CLINICAL STUDY

Growth hormone isoforms in newborns and postpartum women

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Abstract

The neonatal and postpartum periods are characterized by alterations in pituitary GH secretion. We have investigated the proportion of circulating non-22 kDa GH isoforms in newborns, in women within the early postpartum phase (just after the disappearance of placental GH from the maternal circulation) and in women during late postpartum (during the somatotroph recovery phase). We studied 10 newborns (7 males; 3 females; median postnatal age, 45 h), who had been admitted because of polycythaemia, 10 women in the early postpartum phase (median, 48 h after delivery; range, 42–54 h), 18 women in the late postpartum phase (median, 10 weeks after delivery; range, 3–25 weeks) and 9 healthy non-pregnant women. The proportion of non-22 kDa GH isoforms was determined by the 22 kDa GH exclusion assay, which is based on immunomagnetic extraction of 22 kDa GH from serum, and quantitation of non-22 kDa GH isoforms using a polyclonal GH assay. In newborns, non-22 kDa GH isoforms were measured in two arterial blood samples obtained with a 5–6 h interval. In the other groups, serum samples were obtained 40 min after an i.v. bolus administration of the GH secretagogue, GH releasing peptide-1 (GHRP-1).

In newborns, the median proportion of non-22 kDa GH isoforms was 10% (range, 7.2–19.4%) and the values were similar in samples collected at different times. In early postpartum women, total GH levels after GHRP-1 were lower and the proportion of non-22 kDa GH isoforms was higher compared with the values in non-pregnant and late-postpartum women. In late postpartum, there was a partial recovery of GH response to GHRP-1, as shown by an increment in total GH levels, which was associated with a decrease in the fraction of non-22 kDa GH isoforms.

In conclusion, we found that (i) the proportion of non-22 kDa GH isoforms in the newborn is comparable to that in the adult (non-pregnant women), (ii) in early postpartum, the non-22 kDa fraction is high within the small pool of readily releasable GH, (iii) in late postpartum, recovery of pituitary GH responsiveness is associated with a relative decrease in the release of non-22 kDa GH isoforms.

European Journal of Endocrinology 142 353–358

Introduction

Human growth hormone (GH) consists of a complex mixture of several structurally modified isoforms and fragments that have been detected in blood, pituitary extracts and placenta (for reviews, see 1–3), in addition to the main 22 kilodalton (kDa) GH. The presence of circulating non-22 kDa GH isoforms and fragments contributes to the discrepancies in GH measurements, since the free monomeric 22 kDa GH accounts for only about 25% of the GH immunoreactivity in serum (2). The physiological role of multiple GH isoforms is not

clear, but this mixture of molecules may intermodulate their activities by acting as partial agonist or antagonists of the GH receptor (3–5). In addition, it has been suggested that some GH isoforms might act through specific receptors (3, 6, 7).

The GH heterogeneity arises from different sources, one of which is the presence of two related genes in the human GH gene cluster with tissue-specific expression: the GH-N gene in the anterior pituitary and the GH-V gene in the placenta (8–10). The pituitary secretion of GH is pulsatile and regulated mainly by two hypothalamic peptides, GH-releasing hormone (GHRH)

and somatostatin (SRIH). During pregnancy, the pulsatile release of GH from the pituitary is progressively suppressed and replaced by a continuous secretion of placental GH into the maternal circulation (11–14). Consequently, the somatotrophs are less responsive to GH secretagogues, such as GHRH, in the early postpartum phase, with a gradual recovery of pituitary GH secretion observed at the late postpartum phase (15–17).

In the fetus, circulating GH levels fall towards the end of gestation, while there is an amplification of GH secretion immediately after birth (18, 19). Accordingly, the neonatal period is characterized by a physiological hypersecretion of GH. Using a panel of site-specific monoclonal antibodies, King *et al.* (20) found that 30 to 60% of the circulating GH during the first three months of life represents the 20 kDa GH, an isoform produced by the use of an alternative splice site within exon 3 of the GH-N (21) and GH-V gene (9). However, this finding has not been confirmed by recent studies where 20 kDa GH was measured by specific ELISA and showed a lower proportion of this isoform in cord blood and in serum samples collected at early postnatal life (22, 23).

We have developed the 22 kDa GH exclusion assay (GHEA), in which the 22 kDa GH isoform is extracted from serum and the levels of non-22 kDa GH isoforms, which include 20 kDa GH and other immunoreactive isoforms, fragments and oligomers of GH, are quantitated (24). The GHEA has been conceived as a screening method for application in a large number of clinical samples, allowing the identification of an abnormal proportion of non-22 kDa GH isoforms in different physiological and pathological conditions.

In the present study, we have applied the GHEA to evaluate the proportion of circulating non-22 kDa GH isoforms in periods of life characterized by alterations in pituitary GH secretion: (i) the neonatal period, when there is a physiological hypersecretion of GH; (ii) the early postpartum phase, just after the disappearance of placental GH from the maternal circulation; and (iii) the late postpartum phase, when somatotroph function seems to recover.

Subjects and methods

Neonatal samples

Ten newborns (7 males and 3 females), who had been admitted to the neonatal care unit because of symptoms related to polycythaemia, were studied as previously reported (19). None of the newborns was in severe distress. The range of the gestational ages was 32 to 38 weeks (median, 37.5 weeks), of postnatal ages 3 to 95 h (median, 45 h), and of birthweight 1030 to 2490 g (median, 2100 g). Blood samples (1 ml/kg) were obtained every 20 min for 6 h through an umbilical or peripheral arterial catheter during a standardized

partial exchange transfusion. During the procedure, the infants received an isotonic plasma protein solution and continuous 10% glucose infusion. They were not fed, but were allowed nonnutritive suckling.

Blood was collected into glass tubes; after clotting at 4 °C and centrifugation, the serum was kept frozen at –20 °C until analysis. The total GH and non-22 kDa GH concentrations were measured by the GHEA in two distinct stored samples from the same infant, one collected at the start of the partial exchange transfusion (sample A) and the other collected after a 5–6 h interval (sample B). All samples were assessed in the same assay run. As blood samples were obtained during a therapeutic procedure and would otherwise have been discarded, the study protocol was not submitted for institutional review and parental consent was not requested.

Maternal samples

A total of 37 women, age range 24–40 years, was studied. The control group consisted of healthy non-pregnant women ($n=9$), who were either in the late follicular stage of a spontaneous cycle or between days 11 and 21 of a cycle timed by low dose oestrogen/progestagen contraceptive medication at the time of the study. The study groups consisted of lactating women ($n=10$) in the early postpartum phase (median, 48 h after delivery; range, 42–54 h), and lactating and nonlactating women ($n=18$) in the late postpartum phase (median, 10 weeks after delivery; range, 3–25 weeks).

The samples were obtained as previously described (17). Briefly, an indwelling venous catheter was inserted approximately 20 min before the start of the sampling. Blood was collected every 20 min, from 20 min before until 100 min after an i.v. bolus administration of 100 µg GH releasing peptide-1 (GHRP-1) (Ala-His-Dβ-Nal-Ala-D-Trp-Phe-Lys-NH₂), which was provided by Dr CY Bowers. All studies were started between 08.00 and 10.00 h, at least 2 h after a light breakfast. In the groups of lactating mothers, the last breastfeeding before the study was initiated approximately 90 min (± 30 min) before bolus injection.

The total GH and non-22 kDa GH concentrations were measured in stored samples collected 40 min after GHRP-1 administration, representing the samples obtained at or just after the occurrence of the GH peak. All samples were assessed in the same assay run. The study protocol was approved by the Ethics Committee of the University of Leuven Medical School and all subjects gave their informed consent.

GH exclusion assay (GHEA)

The 22 kDa GHEA, which has been described previously, is an immunomagnetic extraction-based assay for serum measurement of non-22 kDa GH isoforms (24).

Briefly, a 100 μ l aliquot of serum is incubated with an anti-22 kDa GH monoclonal antibody (MCB, Genentech Inc., South San Francisco, CA, USA). Paramagnetic beads coated with rat anti-mouse immunoglobulin G, and a magnet device (Dynal, Oslo, Norway) are used to remove monomeric and dimeric 22 kDa GH from serum. After extraction, the non-22 kDa GH levels are measured by a polyclonal antibody-based GH-IRMA (Pharmacia & Upjohn, Uppsala, Sweden). In the GHEA, total GH concentrations are determined in another 100 μ l aliquot of serum incubated only with assay buffer (without the addition of the MCB), and non-22 kDa GH levels are expressed as a percentage of total GH in the samples. There is no influence of storage, freezing and thawing of the samples in the measurements of non-22 kDa GH levels by the GHEA (24).

Statistical analysis

All the descriptive statistical results are presented as the median and range. Comparisons between groups were carried out using the Mann–Whitney U-test. Correlations were sought by calculating the Spearman rank correlation coefficient. A *P* value less than 0.05 was considered significant.

Results

Neonatal samples

The median total GH concentrations in neonatal samples A and B were 13.5 μ g/l (range, 3.7 to 68.1 μ g/l) and 13.7 μ g/l (range, 4.7 to 50.0 μ g/l) respectively, with a median proportion of non-22 kDa GH isoforms in sample A of 9.7% (range, 7.2 to 19.4%) and in sample B of 10.5% (range, 7.5 to 16.3%) (Table 1). The overall median proportion of non-22 kDa

GH isoforms in all neonatal samples (*n* = 20) was 10.1%. The percentage of non-22 kDa GH isoforms was fairly stable in the two samples of the same newborn collected at different times, with a median intra-individual variation of 1.6% (range, 0.1 to 7.8%), whereas the median intra-individual variation of total GH levels was 7.8 μ g/l (range, 0.4 to 58.1 μ g/l).

Maternal samples

Figure 1 depicts total GH concentrations and the percentage of non-22 kDa GH isoforms in the three study groups. In the control group of non-pregnant women, the median total GH concentration after GHRP-1 administration was 49.5 μ g/l (range, 17.5 to 77.3 μ g/l) and the median proportion of non-22 kDa GH isoforms was 10.7% (range, 7.9 to 11.1%). In postpartum women, there was an opposite pattern of total GH and non-22 kDa GH isoforms in the circulation during early and late phases. The pituitary was less responsive to the GH secretagogue at the early postpartum phase, as shown by significantly lower median total GH concentrations after GHRP-1 stimulation in this group (median 8.6 μ g/l; range, 2.7 to 25.7 μ g/l), compared with that in non-pregnant women (*P* < 0.001). At the late postpartum period, the pituitary GH secretion after GHRP-1 was partly recovered, with the median total GH concentration higher than that in the early postpartum phase (median 19.2 μ g/l; range, 4.6 to 81.0 μ g/l; *P* = 0.01), but still significantly lower than the median value seen in the control group (*P* = 0.02).

In contrast to the total GH concentration, the median proportion of non-22 kDa GH isoforms after GHRP-1 was significantly higher at the early postpartum phase (median 14.2%; range 8.7 to 18.2%) compared with the percentage in non-pregnant women (*P* = 0.003). At the late postpartum phase, the median proportion of non-22 kDa GH isoforms after GHRP-1 was 8.1% (range, 4.3 to 11.9%), which was significantly lower than the value in the control group (*P* < 0.01) and in the early postpartum phase (*P* < 0.01).

Discussion

The pituitary secretion of GH in newborns is intermittent and distinctly pulsatile, indicating that the regulatory system of GHRH and SRIH is operational by the first day of postnatal life. A characteristic finding of the immediate postnatal period is that plasma concentrations of GH are markedly elevated (19). This elevation could be explained by several factors, including a higher sensitivity of somatotrophs to GHRH than to SRIH, an amplifying effect on GH secretion caused by oestrogens of placental origin or by a neonatal surge in thyroid hormones, a fall in serum levels of insulin-like growth factor-I within hours after birth, and a physiological decrease in nutrient supply (19, 25, 26).

Table 1 Total GH concentrations and proportion of non-22 kDa GH isoforms measured by the 22 kDa GHEA in two arterial samples of the same newborn collected with a 5–6 h interval during a therapeutic transfusion.

Newborn	Total GH (μ g/l)		Non-22 kDa GH (%)	
	Sample A	Sample B	Sample A	Sample B
1	8.6	5.8	9.2	9.1
2	44.2	13.5	17.8	16.3
3	55.2	30.0	7.2	12.3
4	8.0	20.5	19.4	11.6
5	13.5	13.9	11.0	8.4
6	68.1	10.0	8.9	10.6
7	26.3	50.0	11.7	12.6
8	12.7	9.7	9.4	7.5
9	3.7	4.7	7.2	7.8
10	24.3	22.7	9.9	10.3
Median	13.5	13.7	9.7	10.5

Figure 1 Total GH concentrations (left panel) and the proportion of non-22 kDa GH isoforms (right panel), as measured by the 22 kDa GHEA, in 9 non-pregnant women (NP), 10 early postpartum women (EP) and 18 late-postpartum women (LP). The GHEA was applied to samples collected 40 min after administration of an i.v. bolus of GHRP-1. The box-and-whisker plots indicate median, lower and upper quartiles and the whiskers indicate the 1st and 99th percentiles. The mean values are depicted as solid circles in each box plot. In the left panel, $P < 0.001$ EP vs NP, $P = 0.01$ EP vs LP, and $P = 0.02$ LP vs NP; in the right panel, $P = 0.003$ EP vs NP, $P < 0.01$ LP vs EP and $P < 0.01$ LP vs NP.

The neonatal hypersomatotrophism is not due to a decreased clearance of GH, since the half-life of GH in the newborn has been shown to be around 18 min, identical to that in children and adults (19). It is known that 20 kDa GH and oligomeric forms of GH are cleared more slowly from blood than is 22 kDa GH (27, 28). Thus, one would expect a prolonged half-life of circulating GH in newborns if the proportion of 20 kDa GH isoforms was as high as 60% of total GH, as previously reported (20). Our results showed that hypersecretion of GH in polycythaemic newborns was not associated with increased amounts of non-22 kDa GH isoforms, which represented in the present study 10% of the total GH in the circulation. Although the newborns in the present study were not completely healthy, our results are in accordance with two other recent studies where only 20 kDa GH was measured by a direct ELISA in cord blood samples and samples collected from preterm and full-term neonates, showing a mean percentage of 20 kDa GH varying from 2.7 to 10.3% of total circulating GH (22, 23). The fraction of non-22 kDa GH isoforms in our newborns was similar to that in our group of non-pregnant women, despite marked differences in total GH concentrations between the groups. In addition, the neonatal values were also comparable to those reported by us in a group of healthy children at different stages of puberty (29). Hence, the normal proportion of circulating non-22 kDa GH isoforms in our study is in agreement with the previously reported half-life of GH in newborns (19). However, higher amounts of non-22 kDa GH isoforms may be present in the circulation of newborns in some circumstances, as it has been shown that serum 20 kDa GH

levels are increased in cord blood of neonates with low birth weight (22).

During pregnancy, serum levels of placental GH rise in association with a fall in serum GH levels of pituitary origin (11, 30). The physiological postpartum phases are characterized by the gradual return of the pulsatile pituitary GH secretion. At early postpartum, the somatotrophs are less responsive to GHRH, with some recovery seen at the late postpartum phase (16). The present study has shown that the same pattern of GH hyporesponsiveness is found in postpartum women through the GHRP pathway. Interestingly, non-22 kDa GH isoforms were present in increased proportion in the early postpartum period, when somatotrophs were less responsive to GHRP-1 and total GH levels were low. At the late postpartum period, the opposite was found, with a partial recovery of the somatotrophic response leading to an elevation of total GH concentrations associated with a lower fraction of non-22 kDa GH isoforms in the circulation. It has been demonstrated that suppression of pituitary GH synthesis during gestation is associated with a reduced number of GH immunoreactive cells in the adenohypophysis, which are, in turn, less abundant in GH messenger ribonucleic acid (31). These observations indicate that the somatotrophs are not fully recovered during the first days after delivery, and that their low GH secretory capacity is associated with increased production of non-22 kDa GH isoforms. At late postpartum, there is a tendency towards normalization of pituitary GH secretion, not only in terms of responsiveness of the somatotrophs to GHRP-1, but also regarding the ratio of GH isoforms secreted by the gland. In fact, the

recovery of pituitary secretion during the late postpartum stage is characterized by a marked decrease in the pituitary release of non-22 kDa GH isoforms and an enhanced production of the predominant 22 kDa GH. Although it is not clear at this moment if this distinct GH secretory pattern has physiological significance in postpartum women, there are previous *in vitro* and *in vivo* studies showing that GH and its isoforms have different binding affinities to somatogenic and lactogenic receptors and that GH might exert some influence on human lactation (32–35).

Acknowledgements

The authors are grateful to the Genentech Inc. for the gift of the MCB monoclonal antibody, to Dr CY Bowers (Tulane University, New Orleans, USA) for the generous gift of GHRP-1, to the Staff of the Neonatal Intensive Care Unit and the Postpartum Ward of the University Hospital Gasthuisberg (Leuven) for their co-operation in the organization of the study, to Danielle Lemmens, RN and Karin Vanweser, RN (Leuven) for sample collection and editorial support, and to Ulla Karlsson (Göteborg) for technical assistance. This work was supported by grants from the Swedish Medical Research Society (N° 11285, 11502, 13141). Francis de Zegher is a Clinical Research Investigator of the Fund for Scientific Research, Flanders, Belgium.

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Received 6 July 1999

Accepted 13 December 1999