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The Effect of Gestational Age and Labor on Placental Growth Hormone in Amniotic Fluid

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Abstract

Objective—Placental Growth Hormone (PGH) is produced by trophoblast. This hormone becomes detectable in maternal serum during the first trimester of pregnancy. Its concentration increases as term approaches and becomes undetectable within one hour of delivery. PGH has important biological properties, including somatogenic (growth promotion), lactogenic, and lipolytic activity. Recently, PGH has been detected in amniotic fluid (AF) of midtrimester pregnancies. The purpose of this study was to determine whether PGH concentrations in AF change with advancing gestational age and in labor.

Design—AF was assayed for PGH concentrations from samples obtained from patients undergoing genetic amniocentesis between 14 and 18 weeks of gestation (n=67), normal patients at term not in labor (n=24), and pregnant women at term in labor (n=51). PGH concentrations were determined by ELISA. Non-parametric statistics were used for analysis.

Results—1) PGH was detected in all AF samples; 2) patients in the midtrimester had a higher median concentration of PGH in AF than those at term (midtrimester: median: 3140.5 pg/ml; range: 1124.2–13886.5 vs. term: median: 2021.1 pg/ml; range: 181.6–8640.8; p < 0.01); 3) there was no difference in the median concentration of PGH between women at term, not in labor, and those in labor (term not in labor: median: 2113.4 pg/ml; range: 449.3–8640.8 vs. term in labor: median: 2004.1 pg/ml; range: 181.6–8531.5; p=0.73).

Conclusions—1) PGH is detectable in AF at both mid- and third trimesters; 2) the median AF concentration of PGH is significantly lower at term when compared to the second trimester; 3) labor at term is not associated with changes in the AF concentration of PGH. The role of this unique placental hormone now found in the fetal compartment requires investigation.

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Keywords

Placental growth hormone; amniotic fluid; gestational age; labor

Introduction

Placental growth hormone (PGH) is a member of the highly homologous growth hormone family [GH, PGH, human chorionic somatotropin (hCS)-A, hCS-B, and hCS-C],¹ and is closely related to pituitary growth hormone (GH), differing by only 13 amino acids.² PGH exerts somatotrophic, lactotrophic, and lipolytic effects in pregnancy similar to that of GH in non-pregnant women,^{3,4,5} but preferentially binds somatogen receptors.^{6,7} It has been proposed to play a role in fetal growth,^{8,9,10,11,12} placental development¹³ and maternal adaptation to pregnancy^{14,15,16} in order to increase substrate availability to the conceptus. This is achieved through different mechanisms including increased GLUT1 mediated glucose transport, and a role in increasing insulin resistance during pregnancy has been proposed. Several studies have reported significantly lower maternal serum PGH concentrations in pathologic conditions such as small-for-gestational-age (SGA) neonates, compared to normal pregnant women,^{8,17,12,18} while pregnancies with chromosomal anomalies are associated with significantly increased maternal serum PGH concentrations, as opposed to normal pregnancies.¹⁹

PGH is only known to be produced by syncytiotrophoblast and extravillous cytotrophoblast.^{20,21,22} During pregnancy, the maternal serum concentration of PGH increases with gestational age and gradually replaces GH as the predominant growth hormone in maternal blood by 20 weeks of gestation.² Consequently, GH is undetectable in maternal serum during the second half of pregnancy.^{23,24} During labor, however, there is conflicting evidence regarding PGH concentrations in maternal serum. Both an abrupt decrease¹⁸ and no changes^{11,25} have been reported. Thus, the relationship between maternal serum PGH concentrations and labor remains unclear.

Of interest, since PGH had not been previously detected in amniotic fluid, it was believed to be secreted exclusively into the maternal compartment. However, a recent study has reported the detection of PGH in amniotic fluid during the midtrimester.²⁶ This evidence has laid the groundwork for further evaluation and description of PGH in amniotic fluid. Thus, the aims of this study were to determine: 1) if there are changes in the amniotic fluid concentration of PGH with advancing gestational age; and 2) if the amniotic fluid concentration of PGH changes with the spontaneous onset of labor.

Material and Methods

Study population

A cross-sectional study was performed by searching our clinical database and bank of biological samples. This study included normal pregnant women in the following groups: 1) midtrimester (n=67); 2) term not in labor (n=24); and 3) spontaneous labor at term (n=51). Normal pregnancy was defined as no obstetrical, medical, or surgical complications of pregnancy, and delivery of a term neonate with appropriate birthweight for gestational age.^{27,28} Exclusion criteria included preterm delivery (<37 weeks of gestation), abnormal fetal karyotype, fetal anomaly, multiple gestation, and chronic maternal medical illness.

Amniotic fluid samples in the midtrimester were obtained from patients enrolled at the Pennsylvania Hospital in Philadelphia, Pennsylvania. Amniotic fluid samples at term were obtained from patients enrolled at the Sotero del Rio Hospital in Puente Alto, Chile. All women

provided written informed consent prior to the collection of amniotic fluid samples. The collection and utilization of 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 Committees of the participating institutions. Many of these samples have been used to study the biology of inflammation, hemostasis, growth factor concentrations, and angiogenesis regulation in normal pregnant women, as well as in those with complicated pregnancies.

Sample collection and placental growth hormone assay

Transabdominal amniocenteses were performed under ultrasonographic guidance for chromosomal analysis in midtrimester patients (14 to 18 weeks) who delivered normal neonates at term, as well as to determine fetal lung maturity in patients approaching term. All patients had intact membranes at the time of fluid collection. Amniotic fluid was centrifuged for 10 minutes at 4°C and then stored at -70°C until assayed.

Amniotic fluid PGH concentrations were determined using specific and sensitive immunoassays obtained from Diagnostics Systems Laboratories, Inc. (Webster, TX, USA). Briefly, standards and unknown amniotic fluid samples were incubated in duplicate wells of the micro titer plates, which have been pre-coated with PGH antibodies. During this incubation, PGH present in the standards or amniotic fluid samples were immobilized by the pre-coated antibodies (form 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 micro titer 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 micro titer plate spectrophotometer (SpectraMax M2 micro plate workstation, Molecular Devices, Sunnyvale, CA). The concentrations of PGH in amniotic fluid 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.

Statistical analysis

The Kolmogorov-Smirnov test was used to determine if the data was normally distributed. Comparisons between groups were performed using Chi-square or Fisher's exact test for categorical variables, as well as Mann-Whitney U test for continuous variables. A p-value <0.05 was considered statistically significant. The statistical package used was SPSS v.12.0 (SPSS Inc., Chicago, IL, USA).

Results

The demographic and clinical characteristics of the study groups are displayed in Table I. No significant differences were found in the gestational age at delivery or birthweight among groups.

PGH was detectable in all samples of amniotic fluid. The median amniotic fluid PGH concentration in midtrimester patients was significantly higher than those at term not in labor (median: 3,140 pg/mL, range 1,124-13,886 vs. median: 2,113 pg/mL, range 449-8,640, respectively; p=0.004) (Figure 1).

No significant differences were observed in the median amniotic fluid PGH concentrations in patients at term in labor, compared to those at term not in labor (median: 2,004 pg/mL; range 181-8531 vs. median: 2,113 pg/mL, range 449-8,640, respectively; $p=0.73$) (Figure 2).

Discussion

Principal findings of this study

1) Placental growth hormone was detectable in all samples of amniotic fluid; 2) the amniotic fluid concentration of placental growth hormone decreases with advancing gestational age; and 3) the concentration of placental growth hormone in amniotic fluid from patients at term did not significantly change in the presence of spontaneous labor.

Maternal serum PGH concentrations in normal pregnancy

PGH has been identified in maternal serum as early as the 5th week of gestation.¹⁰ It gradually replaces GH as the main form of growth hormone in maternal serum by 20 weeks of gestation^{29,23,24,2,18} and, unlike GH, is secreted in a non-pulsatile manner.³⁰ Conflicting evidence has been reported regarding the effect of labor on maternal serum PGH concentration. Mirlesse et al¹⁸ reported an abrupt decrease in PGH with the onset of labor. However, recent studies have described no differences in maternal serum PGH concentrations between patients in the late third trimester and those in labor.^{11,25} In addition, PGH concentration in maternal serum falls abruptly after delivery of the placenta. The half-life of PGH is approximately 15 minutes,^{23,24,25} resulting in the clearance of 75% of PGH from maternal serum within 30 minutes after delivery.²⁵

Maternal serum PGH concentrations in pregnancy complications

Given the growth promoting activity of PGH, many studies have focused on evaluating maternal serum PGH concentrations in mothers who deliver SGA neonates. In a retrospective study, Mirlesse et al¹⁸ measured the PGH concentrations in maternal serum of 22 pregnancies for which the diagnosis of SGA was made after 31 weeks. When compared to 31 normal pregnancies at the same gestational age, the maternal serum PGH concentration was significantly lower in the SGA group. Subsequent studies have confirmed this observation.^{8, 12}

Limited evidence has been reported concerning the association between maternal serum PGH concentrations and preeclampsia. However, there is evidence that patients who subsequently develop either preeclampsia¹⁹ or preeclampsia with SGA²⁶ had an increased maternal serum concentration of PGH in the midtrimester.

Proteohormones in amniotic fluid

The findings reported herein that the amniotic fluid PGH concentrations decrease with advancing gestational age, and that no significant changes are observed in the amniotic fluid PGH concentration at term in the presence of labor, are novel. In addition, PGH was identified in all amniotic fluid samples, which is in agreement with a recent study reporting the identification of PGH in amniotic fluid. Papadopoulou et al²⁶ retrospectively analyzed gestational-age-matched pairs of maternal serum and amniotic fluid samples obtained between 16 and 22 weeks in patients who subsequently developed preeclampsia and SGA. Compared to controls, the amniotic fluid and maternal serum PGH concentrations were significantly higher in patients who subsequently developed preeclampsia and SGA in the index pregnancy.

The pattern of PGH concentration in amniotic fluid differs from the pattern of the same hormone in maternal serum, which has been shown to increase with gestation, reaching peak concentrations between 34 and 37 weeks of gestation.^{9,10} Other similar polypeptide hormones,

such as prolactin and GH, also exhibit a lower amniotic fluid concentration at term when compared to the second trimester.^{31,32} The dichotomy between low amniotic fluid and high maternal serum concentrations of PGH may be explained by the same mechanisms described for other proteohormones. In 1978, Riddick et al³³ demonstrated the novel finding of *de novo* prolactin production by the decidua capsularis. It has been proposed that this peripheral site of prolactin production is the major contributor to prolactin concentration in amniotic fluid. The presence of GH in amniotic fluid is also attributed to production outside the maternal pituitary gland. In fact, GH in amniotic fluid is derived almost solely from the fetal pituitary gland.^{31,32} The decrease in concentration of these hormones with advancing gestational age has been proposed to be due to fetal swallowing, the predominant method of protein clearance in amniotic fluid.^{32,34,35}

How PGH reaches the amniotic cavity and the mechanisms involved in its clearance are matters for further investigation. Currently, the only described sites of PGH production are the syncytiotrophoblast and extravillous cytotrophoblast,^{20,21,22} and no active or facilitated transport mechanisms for PGH into the fetal compartment have been described. Therefore, as in the case of prolactin, one could suggest that another site of PGH production exists within the fetal compartment.

Conclusions

1) The amniotic fluid concentration of PGH decreases with advancing gestational age; and 2) Spontaneous labor at term does not affect the PGH concentration in the amniotic fluid. These findings are novel, and differ from those reported in maternal serum concentration of PGH. Lack of knowledge concerning direct or indirect transport of PGH from its known sites of production to the amniotic cavity indicates that an unidentified site of PGH production should be considered.

Further investigation concerning the production and function of this integral pregnancy-specific hormone is needed.

Acknowledgments

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Figure 1.

Median placental growth hormone concentration in the amniotic fluid of patients at midtrimester (14-18 weeks of gestation) and of patients at term, not in labor. The median amniotic fluid concentration of placental growth hormone of midtrimester patients was significantly higher than patients at term, not in labor (median 3140 pg/mL; range 1124-13,886 vs. median 2113 pg/mL; range 449.26-8640; $p=0.004$). **PGH:** placental growth hormone.

Figure 2.

Placental growth hormone concentrations in the amniotic fluid of term patients not in labor, and term patients in labor. Labor was not associated with a change in median amniotic fluid placental growth hormone concentration (term in labor, median: 2,004 pg/mL; range 181-8,531 vs. term not in labor, median: 2,113 pg/mL; range 449-8,640; $p=0.73$). **PGH**: placental growth hormone; **NS**: not significant.

Table 1
Demographic and clinical characteristics of the study groups

	Midtrimester (n=67)	Term not in labor (n=24)	p [*]	Term in labor (n=51)	p [†]
Maternal age (years)	36 (24-42)	28 (17-40)	<0.001	23 (16-37)	0.01
Parity	1 (0-3)	1 (0-7)	NS	1 (0-3)	NS
Gestational age at amniocentesis (weeks)	16.0 (14-18)	39.2 (37-40)	<0.001	39.3 (37-42)	NS
Gestational age at delivery (weeks)	39.0 (37-41)	39.3 (38-42)	NS	39.3 (37-42)	NS
Birthweight (grams)	3377 (2809-4180)	3400 (2810-4530)	NS	3235 (2540-4440)	NS

Values are expressed as percentage (number) or median (range).

BMI: body mass index; NS: not significant.

p^{*}: Comparison between patients in the midtrimester and those at term not in labor.

p[†]: Comparison between patients at term not in labor and those in labor at term.