Serum and Urinary Divalent Cations and Plasma Renin Activity in Women with Mild Pregnancy-Induced Hypertension

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Summary
Serum concentrations and urinary output of calcium, magnesium, sodium and potassium were analysed in normotensive pregnant women and in women with pregnancy-induced hypertension during the third trimester. In addition, plasma renin activity (PRA) was also determined. Significantly lower serum total calcium, urinary calcium and magnesium excretions and plasma renin activity were evident in women with PIH. Urine output and creatinine clearance were not significantly different between the two groups. No significant correlation was evident between serum calcium, magnesium and PRA. The relationship between these parameters and high blood pressure is not immediately apparent. They nevertheless suggest a disturbance in electrolyte metabolism in women with PIH, that may underly the pathogenesis of this disorder.

Key Words: PIH, Calcium, Magnesium, Plasma renin activity

Introduction
Numerous epidemiological studies have indicated inverse relationships between dietary magnesium intake and systemic blood pressure, and between dietary calcium intake and pregnancy-induced hypertension (PIH). Altered magnesium metabolism in hypertension has long been recognized. Little data, however, exists on magnesium status in women with PIH despite its use in pregnancy-induced hypertensive syndromes. Studies on calcium supplementation during pregnancy report a lower incidence or risk of hypertension during pregnancy.

Whilst a number of epidemiological and calcium supplementation studies reveal an inverse relationship between dietary calcium intake and pregnancy-induced hypertension, results of direct analysis of total and ionised calcium in humans have, however, been conflicting. Although significantly reduced ionised calcium concentration has been reported in hypertensive non-pregnant women, Richards and colleagues however found no difference in serum ionised calcium concentration between normotensive pregnant women and women with PIH. Decreased plasma total calcium and either hypocalciuria or hypercalciuria nevertheless, have been observed in women with mild PIH. To clarify this discrepancy we measured erythrocyte magnesium concentration, serum and urinary magnesium and calcium levels in normotensive pregnant and pregnancy-induced hypertensive non-proteinuric women in the third trimester of pregnancy. In addition, we also measured plasma renin activity in some of these women.

Methodology
Subjects and sample collection
Participants were volunteers from women attending our antenatal care clinic and consuming an unrestricted diet. Women with a history of hypertension, diabetes...
and renal disease or proteinuria prior to the pregnancy were excluded from the study. A written informed consent was obtained and the procedure for 24-hour urine collection was explained. A 24-hour urine sample and 10 to 15 ml of venous blood (collected within the period of urine collection) were collected once in each trimester. There were two groups of subjects; normotensive pregnant (NP) (n=120) and pregnancy-induced hypertensive (PIH) (n=60) subjects.

**Blood pressure measurements**

All blood pressures were measured using a mercury sphygmomanometer. Subjects were seated for about 10 minutes before measurements and on each occasion three measurements were made over at least five minutes and the lowest reading was recorded. All measurements were made by the same observer. The point of disappearance of the Korotkoff sound (K5) was taken as the diastolic pressure. The criteria used for the diagnosis of hypertension, recommended by the American College of Obstetricians and Gynaecologists, were (1) a systolic pressure of > 140 mmHg (2) a diastolic pressure > 85 mmHg (3) an increase of > 30 mmHg in systolic pressure or (4) an increase of >15 mmHg in diastolic pressure. Any of these criteria were taken to indicate a raised blood pressure when present on at least two occasions separated by at least 6 hours.

**Serum and urinary analyses**

Serum, erythrocyte and urinary magnesium and calcium concentrations were determined spectrophotometrically (Micro-flow CL-750). Sodium and potassium concentrations in urine and serum were determined by flame photometry (Corning model 406). Serum ionised calcium was determined using a calcium ion-selective electrode (AVL 984-5). Creatinine concentrations in urine and serum were determined enzymatically (Cobas Mira). Plasma renin activity determined by RIA. Statistical significance was evaluated by Student's t-test with a 'p' < 0.05 considered significant. All values are presented as mean ± standard error of mean (SEM).

**Results**

**Age & Gestation and Body Weight**

No statistically significant differences were evident in the mean ages or in mean periods of gestation between the two groups. Women with PIH however had a greater body weight (p<0.02 ; Table I).

<table>
<thead>
<tr>
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<th>Normotensive (NP)</th>
<th>Pregnancy-induced hypertension (PIH)</th>
</tr>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>28.76 ± 0.38</td>
<td>32.22 ± 1.72</td>
</tr>
<tr>
<td>Gestation (weeks)</td>
<td>33.90 ± 0.29</td>
<td>33.50 ± 0.43</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>62.40 ± 1.12</td>
<td>67.70 ± 1.83*</td>
</tr>
</tbody>
</table>

*; *p*<0.05

**Systolic and Diastolic Blood Pressures**

Mean systolic and diastolic pressures were significantly higher in women with PIH (142.3 ± 1.9/92.9 ± 1.12 mmHg vs 109.2 ± 0.99/68.5 ± 0.73 mmHg, (p < 0.001; Fig. 1).
Serum total and ionised calcium concentrations

Mean serum total calcium concentration was significantly lower in women with PIH (2.13 ± 0.03 vs 2.05 ± 0.04 mmol L⁻¹; p<0.05). Mean serum ionised calcium concentration too was slightly lower in women with PIH (1.20 ± 0.01 vs 1.16 ± 0.02 mmol L⁻¹). The difference, however, was not statistically significant (Fig. 2).

Serum and erythrocyte magnesium concentrations

Magnesium concentrations in serum and erythrocytes of the two groups are presented in Figure 3 and Table II respectively. No statistically significant differences were evident between the two groups in these two parameters.

Packed Cell Volume (PCV) and serum concentrations of sodium and potassium

Serum sodium and potassium concentrations and PCV were not significantly different between the two groups.

Urine output, creatinine clearance and electrolyte excretion

No statistically significant differences were evident in the rates of creatinine clearance, urine output, sodium and potassium excretions between the two groups (Table III).

Urineary calcium and magnesium excretions

Urineary calcium and magnesium excretions in 24 hours are presented in Figure 4. Mean urinary calcium excretion in women with PIH was significantly lower than in normotensive pregnant women (3.17 ± 0.40 vs 5.22 ± 0.44 mmol day⁻¹, p<0.001). Similarly, urinary magnesium excretion was also significantly
**Table III**  
Mean serum concentrations of sodium and potassium excretions in the two groups

<table>
<thead>
<tr>
<th></th>
<th>Normotensive (NP)</th>
<th>Pregnancy-induced hypertension (PIH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine output (l day⁻¹)</td>
<td>0.99 ± 0.05</td>
<td>1.02 ± 0.08</td>
</tr>
<tr>
<td>Creat. cl (ml min⁻¹)</td>
<td>93.60 ± 17.6</td>
<td>80.30 ± 12.4</td>
</tr>
<tr>
<td>U NaV (mmol day⁻¹)</td>
<td>91.29 ± 5.30</td>
<td>75.40 ± 7.20</td>
</tr>
<tr>
<td>U KV (mmol day⁻¹)</td>
<td>25.20 ± 1.25</td>
<td>22.17 ± 1.75</td>
</tr>
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**Fig. 4:** Mean urinary calcium and magnesium excretions in normotensive (NP) and pregnancy-induced hypertensive (PIH) subjects  
(∗∗, p<0.01; ∗∗∗, p<0.001)

lower in women with PIH (2.21 ± 0.27 vs 3.31 ± 0.25 mmol day⁻¹, p<0.01).

**Plasma renin activity**

Plasma renin activity (PRA) was estimated in some of the latter subjects (n=13 and 10 for NP and PIH respectively). It was significantly lower in women with PIH (4.46 ± 0.23 vs 5.79 ± 0.53 ng ml⁻¹ h⁻¹, p<0.05; Fig. 5).

**Fig. 5:** Mean plasma renin activity in normotensive (NP) and pregnancy-induced hypertensive (PIH) subjects  
(*; p<0.05)

**Discussion**

Systemic arterial pressure was significantly higher in women with PIH (Fig.1). The exact cause for the rise in pressure during gestation is not clearly understood. Cardiac output increases whereas total peripheral resistance, as indicated by a fall or unaltered systemic blood pressure, falls during normal gestation. The increase in blood pressure evident in some pregnancies may therefore indicate an inappropriate peripheral vascular resistance. Pre-eclamptic patients have been shown to have a low pregnancy-associated refractoriness to angiotensin II¹⁵,¹⁶. The cause of this is uncertain. Vascular tone is influenced by a number of factors including calcium and magnesium. Raised intracellular calcium is known to increase smooth muscle tone whereas magnesium, considered a nature's antagonist of calcium, decreases vascular tone¹⁷,¹⁸. A disturbance in the metabolism of either of these two divalent cations can affect vascular tone and thereby the blood pressure.

Evidence presented over the last few years suggests a hypotensive effect for calcium. It has been shown to relax smooth muscles, often referred to as the
“membrane stabilising effect” of calcium\textsuperscript{19}. In addition, calcium has also been linked to endothelial dependant factor release\textsuperscript{20} and relaxation\textsuperscript{21}. In view of the epidemiological and clinical studies could it therefore be possible that pregnancy-induced hypertension in some instances may be due to a disturbed extracellular and possibly intracellular calcium concentration?

In this study, serum total calcium concentration was significantly lower in women with PIH (Fig. 2; \(p<0.05\)). Comparison of serum ionised calcium concentrations between NP women and women with PIH in this study revealed a slightly lower mean in the latter group (Fig. 2). Little data exists on serum ionised calcium concentration in women with pregnancy-induced hypertension, although Seely \textit{et al.}\textsuperscript{22} do report of a significantly lower serum ionised calcium in women with preeclampsia. Richard \textit{et al.}\textsuperscript{11} however found no significant differences in serum ionised calcium concentration between their normotensive pregnant and pregnancy-induced hypertensive subjects. The reason for the lower serum calcium concentration in women with PIH in this study is uncertain. No assessment of dietary intake of this cation was however made. Urinary calcium excretion in women with PIH was also significantly lower (Fig. 4) and therefore could not explain the lower serum total calcium. The mechanism for the lower urinary excretion of calcium in women with PIH is also uncertain. Ultrafiltrable fraction of calcium in serum was not estimated in the present study. As creatinine clearance was not significantly different between the two groups, it may be suggested that the decreased filtered load and perhaps an increased tubular reabsorption of this cation. In this respect significantly lowered urinary excretion of calcium with increased renal fractional reabsorption has been reported in women with preeclampsia\textsuperscript{23}. The cause for the evident hypocalcaemia therefore remains unclear.

Precisely how lowered serum calcium concentration increases blood pressure is uncertain. The role of various calciotrophic hormones has been proposed. Serum PTH has been found to be elevated in some women with PIH\textsuperscript{22}, and its possible mechanism of involvement in hypertension has also been proposed\textsuperscript{25}. It may however be argued that an increase in PTH associated with a low calcium concentration may be more coincidental rather than being involved in the hypertensive effect. Moreover a vasorelaxant effect for PTH has also been observed \textit{in vitro}\textsuperscript{26}. Besides, if the homeostatic adjustments have effected then normocalcaemia should have been restored. The concomittant presence of hypocalcaemia and elevated PTH in the study of Seely \textit{et al.}\textsuperscript{22} may suggest of a possible resistance to PTH in women with PIH.

More recently, however a circulating parathyroid hypertensive factor (PHF) has been reported both in spontaneously hypertensive rats (SHR) and in some patients with hyperparathyroidism\textsuperscript{26}. It is released concomitantly with PTH with its plasma level relating directly to the level of calcium intake and blood pressure\textsuperscript{27}. Its presence in PIH has not been demonstrated.

Plasma concentration of 1,25 (OH)\textsubscript{2}D\textsubscript{3} increases during gestation in humans\textsuperscript{29} as may be expected with
the increased calcium demand. Changes in plasma levels of 1,25 (OH)2D3 in PIH have not been extensively investigated although evidence published recently suggests of an impaired or abnormal 1,25(OH)2D3 production in preeclampsia and PIH. Pregnancy or gestation is associated with alterations in calcium demand as such perhaps also the calcium regulating hormones. It is therefore possible that these hormonal deviations may also be involved in the pathogenesis of PIH. Further studies are clearly needed to elucidate the role of calcium and its regulating hormones in hypertension.

From a small number of studies in essential hypertensives it seems that short-term calcium supplementation was of benefit most to individuals with low-renin form of hypertension. High-renin hypertensives benefited only when 1,25(OH)2D3 was given along with the calcium, indicating perhaps the importance of measuring PRA.

Estimation of PRA in a latter group of subjects in this study, revealed a significantly lower renin activity in serum of women with PIH (Fig. 6). The precise reason for this is uncertain. Plasma renin activity and plasma renin concentrations have been found to be suppressed in pregnant women with preeclampsia. The mechanism for this is uncertain although elevated plasma angiotensin II of utero-placental origin has been implicated. There is however also considerable doubt if intact angiotensin II is able to cross the placenta and into the maternal circulation.

Sodium retention with increased extracellular fluid volume could also suppress PRA. In this respect urinary sodium excretion was lower in women with PIH in this study (Table II). In addition, women with PIH also had a significantly higher body weight. Packed cell volume (PVC) and serum sodium concentration were however not different between the two groups (Table II). The reason for the slightly lower urinary sodium excretion is therefore not immediately evident. Dietary intake of sodium was not estimated and all subjects were on an unrestricted diet. In view of the absence of any significant differences in plasma sodium concentrations and creatinine clearances it seems that the slightly lowered urinary excretion in women with PIH may be a consequence of an increased renal tubular reabsorption of this cation. Increased sodium retention has been demonstrated in women with established proteinuric pregnancy-induced hypertension. The increased absolute amount of sodium in the body and the consequent increase in ECF volume could lead to a suppression of renin release and a lowered PRA.

In summary so far, the data from this study suggest of a lowered serum calcium, lowered urinary calcium, magnesium and perhaps also sodium excretions and a lowered PRA in the third trimester of pregnancy in women with PIH. The relationship between the lowered plasma renin activity, hypocalcaemia, and blood pressure is not clear. We found no significant correlation between serum calcium and plasma renin activity in our study. However, low-renin hypertensives are also salt-sensitive hypertensives. Sodium surfeit and expanded ECFV can result in a lowered PRA. Sodium and water retention are normal accompaniments of pregnancy. It is possible that in some women with PIH there exists an increased retention of sodium and water, consequently suppressing PRA. Increased salt retention leads to increased intracellular sodium. This may then increase Na:Ca exchange between intracellular and extracellular compartments, consequently increasing intracellular calcium, whilst decreasing extra-cellular calcium concentration. This may explain the hypocalcaemia evident in some women with PIH. Calcium supplementation not only restores serum calcium to normal, but also causes natriuresis. Sodium excretion decreases the sodium surfeit in the body consequently reducing Na:Ca exchange thereby normalising intracellular calcium and consequently vascular smooth muscle tone.

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References


