Calcium-regulating hormones and parathyroid hormone-related peptide in normal human pregnancy and postpartum: a longitudinal study

M Salleh M Ardawi, Hassan A N Nasrat, Hasan S BA’Aqueel

Departments of Clinical Biochemistry and Obstetrics and Gynaecology, College of Medicine and King Abdulaziz University Hospital, King Abdulaziz University, PO Box 9029, Jeddah 21413, Saudi Arabia

(Correspondence should be addressed to M S M Ardawi, PO Box 9029, Jeddah 21413, Saudi Arabia)

Abstract

Objectives: To evaluate calcium-regulating hormones and parathyroid hormone-related peptide (PTHrP) in normal human pregnancy and postpartum in women not deficient in vitamin D.

Design: A prospective longitudinal study was conducted in pregnant Saudi women during the course of pregnancy (n = 40), at term and 6 weeks postpartum (n = 18). Maternal concentrations of serum calcidiol and calcitriol were determined, together with those of serum intact-parathyroid hormone (PTH), PTHrP, calcitonin, osteocalcin, human placental lactogen (hPL), prolactin, vitamin D binding protein, alkaline phosphatase, calcium, phosphate and magnesium. A group of non-pregnant women (n = 280) were included for comparative purposes.

Results: The calcidiol concentrations decreased (mean±S.D.) significantly from 54±10 nmol/l in the first trimester to 33±8 nmol/l in the third trimester (P < 0.001) and remained decreased at term and postpartum (both P < 0.001). The calcitriol concentration increased through pregnancy, from 69±17 pmol/l in the first trimester to 333±83 pmol/l at term (P < 0.001). Intact-PTH concentrations increased from 1.31±0.25 pmol/l in the first trimester to 2.26±0.39 pmol/l in the second trimester, but then declined to values of the first trimester and increased significantly postpartum (4.02±0.36 pmol/l) (P < 0.001). PTHrP concentration increased through pregnancy from 0.81±0.12 pmol/l in the first trimester to 2.01±0.22 pmol/l at term and continued its increase postpartum (2.63±0.15 pmol/l) (P < 0.001). Significant positive correlations were evident between PTHrP and alkaline phosphatase up to term (r = 0.05, P < 0.001) and between PTHrP and calcitriol (r = 0.46, P < 0.001), osteocalcin (r = 0.23, P < 0.05) and prolactin (r = 0.41, P < 0.05) during pregnancy. Osteocalcin started to increase from 0.13±0.01 nmol/l in the second trimester, through pregnancy and postpartum (P < 0.001). Calcitonin was increased more than twofold by the second trimester compared with the first trimester (P < 0.001) and subsequently decreased (P < 0.001). Prolactin concentrations were significantly greater in the second trimester compared with values before pregnancy (P < 0.001). hPL increased throughout the course of pregnancy, reaching a maximum at term (7.61±2.57 mU/ml). There was no direct correlation between serum calcitriol concentrations during pregnancy and serum prolactin (r = −0.12, P < 0.19) or serum hPL (r = 0.17, P < 0.21). Significant changes were observed in the serum concentrations of calcium and phosphate, but not in that of magnesium, during the course of pregnancy; calcium concentrations showed a maximal decrease at term.

Conclusions: Changes in serum PTHrP during the course of pregnancy, at term and postpartum have been demonstrated, suggesting that the placenta (during pregnancy) and mammary glands (postpartum) are the main sources of PTHrP. No support for the concept of 'physiological hyperparathyroidism' of pregnancy could be demonstrated in the present work. The pregnancy-induced increase in calcitriol concentration may thus be the primary mediator of the changes in maternal calcium metabolism, but the involvement of other factors cannot be excluded.

European Journal of Endocrinology 137 402–409

Introduction

During pregnancy, the requirement of the growing fetus for calcium generally results in profound changes in maternal calcium homeostasis to allow for the active transport of calcium across the placenta (1). Similarly, lactation requires the active transport of large quantities of calcium for the production of milk (2). The exact regulatory mechanisms behind calcium homeostasis in pregnancy and lactation require further study, but several factors are known to be involved in maintaining a balanced relationship between the large pool of...
calcium in the skeleton and the much smaller pool in the extracellular fluid. Among these factors, in addition to calcium itself, are other related minerals such as magnesium and phosphate, and hormones such as calcitriol and parathyroid hormone (PTH) (3, 4). Other hormones such as calcitonin, oestrogen, growth hormone and prolactin are also known to affect calcium homeostasis (1, 5–7). They are involved directly or indirectly to increase the formation of calcitriol and might thus have a role in calcium homeostasis, particularly during pregnancy and lactation.

Recent studies indicated that the PTH-related peptide (PTHrP), which is believed to be responsible for humoral hypercalcaemia of malignancy (8, 9), may also regulate fetal calcium homeostasis, uterine contraction and fetal tissue development and thus have a physiological role in regulating the transport of calcium from the maternal to the fetal circulation (10–12). Moreover, very high concentrations of PTHrP have been found in human and other mammalian milk and evidence now exists to suggest that PTHrP is the principal factor involved in the transfer of calcium from the mammary glands into milk (13, 14). More recently, increases in the plasma concentrations of PTHrP associated with lactation have been described, even though its concentration was undetectable in maternal blood during the course of pregnancy (15).

There are few well-designed longitudinal studies that have examined the alterations in calcium metabolism during the course of pregnancy and postpartum, among them, the study by Gallacher et al. (16). In addition, reports on calcium-regulating hormones are either from pregnancy or from lactation, but not from the same women during pregnancy and lactation (4).

The main objectives of the present study were to evaluate, in a group of women who were not vitamin D-deficient, but who were from an ethnic group (Asian) different than that reported previously (16), the changes in calcitropic hormones (calcitriol, PTH and calcitonin), osteocalcin and PTHrP, in relation to calcium and other minerals, that occurred through the course of pregnancy, at term and 6 weeks postpartum. In addition, the relationships between prolactin, human placental lactogen (hPL) and calcitropic hormones were also evaluated. The results are discussed in relation to calcium homeostasis during pregnancy and postpartum.

### Subjects and methods

#### Subjects

A total of 40 pregnant Saudi women living in the Jeddah area participated in the present study. All had resided in the area for more than 4 years and were recruited randomly from those attending antenatal clinics at King Abdulaziz University Hospital, Jeddah. Women with hepatic, renal or evident endocrine disorders, and those with a history of immunosuppressive therapy or disorders of vitamin D or calcium were excluded from the study. The ages and anthropometric data of the women, together with pregnancy outcomes, are presented in Table 1. The group was studied at presentation to the antenatal clinics (range 8–14 weeks gestation; mean ± s.d. 11.4 ± 1.43 weeks); at the second trimester (17–27 weeks; mean 22.0 ± 2.78 weeks), the third trimester (29–35 weeks; mean 31.4 ± 1.72 weeks), at term (36–42 weeks; mean 39.7 ± 1.29 weeks) and 6 weeks after delivery. At the first appointment, all these pregnant women had a general physical and obstetric examination and measurements of their height and weight were made. In addition, they each underwent an ultrasound scan in order to confirm the pregnancy and assess fetal age and maturity.

At each visit and at delivery, blood samples were collected for the measurement of calcidiol, calcitriol, intact-PTH, PTHrP, calcitonin, hPL, prolactin, osteocalcin, calcium, phosphate, magnesium, vitamin D-binding protein (DBP) and albumin.

Babies were examined at birth and thereafter daily for craniotabes, hypotonia and signs of tetany or any other abnormality.

A total of 280 non-pregnant Saudi women who were randomly selected also participated in the study, as a reference population for comparative purposes. They were healthy and were not lactating and had not been lactating during the previous 2 years, were not pregnant and had not been pregnant during the previous 2 years, were not using oral contraceptives, and had regular menstrual cycles. The mean (± s.d.) age was 27.8 ± 5.3 years and mean body mass index 23.19 ± 2.88 kg/m².

#### Methods

Samples of maternal and cord blood and from non-pregnant women were collected as indicated above and immediately transferred to the laboratory, where sera were separated by centrifugation. Collected sera were divided into multiple aliquots and stored at −130°C until required for analysis. All assays and determinations were performed in batches, to eliminate variability within assays.

### Table 1 Maternal age, body mass index, gravida, week of delivery, fetal birthweight, fetal length and fetal head circumference of pregnant women. Values are means ± s.d. for 40 pregnant women at delivery.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>26.78 ± 5.82</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.56 ± 3.62</td>
</tr>
<tr>
<td>Gravida</td>
<td>4.25 ± 2.79</td>
</tr>
<tr>
<td>Week of delivery</td>
<td>39.65 ± 1.29</td>
</tr>
<tr>
<td>Fetal birthweight (g)</td>
<td>3392 ± 434</td>
</tr>
<tr>
<td>Fetal length (cm)</td>
<td>51.68 ± 3.40</td>
</tr>
<tr>
<td>Fetal head circumference (cm)</td>
<td>34.48 ± 2.05</td>
</tr>
</tbody>
</table>
Determination of calcidiol  Calcidiol (25(OH)D₃) was determined by a modified competitive protein-binding assay (Bullman Laboratories, AG, Postfach, Allschwil, Switzerland). The assay is based on competition between unlabelled 25(OH)D₃ and ³H-labelled 25(OH)D₃ (0.5 µCi) for binding to the 25(OH)D₃-binding protein from rat serum (17). Unbound ligand was removed by absorption with dextran-coated charcoal and the protein–ligand complex remaining in the supernatant was decanted and the radioactivity measured by liquid scintillation spectrometry (LKB 1211 rack-βeta scintillation Counter, Wallacoy, Turku-10, Finland). The concentration of calcidiol in the samples was determined from a calibration curve constructed using a range of standard solutions of calcidiol and was inversely related to the amount of labelled calcidiol that was bound. A purification single-step solvent extraction procedure with a Sep-Pak C₁₈ reverse-phase cartridge was included (17). To check for possible underestimations, controls were monitored, in which known quantities of calcidiol were added to serum samples. The assays of such controls resulted in good quantitative recoveries (85–92% for calcidiol). The inter- and intra-assay coefficients of variation (CV) were 9.6% and 6.5% respectively. The method described had a sensitivity of 2.5 ng/ml (6.25 nmol/l) for detecting calcidiol.

Determination of calcitriol, intact-PTH, PTHrP, calcitonin, osteocalcin, prolactin and hPL  Calcitriol (1,25(OH)₂D₃) was determined by competitive protein receptor-binding assay using the high specificity and avidity of a rachitic chick intestinal receptor protein—receptor-binding assay using the high specificity and avidity of a rachitic chick intestinal receptor protein with dextran-coated charcoal and the protein–ligand complex remaining in the supernatant was decanted and the radioactivity measured by liquid scintillation spectrometry (LKB 1211 rack-βeta scintillation Counter, Wallacoy, Turku-10, Finland). The concentration of calcitriol in the samples was determined from a calibration curve constructed using a range of standard solutions of calcitriol and was inversely related to the amount of labelled calcitriol that was bound. A purification single-step solvent extraction procedure with a Sep-Pak C₁₈ reverse-phase cartridge was included (17). To check for possible underestimations, controls were monitored, in which known quantities of calcitriol were added to serum samples. The assays of such controls resulted in good quantitative recoveries (85–92% for calcitriol). The inter- and intra-assay coefficients of variation (CV) were 9.6% and 6.5% respectively. The method described had a sensitivity of about 0.04 IU/ml.

Statistical analysis  Results are presented as means±S.D. Data were analysed using the SPSS-statistical package (SPSS Inc., Microsoft Corp., Chicago, IL, USA). Results that were not normally distributed were log-transformed before analysis. ANOVA was used to examine differences among the groups for different variables, and the Bonferroni criterion was used when multiple significance tests were made. Correlations were carried out using regression analysis.

Results  A total of 40 Saudi women were studied longitudinally during the course of pregnancy, with successful delivery of their neonates. Relevant clinical and anthropometric data on the mothers and neonates are presented in Table 1. None of the babies delivered showed any form of neonatal complication or morbidity.

The serum concentrations of maternal calcidiol, calcitriol and osteocalcin during the course of pregnancy, at term and 6 weeks postpartum are presented in Fig. 1. The mean serum calcidiol concentration decreased significantly, from 54±10 nmol/l (mean±S.D.) in the first trimester to 33±8 nmol/l by the third
trimester and remained decreased at term (35 ± 11 nmol/l) and postpartum (33 ± 8 nmol/l). In contrast, the mean serum concentrations of calcitriol exhibited proportional increases during the course of pregnancy and reached a maximum at term (69 ± 17 pmol/l in the first trimester, 333 ± 83 pmol/l at term), but then declined postpartum (232 ± 76 pmol/l) to concentrations similar to those present during the third trimester values. In the pregnant women, the maternal serum calcidiol concentrations were correlated positively with serum calcitriol (r = 0.52, P < 0.001), serum PTHrP (r = 0.51, P < 0.001), serum calcium (r = 0.23, P < 0.001) and serum magnesium (r = 0.62, P < 0.001), and negatively with intact-PTH (r = −0.62, P < 0.001). The serum concentration of osteocalcin was slightly decreased during the second trimester (0.13 ± 0.01 nmol/l) compared with the first trimester values (0.16 ± 0.02 nmol/l), but then increased significantly, by 59.8% over the second trimester values (P < 0.001), and remained increased at term and 6 weeks postpartum (P < 0.001; Fig. 1).
Figure 2 shows the maternal concentration of serum intact-PTH, PTHrP and calcitonin in these pregnant women. Serum intact-PTH increased during the second trimester (2.26 ± 0.39 pmol/l) compared with the first trimester values (1.31 ± 0.25 pmol/l), but then declined to values markedly lower than those of the second trimester, reaching values at term (1.86 ± 0.87 pmol/l) that were similar to those of the first trimester. However, intact-PTH concentrations showed a significant increase 6 weeks postpartum (P < 0.001) compared with other values during pregnancy (Fig. 2). While the pattern of change of intact-PTH and osteocalcin through pregnancy was similar, a weak correlation was evident between these two parameters (r = 0.29, P < 0.05).

Among the non-pregnant women, PTHrP was non-detectable in 95 (33.9%) subjects and very low in the remainder (0.45 ± 0.28 pmol/l) compared with values measured during pregnancy or postpartum. Among the pregnant women, PTHrP concentrations increased gradually, from 0.81 ± 0.12 pmol/l in the first trimester to 2.01 ± 0.22 pmol/l at term, and had increased further at 6 weeks postpartum (2.63 ± 0.15 pmol/l). Five of the pregnant women had no detectable PTHrP in their sera in the first trimester. PTHrP and alkaline phosphatase were correlated significantly up to term (r = 0.51, P < 0.001), and PTHrP exhibited positive correlations during the course of pregnancy with calcitriol (r = 0.46, P < 0.001), osteocalcin (r = 0.23, P < 0.05) and prolactin (r = 0.41, P < 0.05).

Serum calcitonin was increased more than twofold by the second trimester compared with the first trimester, and had declined slightly at term. Values similar to those obtained at the second and third trimesters were also obtained 6 weeks postpartum (Fig. 2).

Prolactin concentrations were significantly higher during the second (6724 ± 1459 pmol/l) and third (8394 ± 2086 pmol/l) trimesters than before pregnancy (P < 0.001, in each case) and remained above normal postpartum (Fig. 3). hPL serum concentrations increased throughout the course of pregnancy, reaching a maximum at term (7.61 ± 2.57 mIU/ml), but decreased postpartum, to prepregnancy values (Table 2). There was no direct correlation between serum calcitriol concentrations during pregnancy and serum prolactin (r = −0.12, P < 0.19) or serum hPL (r = 0.17, P < 0.21).

Table 2 shows the maternal concentrations of serum calcium, phosphate, magnesium, alkaline phosphatase, albumin and DBP during the course of pregnancy, at term, and 6 weeks postpartum. Significant changes were observed in the serum concentrations of calcium and phosphate, but not of magnesium, during the course of pregnancy. Serum calcium showed a tendency to decrease during the course of pregnancy and reached a nadir at term (Table 2). In contrast, serum alkaline phosphatase exhibited a gradual but significant increase during the course of pregnancy, reaching a maximal value at term (163.68 ± 54.9 U/l), but then declining to 115.16 ± 26.10 U/l 6 weeks postpartum (Table 2).

Serum DBP showed a significant tendency to increase during the course of pregnancy (Table 2).

**Discussion**

There is a considerable variation in maternal serum calcidiol that does not bear any relation to the duration of pregnancy. If dietary vitamin D intake and exposure to sunlight are taken into consideration, serum concentrations of calcidiol during pregnancy do not seem to differ extensively from those of the
Maternal concentrations of serum calcium, phosphate, magnesium, alkaline phosphatase, albumin and DBP studied during the first, second and third trimesters, at term and 6 weeks postpartum. Values are means ± S.D.

<table>
<thead>
<tr>
<th>Sampling visit</th>
<th>NP (n = 280)</th>
<th>P1 (n = 40)</th>
<th>P2 (n = 40)</th>
<th>P3 (n = 40)</th>
<th>Term (n = 40)</th>
<th>PP (n = 18)</th>
<th>Probability (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>2.25 ± 0.13</td>
<td>2.38 ± 0.09*</td>
<td>2.29 ± 0.09*</td>
<td>2.20 ± 0.09*</td>
<td>2.14 ± 0.14*</td>
<td>2.20 ± 0.10*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum phosphate (mmol/l)</td>
<td>1.19 ± 0.16</td>
<td>1.36 ± 0.15*</td>
<td>1.23 ± 0.10*</td>
<td>1.16 ± 0.12*</td>
<td>1.22 ± 0.13*</td>
<td>1.25 ± 0.14*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum magnesium (mmol/l)</td>
<td>0.85 ± 0.10</td>
<td>0.80 ± 0.11</td>
<td>0.79 ± 0.06</td>
<td>0.81 ± 0.08</td>
<td>0.78 ± 0.13</td>
<td>0.83 ± 0.07</td>
<td>0.532</td>
</tr>
<tr>
<td>Serum alkaline phosphatase (U/l at 25°C)</td>
<td>92.1 ± 24.8</td>
<td>61.30 ± 20.96*</td>
<td>96.15 ± 29.74*</td>
<td>126.33 ± 34.47*</td>
<td>163.70 ± 54.90*</td>
<td>115.20 ± 26.10*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>47.11 ± 3.18</td>
<td>46.02 ± 4.52*</td>
<td>41.41 ± 3.85*</td>
<td>38.51 ± 3.40*</td>
<td>37.62 ± 3.56*</td>
<td>45.20 ± 3.60*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum DBP (g/l)</td>
<td>0.31 ± 0.08</td>
<td>0.33 ± 0.07*</td>
<td>0.37 ± 0.09*</td>
<td>0.36 ± 0.06*</td>
<td>0.37 ± 0.09*</td>
<td>0.35 ± 0.10*</td>
<td>0.010</td>
</tr>
</tbody>
</table>

n, Number of women studied. NP, non-pregnant non-lactating. P1, P2 and P3 = first, second and third trimesters of pregnancy; PP, 6 weeks postpartum; DBP, vitamin D binding protein.
* Statistically significant from corresponding controls.
postpartum (Fig. 2), the latter being consistent with the findings of Gallacher et al. (16). The increased concentrations of PTHrP postpartum could be explained by the lactation process itself (i.e. suckling), by the associated increases in prolactin concentrations, or a combination of both. Some evidence for the second of these was afforded by the positive significant correlation that we found between prolactin and PTHrP ($r = 0.41$, $P < 0.05$). Because PTHrP exhibits PTH-like effects, PTHrP may inhibit secretion of PTH during lactation (16). During pregnancy, the major source of PTHrP is the fetal parathyroid gland, although some is provided by the placenta (for review see (5)), as suggested by the close relationship, up to the time of delivery, between PTHrP and alkaline phosphatase ($r = 0.51$, $P < 0.001$), which is known to be manufactured by the placenta (29). This would be consistent with the findings of the study of Gallacher et al. (16), and it may be this particular placental component that passes into the maternal circulation and has a role in calcium homeostasis by acting through the PTH receptor (5). In addition, consistent with the work described by Grill et al. (15), the postpartum increase in PTHrP could be produced by the mammary glands in the lactating mothers.

Our finding that maternal serum calcitonin concentrations were increased during the course of pregnancy compared with the non-pregnant state (Fig. 2) is in agreement with most [e.g. (30)], but not all [e.g. (22)], previous reports.

Osteocalcin is a bone-specific protein released into circulation in relation to the rate of new bone formation (31). Several studies have demonstrated the possibility of using serum osteocalcin as a clinical index of bone turnover (32). We found that serum osteocalcin concentrations were moderately decreased at the second trimester, but increased significantly thereafter (Fig. 1), which is in general agreement with a previous study (16). Such changes are consistent with the expected changes in maternal bone turnover during the course of pregnancy: bone turnover is decreased during early pregnancy, further decreased by mid pregnancy and increased in late pregnancy, compared with the changes in early pregnancy (4).

Among our group of pregnant women, total serum calcium showed a significant tendency to decrease toward the end of pregnancy, reaching a nadir by term (Table 2). Previous studies have shown a decrease in total maternal serum calcium concentration during pregnancy (33, 34) that paralleled a concomitant decrease in serum albumin concentration. The physiological hypoalbuminaemia of pregnancy caused by haemodilution thus appears to be largely, if not entirely, responsible for this decrease.

Some previous studies [e.g. (35)] have documented lower serum magnesium and phosphate concentrations during pregnancy, and our findings of marked changes in serum phosphate concentration, but not in magnesium concentration, during the course of pregnancy (Table 2) are consistent with this. However, others have reported normal serum phosphate concentrations during pregnancy [e.g. (33)].

In conclusion, the present work shows that, in women who are not deficient in vitamin D, hormonal mechanisms during the course of pregnancy do not confirm the ‘physiological hyperparathyroidism’ reported previously; however, an increase in the concentration of intact-PTH was evident postpartum in lactating women, and was associated with a decline in serum calcitriol concentrations. The suggested roles of prolactin and hPL in the regulation of calcitriol during pregnancy could not be confirmed in the present work. Although understanding of the normal physiological roles of PTHrP in its infancy, it appears that the changes in placental and mammary gland production of PTHrP that are observed in pregnancy and postpartum are reflected by changes in the serum concentrations of PTHrP. It is not clear, however, whether such observations have a significant effect on maternal or fetal calcium homeostasis, independent of other calcitrophic hormones – mainly PTH and calcitriol.

Acknowledgements
We are grateful to King Abdulaziz University for financial support (grant No. 132/412) to Professor M S M Ardawi at the Department of Clinical Biochemistry and the Clinical Endocrine and Metabolic Research Laboratory, King Fahd Medical Research Centre, Jeddah, Saudi Arabia. We also thank Dr H AlBajori, Dr J Zaharna and Mr M AbdulGhani for their excellent technical assistance. Special thanks are due to Mrs H Khoja for her excellent secretarial help.

References