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# Calcium homeostasis in second trimester fetuses

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SUMMARY The concentrations of ionised calcium ions ($Ca^{++}$), total calcium, parathyroid hormone, pH, total protein, albumin, sodium, and potassium were measured in paired fetal and maternal blood from pregnancies at 15 to 24 weeks' gestation. Pure fetal blood samples were obtained fetoscopically.

The concentrations of fetal ionised calcium ions (n = 26); mean (SD) 1·33 (0·12) mmol/l (5·32 (0·48) mg/100 ml) and those of parathyroid hormone (n = 9); 68 (19) pmol/l (58 (16) μg/100 ml) were significantly higher than those of the mothers: 1·18 (0·09) mmol/l (4·7 (0·4) mg/100 ml), and 40 pmol/l (<34 μg/100 ml), respectively. There was no difference between measured fetal and maternal total calcium, pH, and electrolytes. The fetal total protein and albumin concentrations increased with gestation but were always lower than the equivalent maternal values. The calculated total calcium was 0·23–0·45 mmol/l (0·9—1·8 mg/100 ml) higher in the fetal than in maternal blood from the same pregnancy. There were no fetal arteriovenous differences in ionised calcium ions despite higher venous pH.

Calcium metabolism in the pregnant woman and her fetus is a complex process with several intricate and interrelated components.[1] As long ago as 1923, Boget and Plass reported that the total serum calcium in the fetus was higher than that of the mother.[2] This observation was subsequently confirmed and elaborated on by other investigators, who found that both total and ionised calcium were higher in the fetus.[3–6] Data on parathyroid hormone concentrations in the fetus are contradictory, being reported as either low,[7] normal,[8] or high.[9] Reports on calcitonin concentrations are sparse and equally contradictory.[6]

All existing data, however, are derived from studies on animals, or in man, from blood samples obtained from the umbilical cord at the time of delivery. The technique of fetoscopy, which is used for the diagnosis and treatment of various fetal conditions,[10] has given us the unique opportunity to obtain pure fetal blood from second trimester pregnancies (as early as 15 weeks' gestation) and to study the calcium homeostasis in the unstressed normal fetus in utero.

## Patients and methods

Fetal and maternal blood, 1 ml and 5 ml, respectively, were obtained from 36 pregnancies undergoing diagnostic fetoscopy at 15 to 24 weeks' gestation. The data used for the determination of the reference values were derived from pregnancies in which the diagnostic outcome was normal. Pure fetal blood samples were obtained by umbilical cord puncture under direct vision through an Olympus Selfoscope that was introduced transabdominally into the amniotic cavity.[11–13] Either arterial or venous blood was taken; in five cases paired arterial and venous samples were obtained. One millilitre of fetal blood was sufficient for the diagnostic investigation, and the additional sample required for this study did not increase the fetal risk. The maternal samples were obtained from the antecubital vein without venous stasis.

The samples were collected in sodium heparin saturated with calcium chloride (580 heparin, Radiometer Ltd) to a final concentration of 17–50 IU/ml. Whole blood was analysed immediately for ionised calcium and pH, by using an ion selective electrode (ICA 1 Radiometer, Cohenhagen). The samples were then centrifuged at 500 *g* for 10 minutes and the plasma analysed within four hours of collection. The maintenance schedule for the electrode system included a daily calibration with aqueous standards CAL 1 and CAL 2 (Radiometer Ltd) and a one point calibration with CAL 1 before introducing the samples. The sensitivity of the calcium electrode was accepted when the theoretical Nerst response was between 0·90 and 1·05. The pH electrode was very stable and showed no drift.

Quality control for the ICA 1 Radiometer was performed using aqueous controls, Qualicheck Low and

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Table 1 *Methods and performance of assays*

| *Analyte* | *Method* | *Mean (SD)* |
|---|---|---|
| Ionised calcium (mmol/l) | Ion selective electrode | 1·10 (0·001) |
| Total calcium (mmol/l) | Cresophthalein complexonate | 2·65 (0·03) |
| Sodium (mmol/l) | Flame photometry | 131 (1·7) |
| Potassium (mmol/l) | Flame photometry | 4·0 (0·1) |
| Total protein g/l | Biuret | 54 (1·10) |
| Albumin g/l | Turbimetry | 34 (0·90) |
| pH | Ion selective electrode | 7·45 (0·004) |

High (Radiometer Ltd) propriety and external quality assurance was achieved by participation in the three monthly external quality control scheme run by Radiometer Ltd. Total calcium, total protein, sodium, and potassium were determined by analysis of the plasma on a multichannel continuous flow analyser (SMAC, Technicon, Basingstoke). Table 1 shows the methods used and the performance obtained. The albumin was measured on a CobasBio centrifugal analyser (Roche Diagnostica, Welwyn Garden City, Hertfordshire, United Kingdom). The accuracy of the methods was monitored by comparing the results of the UK External Quality Assurance Scheme (UKEQAS) with the mean value obtained by the assay.

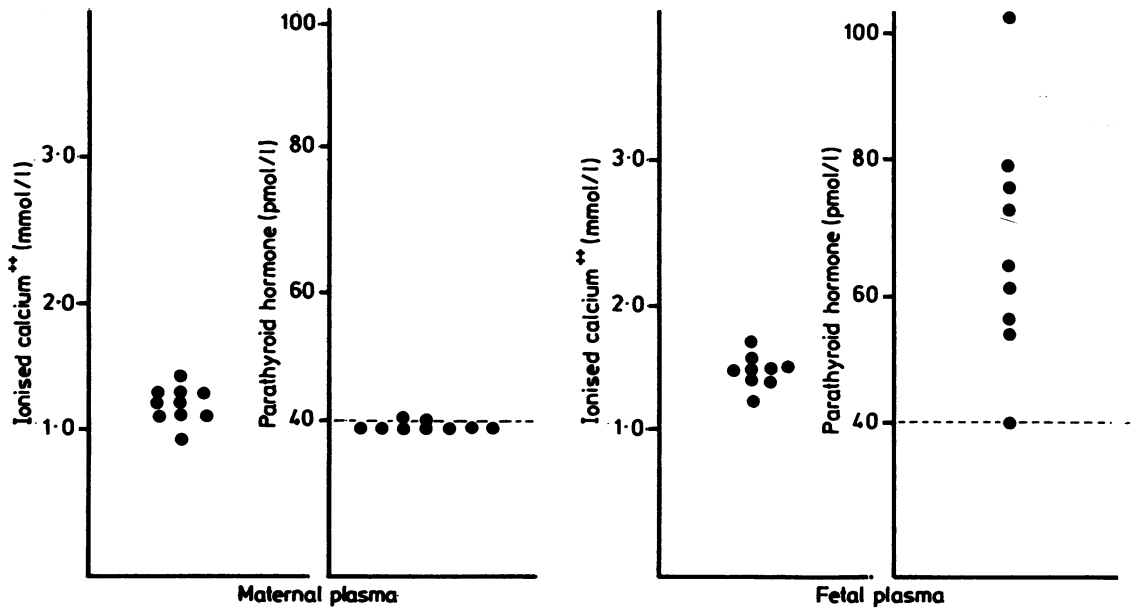
In nine cases parathyroid hormone was assayed using a mid molecule assay (PTH—MM, Immunonuclear Inc), in which the antibody is sensitive to the 46–68 amino acid region of human parathyroid hormone. The standards are calibrated with several reference preparations, including World Health Organisation 79/500. The coefficient of variation between and within assay was never more than 8·9%.

Table 2 *Concentration of analytes in fetal and maternal blood*

| *Analyte* | *Maternal blood* | *Fetal blood* |
|---|---|---|
| | *Mean (SD)* | *Mean (SD)* |
| Ionised calcium (mmol/l) | 1·18 (0·09) | 1·33 (0·12) |
| Total calcium (mmol/l) | 2·17 (0·08) | 2·27 (0·13) |
| Sodium (mmol/l) | 135 (1·3) | 135 (1·7) |
| Potassium (mmol/l) | 3·5 (0·9) | 3·4 (1·0) |
| Total protein g/l | 62 (3·00) | 34 (3·50) |
| Albumin g/l | 34 (2·60) | 19 (3·70) |
| pH | 7·41 (0·03) | 7·38 (0·04) |

## Results

Table 2 shows the fetal and maternal blood concentrations (mean (SD)) of total calcium, ionised calcium, total protein, albumin, sodium, and potassium, and the figure shows the individual values of ionised calcium and parathyroid hormone. There was no difference between the sodium or potassium concentrations of the two compartments. Of all the substances measured, the only one that showed a significant change with gestation in the gestational range studied, was albumin in fetal blood (albumin = 0·97 gestation + 0·79; correlation coefficient = 0·57,




*Concentrations of ionised calcium ($CA^{++}$) and parathyroid hormone (PTH) in fetal and maternal plasma. Detection limit of immunoreactive porathyroid hormone.*

Table 3 *Calcium and pH values in umbilical artery and vein*

| *Blood sample* | *Total calcium (mmol/l)* | *Ionised calcium (mmol/l)* | *Ph* |
|---|---|---|---|
| Umbilical vein | 2·27 (0·19) | 1·34 (0·11) | 7·36 (0·05) |
| Umbilical artery | 2·30 (0·16) | 1·34 (0·10) | 7·31 (0·05) |
| Maternal vein | 2·20 (0·09) | 1·20 (0·04) | 7·34 (0·04) |

$p < 0·005$).

Although the ionised calcium was higher in fetal than in maternal blood from the same pregnancy (Student's paired $t$ test; $t = 2·7$, $p < 0·05$), there was no significant difference in the measured total calcium (Table 2). The calculated total calcium, however (taking into account the lower fetal albumin), was 0·23–0·45 mmol/l (0·9–1·8 mg/100 ml) higher in fetal than in maternal blood from the same pregnancy. The calculated ionised calcium, using the algorithm of MacCleans and Hastings,[14] which incorporates the measured total calcium, pH, and proteins was also higher in the fetal compartment, indicating that fetal plasma proteins have similar binding characteristics to adult plasma proteins.

Table 3 shows the ionised calcium and pH in the five paired umbilical arterial and venous samples. Although the venous pH was higher (Student's paired $t$ test; $t = 8·7$, $p < 0·001$), there was no difference between measured ionised calcium ($t = 0·75$, not significant).

The fetal parathyroid hormone was higher than that of the mothers's (Student's paired $t$ test; $t = 3·4$, $p < 0·05$).

## Discussion

In this study a normal range of variables relating to calcium homeostasis was established in samples obtained fetoscopically from fetuses in the second trimester of pregnancy. The results confirm the findings of experiments on animals and previous studies on samples obtained at the time of delivery.[4 5 14 15] Thus in both the second and third trimesters of intrauterine life the fetus exhibits relative hypercalcaemia. Moreover, by measuring the protein and electrolyte concentrations in fetal and maternal blood, we showed that there was good agreement between the measured and derived ionised calcium concentrations in the two compartments.

Studies on animals suggest that the placental transfer of calcium entails both active and passive processes[16 17] and results in the net intrauterine accumulation of 25–30 g, which is mostly incorporated in the fetal skeleton. We found no fetal arteriovenous difference between the measured ionised calcium, but when the correction for the pH gradient, which exists between the two vessels, was incorporated, the venous samples were hypercalcaemic, indicating a net placental transfer of calcium to the umbilical venous blood of 0·1 mmol/l (0·14 mg/100 ml).

In contrast to calcium, parathyroid hormone does not cross the placenta.[18 19] The fetal parathyroid gland contains detectable hormone from 10 weeks onwards.[20] Our findings of paradoxically high fetal parathyroid hormone values, despite relative hypercalcaemia, support the hypothesis that the release of hormones in utero is regulated by some means other than that of the calcium concentration.[21] Indeed, discrepant results between bioactive and immunoreactive parathyroid hormone in cord blood[22] suggest a changed structure and function correlation for this hormone in fetal life when compared with that of adults. Experiments on animals, however, have shown a prompt fetal parathyroid hormone response to hypocalcaemia.[23 24] It is therefore more likely that the negative feedback mechanism regulating secretion of parathyroid hormone in utero is set at a higher calcium concentration than in postnatal life.

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