purposes was approved by the Institutional Review Board of the National Institute of Child Health and Human Development (NICHD/NIH/DHHS, Bethesda, Maryland), as well as the Human Investigation Committee of Wayne State University (Detroit, Michigan). Many of these samples have been previously used to evaluate the biology of inflammation, angiogenesis regulation, hemostasis, and growth factor concentrations in normal and complicated pregnancies.

Statistical Analysis

Kolmogorov-Smirnov tests were used to determine if the data were normally distributed. Comparisons among the groups were performed with a Kruskal-Wallis test, followed by post-hoc analysis for continuous variables. The statistical package employed was SPSS v.12 (SPSS Inc., Chicago, IL, USA). A p value of less than 0.05 was considered significant.

Results

Table I displays the demographic and clinical characteristics of the study groups. There were no significant differences among the groups in gestational age at delivery. The patients with normal pregnancies delivered larger neonates at a later gestational age than the preeclampsia group. All patients with preeclampsia were diagnosed with severe preeclampsia according to the diagnostic criteria previously noted.
Maternal serum PGH results

Patients with preeclampsia had a significantly higher maternal serum concentration of PGH than women with normal pregnancies (preeclampsia: median 23,076 pg/mL, range 3,473-94,256 vs. normal pregnancy: median 12,157 pg/mL, range: 2,617-34,016; \( p < 0.05 \)). The median PGH concentration in the maternal serum of patients with preeclampsia and SGA was significantly lower than those with preeclampsia group (preeclampsia+SGA: median 11,027 pg/mL, range 1232-61,702; \( p < 0.05 \)), but comparable to the median PGH concentration of the normal pregnancy group (\( p > 0.05 \)). Patients with SGA alone had a significantly lower concentration of PGH in maternal serum (SGA alone: median 10,206 pg/mL, range 1816-34,705; \( p < 0.05 \)) than those with preeclampsia. While a trend was observed for the median serum concentration of PGH in the SGA alone group to be lower than the median serum concentration in both the normal pregnancy and preeclampsia+SGA groups, the difference was not significant (\( p > 0.05 \)) (Figure 1).

Umbilical cord serum PGH results

PGH was measurable in all samples of umbilical cord blood. The median concentration of PGH in umbilical cord serum from neonates of women with preeclampsia was higher than the concentration detected in the case of normal pregnancies (preeclampsia: median 356.1 pg/mL, range 72.6-20,946 vs. normal pregnancy: median 128.5 pg/mL; range 21.6-255.9; \( p < 0.01 \)). (Figure 2)

The median birth weight of those of normal pregnancies was greater than neonates of women with preeclampsia (normal pregnancy: median 3425 g, range 2850-3910 vs. preeclampsia: median 3105 g, range 1960-3780; \( p = 0.2 \)). Of the 16 neonates from women with preeclampsia four were SGA (median 2170 g, range 1960-2400).

Discussion

Principal findings of this study

(1) Patients with preeclampsia had a significantly higher concentration of PGH in both the maternal and fetal serum. (2) The maternal serum concentration of PGH in pregnancies complicated by both preeclampsia and SGA, as well as SGA alone, was significantly lower than in the case of pregnancies with preeclampsia alone. However, there were no significant differences in serum PGH concentration between normal pregnancies and those with preeclampsia and SGA, or SGA alone. (3) Contrary to previous reports, PGH is detectable in cord blood. Furthermore, the concentration of PGH in cord blood is also significantly higher in patients with severe preeclampsia.

What is PGH?

PGH was first described in 1985 as a product of the growth hormone variant (GH-V) gene [27]. GH-V belongs to a family of five highly related genes, located on chromosome 17, q22-24, which demonstrate 91-99% structural homology [28]. This family includes GH, GH-V, and three placental lactogens, or chorionic somatomammotropins (hCS-A, hCS-B, and hCS-L) [29]. PGH is a 22 kD polypeptide structural variant of pituitary growth hormone and differs from GH by 13 amino acids. In 1989, Liebhaber et al. demonstrated that unlike GH, GH-V mRNA is expressed in the placenta, but not in the pituitary [14]. Except for GH, all genes in this family are expressed by the placenta.

In situ hybridization studies originally localized the GH-V transcript to the syncytiotrophoblast [14,15,30]. Unlike the homogenous expression of placental lactogen, GH-V yielded “dense but highly dispersed spots” [14]. GH-V mRNA has been detected in the placenta as early as 9 weeks of gestation [14]. Recently, LaCroix et al. demonstrated the effect of PGH on EVCT
invasiveness using an *in vitro* model, as well as its expression by EVCT. While both GH and PGH stimulated EVCT to invade in a dose-dependent manner, PGH did so more effectively and to a greater extent. Furthermore, EVCT expressed PGH as they acquired a more invasive phenotype [1] This suggests a direct role of PGH in trophoblast invasion.

**PGH in angiogenesis**

PGH has also been implicated in angiogenesis. In *in vitro* and *in vivo* models, Struman et al. describe both the pro-angiogenic and anti-angiogenic functions of the growth hormone family [31]. Specifically, intact PGH increased blood vessel formation while its 16-kDa fragment prevented angiogenesis. The exact function of PGH in angiogenesis has yet to be described, but this data supports a role for PGH in the development of pathologic pregnancies, as both preeclampsia and SGA have been associated with an imbalance between pro- and anti-angiogenic factors [32-63].

**Metabolic effects of PGH**

PGH demonstrates somatotrophic effects, as is observed with GH. Transgenic mice expressing GH-V showed a 40-90% increase in growth over controls [13], similar to the effect of mice compared to expressing GH. PGH, however, has a lower lactogenic effect than GH, and preferentially binds to the somatogen receptor [11,12]. Overall, PGH affects all target cells that express the GH receptor [67]. In *in vitro* studies utilizing the adipose tissue of rats, Goodman et al. demonstrated that PGH binds to adipocytes and engenders a lipolytic response [10]. In addition, transgenic mice models and *in vitro* studies have suggested that PGH is likely to play a major role in the insulin resistance normally seen in pregnancy, thus increasing the amount of substrate available to the fetus [8,69]. However, these effects have not yet been confirmed in human *in vivo* studies.

**PGH and fetal growth**

Based on the observations that maternal serum concentrations of PGH and insulin-like growth factor-1 (IGF-1) are strongly correlated throughout gestation, it has been proposed that PGH influences fetal growth through IGF-1 [2,4,6,18]. Moreover, the role of PGH and IGF-1 in fetal growth is supported by investigations of patients with GH deficiency. These pregnancies generally proceed normally [71], with increasing concentrations of both PGH and IGF-1 akin to controls, and result in average weight neonates [72,73]. But while the expression and basic physiology of PGH has been described, its regulation is not currently understood.

**PGH in normal pregnancies**

In longitudinal studies, Chellakooty et al. demonstrated that PGH is detectable in maternal serum as early as the fifth week of gestation, and increases to peak at 34-37 weeks [3,4]. As PGH production increases, it gradually replaces GH as the main form of growth hormone, to the point where GH becomes undetectable in maternal serum by the third trimester [20,21, 27]. Of interest, an association between the time at which PGH maternal serum concentration peaks and the interval to delivery has been reported. Specifically, the earlier the peak PGH concentration was reached, the earlier labor began [3]. However, the cohort of patients observed, all delivered at greater than 37 weeks of gestation. At this time, no studies have been performed specifically to investigate any relationship between PGH peak concentrations and delivery at less than 37 weeks of gestation.

Though there is a wide inter-individual variation of maternal serum PGH concentrations among patients at any given gestational age, the individual’s increase in PGH is positively associated with fetal growth [4]. Furthermore, patients who experienced a greater fall in maternal serum PGH concentration after the peak level delivered smaller neonates [4]. Following delivery,
maternal serum PGH values rapidly decline. With a half-life of approximately 15 minutes [20], 75% of PGH is cleared from maternal serum within 30 minutes after delivery.

PGH in fetal growth restriction and preeclampsia

Given the observations asserting the association between PGH and fetal growth, the majority of studies concerning PGH in pathologic pregnancies have focused on fetal growth restriction (FGR). Mirlesse et al first investigated this association measuring PGH in maternal serum from 22 cases of FGR after 31 weeks of gestation [21]. The cases were characterized as idiopathic (n=14), in conjunction with preeclampsia (n=6), chromosomal (n=1), and materno-fetal infection (n=1). When compared with 31 normal pregnancies, the PGH concentration in maternal serum at term was significantly lower in patients with FGR. These results were supported by those of Caufriez et al. in a cross-sectional study including 80 patients with disorders of the ‘fetoplacental unit.’ Within this group were 32 pregnant women with FGR and 42 patients who experienced preterm contractions. The concentration of PGH in patients with disorders of the ‘fetoplacental unit’ was significantly lower (p=0.003) than in the case of the control group (n=93)[81]. More recently, McIntyre et al. reported a significant reduction of maternal serum PGH in 16 cases of FGR, when compared with 23 controls [6].

The observation that severe preeclampsia is associated with a significantly higher maternal serum plasma concentration of PGH than normal pregnancies is novel and suggests that PGH may participate in the mechanism of disease of preeclampsia. These results are consistent with two previous reports indicating that patients destined to develop preeclampsia have significantly higher maternal serum concentrations of PGH in the mid-trimester than those with normal pregnancies. It is possible that the high maternal serum plasma concentration of PGH observed in women with preeclampsia may be a compensatory mechanism to preserve fetal growth. Failure of this mechanism may result in the delivery of an SGA neonate, as indicated by the observation that patients with preeclampsia and SGA had a lower maternal serum concentration of PGH than patients with preeclampsia alone. Alternatively, the lower maternal serum concentration of PGH may be a reflection of a lower placental mass that is frequently associated with FGR.

PGH in the fetal circulation

The observation that PGH was detected in all cord blood samples is novel. Prior investigations have suggested that PGH is secreted by the placenta only into the maternal circulation and, therefore, acts indirectly to affect fetal growth [20]. In this study, not only have we shown that PGH is measurable in cord blood, but also that the concentration is increased in pregnancies affected by preeclampsia, which is in concordance with the results in maternal serum in preeclampsia. Original studies assessing the presence of PGH in the fetal circulation utilized a sensitive solid-phase immunoradiometric assay (Biocode, Liege, Belgium), whose lowest limit of detection was 0.4 ng/mL. In contrast, the highly sensitive enzyme-linked immunosorbent chemiluminiscent assay used in this investigation has a lowest limit of detection of 0.01 ng/mL, and is not affected by the presence of growth hormone binding protein [22], a potential confounder in earlier assays. Given this original finding, the current understanding of PGH physiology and function in the fetus needs to be re-evaluated.

Conclusions—The concentration of PGH in maternal serum is significantly increased in patients with preeclampsia who delivered infants with appropriate growth for gestational age, compared to normal pregnancies. In contrast, patients with preeclampsia delivering SGA infants have a decreased concentration of PGH compared with preeclampsia alone. This finding supports the relationship between PGH and fetal growth, while suggesting an increase of PGH production in preeclampsia in order to enable continued fetal growth. In light of the finding that PGH does enter the fetal circulation and is not limited to the maternal circulation, the
physiological role and effect of PGH on fetal growth needs to be re-examined. Further prospective clinical and histological studies will clarify the role of PGH in preeclampsia and fetal development.

Acknowledgements

This research was supported by the Intramural Research Program of the National Institute of Child Health and Human Development, NIH, DHHS.

Reference List


34. Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Mee KY, Goncalves LF, Gomez R, Edwin S. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the


Figure 1.
Maternal serum placental growth hormone (PGH) concentrations among the study groups. Patients with severe preeclampsia had a significantly higher median maternal serum PGH concentration than women with normal pregnancies (severe preeclampsia: median 23076 pg/mL; range 3473-94,256 vs. normal pregnancy: median 12157 pg/mL; range: 2617-34016; p<0.05), women with preeclampsia + small for gestational age (median: 11,027.3 pg/mL; range 1232-61,702; p<0.05), as well as women with pregnancies complicated by SGA alone (median 10,206 pg/mL; range: 1816-34,705; p<0.05). Multiple comparisons among normal pregnancy, preeclampsia + SGA, and SGA showed no significant differences in median maternal serum PGH concentration among these groups.
Figure 2.
Umbilical cord blood median placental growth hormone (PGH) concentrations from normal pregnant women and women with severe preeclampsia. The median umbilical cord serum concentration of PGH was significantly higher in pregnancies complicated by severe preeclampsia when compared to normal pregnancies (preeclampsia: median 356.1 pg/mL; range 72.6-20,946 vs. normal pregnancy, median: 128.5 pg/mL; range 21.6-255.9; p<0.01).
Table I
Demographic and clinical characteristics of the study groups

<table>
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<th>Normal pregnancy (n=61)</th>
<th>Severe preeclampsia (n=48)</th>
<th>PE+SGA (n=30)</th>
<th>SGA (n=41)</th>
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<td>Maternal age (years)</td>
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<td>27 (16 - 40)</td>
<td>23 (16 - 43)</td>
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<tr>
<td></td>
<td>25.5 (16.3 – 51.6)</td>
<td>26.3 (18.3 – 44.5)</td>
<td>24.9 (14 - 36)</td>
<td>25.9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 (16.3 – 51.6)</td>
<td>26.3 (18.3 – 44.5)</td>
<td>24.9 (14 - 36)</td>
<td>25.9</td>
<td>NS</td>
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<td>Nulliparity</td>
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<td>20 (11/48)</td>
<td>26 (9/30)</td>
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<td>NS</td>
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<td>16.7 (5/30)</td>
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<td>Gestational age at blood draw (weeks)</td>
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<td>32.6</td>
<td>NS</td>
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<td>Gestational age at delivery (weeks)</td>
<td>39.6 (37 – 42)</td>
<td>33.1 (23.7-38.6)</td>
<td>33.9 (25.0-37.3)</td>
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<td>Birthweight (g)</td>
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<td>1685 (510-2500)</td>
<td>1680</td>
<td>&lt;0.05†</td>
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</table>

Values are expressed as a percentage (number) or median (range). BMI, body mass index; PE+SGA: preeclampsia with small for gestational age neonate; SGA: small for gestational age neonate; NS: not significant.

*: Kruskal-Wallis with post-hoc analysis.
†: <0.05 between severe preeclampsia and normal pregnancy, PE+SGA and normal pregnancy, and SGA compared with normal pregnancy.