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Growth hormone binding protein and maternal body mass index in relation to placental growth hormone and insulin requirements during pregnancy in type 1 diabetic women

Jens Fuglsang [a,*], Finn Friis Lauszus [b], Sanne Fisker [c], Allan Flyvbjerg [c], Per Ovesen [a]

[a] *Gynecological/Obstetrical Research Laboratory Y, Aarhus University Hospital, Skejby Sygehus, DK-8200 Aarhus N, Denmark*
[b] *Department of Gynecology and Obstetrics, Holstebro County Hospital, DK-7500 Holstebro, Denmark*
[c] *Medical Research Laboratories, Aarhus University Hospital, Aarhus Kommune Hospital, DK-8000 Aarhus C, Denmark*

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Abstract

In pregnancy, the growth hormone axis is shifted from pituitary growth hormone (GH) to placental growth hormone (PGH). Their common binding protein, GH binding protein (GHBP), displays peak serum levels at mid-gestation in normal individuals. In the non-pregnant state, diabetes is known to be associated with elevated levels of GH and decreased levels of insulin-like growth factors (IGFs) and GHBP. Diabetes in pregnancy may therefore as well be associated with disturbances in the growth hormone axis. In the present study, we aimed at investigating the impact of GHBP and maternal body mass index (BMI) on levels of PGH, thereby enabling estimation of any association between free PGH and weight adjusted insulin requirements.

In 51 type 1 diabetic women, blood samples were collected in gestational week 10+, 16+, 22+, 28+ and 34+, and analysed for their serum content of GHBP, PGH, and GH.

Serum GHBP increased from the first weeks of pregnancy to median 2.07 nmol/l (range 1.17–4.26) in week 22+, then declined to median 1.29 nmol/l (range 0.77–2.35) in week 34+ (ANOVA $P < 0.001$). Serum PGH levels were highest in week 34+ at median 21.3 μg/l (range 5.1–165.4) ($P < 0.001$), whereas a steady decrease in GH values was observed throughout pregnancy to a median 0.17 μg/l (range 0–5.53). The fraction of calculated free PGH to total PGH increased from mid-gestation onwards to 55.2% (37.0–87.1) in week 34+ at a median level of free PGH of 10.4 μg/l (range 1.9–144.0) ($P < 0.001$). Similarly, the molar ratio of total PGH to GHBP increased to a maximum of 0.68 (0.12–6.62) in week 34+. As in normal pregnancies, the correlation between BMI and GHBP was lost in late pregnancy. The newborns birth weight *z*-score correlated with total PGH and derivatives hereof in week 34+. Neither total nor weight adjusted insulin requirements correlated to total PGH, calculated free PGH, nor GHBP.

In conclusion, PGH and GHBP display a similar course during pregnancy in type 1 diabetic women as described in normal women. The well-known association between GHBP and BMI was lost in late pregnancy. Calculated levels of free PGH were positively associated to fetal growth, but not to maternal insulin requirements.

Keywords: Human placental growth hormone; GHBP; Pregnancy; Birth weight; Insulin

1. Introduction

Placental growth hormone (PGH) replaces pituitary GH with advancing gestational age [1,2]. The physiological role of PGH has not been fully identified, but due to an amino acid sequence and tertiary structure highly resembling GH, similar effects have been postulated, but with PGH having less lactogenic properties [3]. Like GH, PGH appears to be involved in the regulation of the serum levels of insulin-like growth factors (IGFs) [2,4], and serum PGH is positively correlated to the birth

* Corresponding author. Tel.: +45 8949 6398; fax: +45 8949 6373.
E-mail address: Fuglsang@ki.au.dk (J. Fuglsang).

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weights of the newborns [4,5]. In the circulation, PGH is transported to GH-binding protein (GHBP) [6], and the amount of GHBP determines the concentration of free and bound PGH. Free PGH is expected to be the biologically active form of PGH. In contrast to GH, PGH displays a more continuous secretory profile [7,8] and this allows for a sustained effect of PGH on peripheral tissues. Furthermore, GHBP levels decline during late gestation in normal pregnancies [9], thereby further interfering in the balance between free and bound PGH.

Advancing gestation induces insulin resistance. In pregnant type 1 diabetic women insulin requirements change with the gestational age. Often a slight decrease is observed in the first trimester, in contrast to a steep increase in insulin requirements from mid-gestation onwards [10,11], and levels of PGH in serum parallels the changes in insulin requirements in the last half of pregnancy. The diabetogenic properties of GH are well known, and some studies have pointed towards diabetogenic properties of PGH as well. Rodents transgenic for human PGH become large and hyperinsulinaemic [12], and insulin receptors and glucose transport mechanisms in skeletal muscle are affected in these animals [13]. Similarly, in diabetic women postprandial glucose levels correlate to PGH levels [5]. In contrast, mean clinic glucose or fasting glucose measurements were not correlated to serum PGH [5], and in type 1 diabetic pregnancies, no significant correlations were found between daily insulin requirements and serum total PGH levels [11].

The present study is an extension of a previous study in type 1 diabetic women, in whom aspects of the PGH–IGF-axis have been reported recently [11]. Here, we investigate the impact of maternal BMI and GHBP on PGH levels, allowing for an estimation of free PGH levels. In this way, we are able to compare the bioactive form of PGH with maternal insulin requirements and foetal growth.

2. Materials and methods

Blood samples were available from 51 pregnant type 1 diabetic women, who were referred to the outpatient maternity ward at Aarhus University Hospital, Skejby Hospital.

All women had pregestational type 1 diabetes. One woman received antithyroid substitution therapy, and before pregnancy two women received an angiotensin converting enzyme inhibitor, which was discontinued at the onset of pregnancy. Two women had microalbuminuria (30–300 mg protein/24 h) before pregnancy, and further three were diagnosed at first visit in the first trimester. A detailed description of the cohort has been given elsewhere together with data for the longitudinal course of serum total PGH, total IGFs and total insulin requirements during pregnancy [11].

Participants were seen at regular interval throughout pregnancy. Until gestational week 30, visits were every second week, thereafter weekly, but with respect to individual needs. To represent the course of GHBP during pregnancy, blood samples were selected at 6 weeks intervals from gestational week 10 to 11 (denoted week 10+), week 16 to 17 (16+), week 22 to 23 (22+), week 28 to 29 (28+) and week 34 to 35 (34+). In case of more than one blood sample per selected time period, the first was chosen.

In contrast to GH, PGH appears to be secreted in a rather continuous fashion [8]. Therefore, non-fasting blood samples were considered informative for PGH secretion.

With this approach, 190 serum samples were available from the 51 type 1 diabetic women in the cohort. A median of 4 (range 2–5) blood samples from each patient were available.

Patient characteristics, HbA1c measurements and insulin requirements were retrieved from journal records, as were data for body weight (BW) whenever possible. If applicable, a weight was estimated from weight recordings at two adjacent visits in the out-patients clinic assuming a linear relationship.

2.1. Blood sample analysis

All hormone analyses were performed in duplicate, and all samples from the same subject were analysed within the same run of a hormone assay. Serum PGH was determined using a commercially available solid phase immunoradiometric assay (PGH IRMA, BC1017, Biocode, Liege, Belgium). In our hands the intra- and interassay variation coefficients (CV) were <4% and <6%, respectively, and the detection limit is lower than 0.4 μg/l.

Serum total GH was measured by Delfia TRIFMA specific to the 22 kDa pituitary GH (Wallac, Turku, Finland). The detection limit is lower than 0.03 μg/l and the intra- and interassay variation is 2% and 3.2%, respectively. An in-house immunofunctional assay was used for determination of serum total GHBP [14]. This assay has an intra-assay CV <4% and an interassay CV of 12% at 0.56 nM and 6.3% at 1.40 nM.

2.2. Calculations and statistics

Approximated values for the serum levels of free growth hormone were calculated according to the law of mass action as exemplified by Barsano and Baumann [15]. A molecular weight of GH of 22000 and non-glycosylated PGH of 22000 Da [16,17] and an affinity constant (K_a) of GHBP of 0.91 l/nmol [18] were assumed to facilitate calculations. Finally, a stoichiometric ratio of 1:1 for GH or PGH binding to GHBP at serum levels of GHBP [19] was assumed, meaning that serum GH