THE EFFECT OF ETHINYL OESTRADIOL ON CALCIUM AND PHOSPHORUS METABOLISM OF POST-MENOPAUSAL WOMEN WITH PRIMARY HYPERPARATHYROIDISM

J. C. GALLAGHER AND R. WILKINSON

M.R.C. Mineral Metabolism Unit, The General Infirmary, Leeds

(Received 25 June 1973)

SUMMARY

1. Eight post-menopausal women with primary hyperparathyroidism were given ethinyloestradiol (0.05 mg daily) and the effects on calcium and phosphorus metabolism were observed.

2. In every patient ethinyloestradiol produced a fall in fasting plasma and urine calcium. Calcium balance improved in seven patients on treatment and there was a significant fall in 24 h urine calcium in all eight patients; however, there was no consistent change in net or true absorption of calcium.

3. Ethinyloestradiol produced a small fall in the fasting plasma inorganic phosphorus and a fall in fasting urine phosphorus in seven cases. There was a decrease in 24 h urine phosphorus in seven of the eight cases, but there was no consistent effect on phosphorus absorption nor on phosphorus balance.

4. Bone mineralization rate and bone resorption rate were determined in seven of the patients. The administration of ethinyloestradiol produced a decrease in both these variables in all seven patients.

5. The 24 h urine hydroxyproline, used as an independent measure of bone resorption, decreased in all eight patients during ethinyloestradiol therapy.

6. It is concluded that ethinyloestradiol produces an improvement in calcium and phosphorus balance and a decrease in plasma calcium and phosphorus in primary hyperparathyroidism by decreasing bone resorption.

7. It is suggested that ethinyloestradiol may be used as a medical treatment for primary hyperparathyroidism in post-menopausal women who are either unsuitable for surgery or on whom operative procedures have failed, or in those cases in whom primary hyperparathyroidism is mild.

Key words: hyperparathyroidism, calcium and phosphorus metabolism, bone formation and resorption, ethinyloestradiol therapy, calcium tracer studies.

Correspondence: Dr J. C. Gallagher, M.R.C. Mineral Metabolism Unit, The General Infirmary, Great George Street, Leeds LS1 3EX.
It has previously been shown that the administration of ethinyloestradiol to post-menopausal women with primary hyperparathyroidism produces a fall in plasma and urine calcium and 24 h urine calcium and hydroxyproline and it was suggested that this might be due to oestrogenic inhibition of parathyroid hormone-induced bone resorption (Gallagher & Nordin, 1972).

The present paper presents more detailed metabolic investigations, including calcium and phosphorus balances and bone turnover studies, on post-menopausal women with primary hyperparathyroidism before and during ethinyloestradiol treatment in an attempt to confirm this hypothesis.

**METHODS**

Investigations were performed in a metabolic ward according to the principles of Reifenstein, Albright & Wells (1945). All procedures were fully explained to the patients who gave their informed consent to the studies which were undertaken.

**Patients**

Eight post-menopausal women with primary hyperparathyroidism were studied. The initial data on these patients is shown in Table 1. Four of the patients presented with renal stone disease, one patient with a fractured neck of femur, the other three patients presented with a long history of backache in whom routine screening revealed hypercalcaemia.

The diagnosis of primary hyperparathyroidism was based on the following criteria. There was fasting hypercalcaemia in all cases, with increased tubular reabsorption of calcium, the values for five patients lying outside the normal relationship as defined by Peacock, Robertson & Nordin (1969). There was a low fasting plasma phosphorus in six cases and a decreased tubular reabsorption of phosphorus in all cases, the relationship between these two variables lying outside the normal range defined by Nordin & Bulusu (1968) in all cases. Immuno-reactive plasma parathyroid hormone was measured on each patient before admission to the study. In every case the plasma parathyroid hormone was greater than the upper limit of normal: 1.5 ng/ml for the method. Each patient was studied in the untreated state, and then during ethinyloestradiol administration. All patients, except one (case 2), were given the ethinyloestradiol cyclically; 3 weeks of administration followed by 1 week without therapy to produce withdrawal bleeding and to prevent endometrial proliferation. The other case who had previously undergone a hysterectomy was given ethinyloestradiol every day.

In five cases the initial balance study was carried out in the untreated state and then repeated during treatment with ethinyloestradiol which had been given for periods varying from 3 to 20 months (Table 2). In the other three cases (nos. 1, 6 and 8) the initial study was carried out on ethinyloestradiol; at the time of the initial study ethinyloestradiol had been given for 3, 10 and 4 months respectively. Ethinyloestradiol was then stopped and the studies were repeated after 3, 4 and 9 months respectively. No other treatment was given during the period of study.

**Balance studies**

Calcium and phosphorus balances were determined by a daily balance technique (Bullamore, Marshall, Nordin, Oldfield & Wilkinson, 1970). Diets were prepared under the supervision
TABLE 1. Data in eight post-menopausal patients with primary hyperparathyroidism. C₈ and P₈, urine calcium and phosphorus excretion respectively as mg/100 ml of glomerular filtrate (G.F.). K.A. units are King-Armstrong units.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Presentation</th>
<th>Age (years)</th>
<th>Years since menopause</th>
<th>Plasma Ca (mg/100 ml)</th>
<th>Urine C₈ (mg/100 ml of G.F.)</th>
<th>P₈ (mg/100 ml of G.F.)</th>
<th>Plasma creatinine (mg/100 ml)</th>
<th>Alkaline phosphatase (K.A. units)</th>
<th>X-ray evidence of bone disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Renal calculi</td>
<td>59</td>
<td>10</td>
<td>13.6</td>
<td>0.33</td>
<td>0.51</td>
<td>0.7</td>
<td>18</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Renal calculi(1)</td>
<td>50</td>
<td>14</td>
<td>11.7</td>
<td>0.24</td>
<td>0.63</td>
<td>1.0</td>
<td>5</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Renal calculi</td>
<td>52</td>
<td>2</td>
<td>11.9</td>
<td>0.19</td>
<td>0.45</td>
<td>0.7</td>
<td>52</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Fractured neck of femur</td>
<td>80</td>
<td>35</td>
<td>11.4</td>
<td>0.34</td>
<td>0.66</td>
<td>1.1</td>
<td>13</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Backache</td>
<td>52</td>
<td>5</td>
<td>11.7</td>
<td>0.23</td>
<td>0.53</td>
<td>0.7</td>
<td>16</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Backache</td>
<td>56</td>
<td>5</td>
<td>11.6</td>
<td>0.25</td>
<td>0.80</td>
<td>1.2</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Backache</td>
<td>76</td>
<td>30</td>
<td>11.8</td>
<td>0.57</td>
<td>1.00</td>
<td>1.1</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Renal calculi</td>
<td>67</td>
<td>17</td>
<td>(8.9–10.2)</td>
<td>(2.5–4.0)</td>
<td>(1.2)</td>
<td>(3–13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Normal range

(1) Hysterectomy and bilateral oophorectomy.
### Table 2. Effect of ethinyloestradiol on calcium balance and bone turnover in primary hyperparathyroidism. All values are expressed as mg kg$^{-1}$ day$^{-1}$.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Wt. (kg)</th>
<th>Months</th>
<th>Intake</th>
<th>Faeces</th>
<th>Urine</th>
<th>Balance ± SEM (n)</th>
<th>Net Absorption (%)</th>
<th>True Absorption (%)</th>
<th>Endogenous faecal calcium (e)</th>
<th>Digestive juice calcium (d)</th>
<th>Bone mineralization rate</th>
<th>Bone resorption rate</th>
<th>$^{45}$Ca pool (mg/kg)</th>
<th>24 h urine hydroxyproline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>54</td>
<td>Control</td>
<td>3½</td>
<td>10·96</td>
<td>2·79</td>
<td>10·74</td>
<td>-2·57 ± 0·41 (7)</td>
<td>74·50</td>
<td>86·70</td>
<td>1·34</td>
<td>5·0</td>
<td>8·25</td>
<td>10·82</td>
<td>98·1</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>68</td>
<td>Treated</td>
<td>4½</td>
<td>9·02</td>
<td>1·96</td>
<td>3·67</td>
<td>3·39 ± 0·26 (4)</td>
<td>78·20</td>
<td>68·80</td>
<td>0·95</td>
<td>3·80</td>
<td>18·05</td>
<td>14·66</td>
<td>175·2</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>55</td>
<td>Control</td>
<td>3½</td>
<td>9·37</td>
<td>6·82</td>
<td>5·78</td>
<td>-3·23 ± 0·71 (4)</td>
<td>27·21</td>
<td>41·62</td>
<td>1·35</td>
<td>2·08</td>
<td>23·21</td>
<td>26·46</td>
<td>213·4</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>68</td>
<td>Treated</td>
<td>4½</td>
<td>6·42</td>
<td>5·49</td>
<td>2·40</td>
<td>-1·47 ± 0·32 (3)</td>
<td>14·48</td>
<td>30·06</td>
<td>1·0</td>
<td>1·34</td>
<td>6·67</td>
<td>8·14</td>
<td>83·3</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>58</td>
<td>Control</td>
<td>4½</td>
<td>7·59</td>
<td>5·98</td>
<td>9·04</td>
<td>-7·43 ± 0·17 (6)</td>
<td>21·21</td>
<td>68·64</td>
<td>3·60</td>
<td>8·64</td>
<td>16·40</td>
<td>23·83</td>
<td>93·6</td>
</tr>
<tr>
<td>6</td>
<td>56</td>
<td>59</td>
<td>Control</td>
<td>4½</td>
<td>7·72</td>
<td>6·40</td>
<td>4·82</td>
<td>-3·50 ± 0·40 (6)</td>
<td>17·10</td>
<td>57·77</td>
<td>3·14</td>
<td>6·17</td>
<td>5·46</td>
<td>8·98</td>
<td>151·0</td>
</tr>
<tr>
<td>7</td>
<td>76</td>
<td>62</td>
<td>Control</td>
<td>6½</td>
<td>7·10</td>
<td>4·26</td>
<td>6·27</td>
<td>-3·43 ± 0·51 (5)</td>
<td>40·00</td>
<td>60·84</td>
<td>1·48</td>
<td>3·06</td>
<td>6·89</td>
<td>10·32</td>
<td>90·8</td>
</tr>
<tr>
<td>8</td>
<td>67</td>
<td>63</td>
<td>Control</td>
<td>9½</td>
<td>5·49</td>
<td>4·07</td>
<td>3·06</td>
<td>-1·64 ± 0·12 (5)</td>
<td>25·90</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9</td>
<td>66</td>
<td>80</td>
<td>Treated</td>
<td>4½</td>
<td>7·26</td>
<td>4·45</td>
<td>1·83</td>
<td>-0·98 ± 0·12 (7)</td>
<td>38·70</td>
<td>57·00</td>
<td>1·33</td>
<td>2·58</td>
<td>1·35</td>
<td>0·37</td>
<td>55·3</td>
</tr>
</tbody>
</table>

Normal range (1·00-2·56) (1·49-4·90) (1·76-6·02) (38-82) (<0·40)

Indicates the first balance study performed.
of a dietician and were gelatin-free. Any given subject ate a constant daily diet for the duration of the study and was given 500 mg of polyethylene glycol 4000 (PEG) three times a day with meals as a non-absorbable faecal marker (Wilkinson, 1971). All food and milk was consumed between 08.00 and 18.00 hours each day but the patient was allowed a non-calcium-containing drink at 21.00 hours.

After 7 days of equilibration on the diet and marker, daily collections of faeces and urine were made for a further 7 days. The excretion of calcium and phosphorus in the faeces was corrected to a 24 h output using the PEG content of each faecal sample. In seven patients each 24 h urine was collected as a 16 h sample from 08.00 to 24.00 hours and an 8 h sample from 00.00 to 08.00 hours, and analysed for calcium, phosphorus and creatinine. The dietary intake of calcium and phosphorus was determined by the analysis of two complete daily diets for each patient. Daily calcium and phosphorus balances were determined for every day on which a faecal sample was obtained and the mean balance plus standard error of the mean was calculated for each patient. During the 7 days of balance collections a 1% aliquot of each urine collection was pooled and the pool analysed for hydroxyproline so that a mean daily urine hydroxyproline output could be calculated. Fasting venous blood samples were taken at the mid-point of a 1 h urine collection between 08.00 and 09.00 hours on day 5 of the equilibration week and on days 1 and 5 of the balance week and were analysed for calcium, phosphorus and creatinine. The mean of these three observations for the control and for the treated periods are reported.

Bone turnover studies

On day 1 of the balance week the patients received an intravenous injection of 10 μCi of ⁴⁷calcium chloride (containing 5 μg of calcium chloride/μCi) in sterile iso-osmotic saline solution. Blood samples were taken at 5, 10, 20, 40 and 90 min, 3, 5, 9 and 22 h and 2, 3, 4, 5 and 7 days after the injection. ⁴⁷Calcium radioactivity was measured in all the plasma samples and in the daily faecal and urine samples of the subsequent 7 days. Bone mineralization rate was calculated by the method of Burkinshaw, Marshall, Oxby, Spiers, Nordin & Young (1969) with the aid of a computer to give the best fit of the data points to the equation. Burkinshaw & Marshall (1971) have shown with the aid of a computer simulating technique that the error of an estimation of the mineralization rate by this method is of the order of no more than 0·6-0·8 mg of calcium kg⁻¹ day⁻¹. Bone resorption rate was calculated as the difference between the mineralization rate and the calcium balance. Since faecal and urine radioactivity were also measured during the balance week this enabled endogenous faecal calcium (e) to be determined and the digestive-juice calcium (d) and the true absorption (α) of calcium to be calculated by the method of Heaney & Skillman (1964).

Chemical methods

Calcium, phosphorus and creatinine in plasma and in acidified samples of urine were estimated by standard AutoAnalyzer techniques.

Diet and faeces were homogenized with three times their own weight of deionized distilled water. Duplicate aliquots of dietary and faecal homogenates (weighed on a Salter top-pan balance to 0·01 g) were dried, ashed at 550°C for 16 h, taken up in 5 ml of HCl (2 mol/l) and made up to a standard volume with deionized distilled water. Aliquots of these faecal and dietary digests were analysed for calcium and phosphorus by standard AutoAnalyzer
techniques. Duplicate aliquots of faecal homogenate, approximately 1-00 g, accurately weighed, were taken for analysis of PEG by a turbidimetric method (Wilkinson, 1971). Analyses of calcium, phosphorus and PEG in the diet and faeces were the mean of duplicate estimations.

Hydroxyproline was determined in urine, after acid hydrolysis, by the method of Grant (1964) modified for use on an AutoAnalyzer.

**Radioactivity measurements**

$^{47}$Calcium radioactivity in plasma, faeces and urine was determined in a well-type automatic gamma counter with a sodium iodide crystal (Nuclear Enterprises Gammamatic) with pulse-height analysis to exclude disintegration due to $^{47}$scandium (as previously described by Bullamore, Nordin, Wilkinson & Marshall, 1971). All counts were corrected for background and decay.

**Plasma proteins**

Plasma proteins were determined on an AutoAnalyzer using a modified biuret reaction (Technicon AAl l-14). Albumin was measured by the quantitative binding of Bromocresol Green adapted for use on an AutoAnalyzer (Northam & Widdowson, 1967).

**Alkaline phosphatase**

The measurement of alkaline phosphatase was carried out by the method of Axelsson, Ekman & Knutsson (1965) on an AutoAnalyzer and the results were expressed in King-Armstrong units.

**Statistics**

Statistical analyses of significance were performed by the paired t-test, each patient acting as her own control.

**RESULTS**

**Plasma and urine calcium**

The effect of ethinyloestradiol on fasting plasma and urine calcium is shown in Fig. 1. In each patient there was a fall in plasma calcium; the mean control value was 11.78 mg/100 ml, and during treatment it fell to 10.72 mg/100 ml. This fall was highly significant ($P<0.001$). The fall in plasma and urine calcium was always sustained throughout the duration of treatment, which varied from 3 to 20 months.

The control values of the fasting urine calcium/creatinine ratio (Ca/Cr) were elevated above the normal range (>$0.15$) in all patients except one. In every patient treated with ethinyloestradiol there was a fall in the fasting urine Ca/Cr (Fig. 1); the initial mean Ca/Cr was 0.30 and it fell to a mean value of 0.09. This fall was highly significant ($P<0.001$).

For seven of the patients during each balance period the urine Ca/Cr was expressed as the mean day Ca/Cr (from 08.00 to 24.00 hours), the mean night Ca/Cr (00.00–08.00 hours) and the mean fasting Ca/Cr (08.00–09.00 hours). During the control balances, there was no significant difference between the day and the night Ca/Cr, but they were slightly higher than the fasting Ca/Cr (Fig. 2). During therapy with ethinyloestradiol, however, the Ca/Cr was significantly decreased during the day ($P<0.01$) and night ($P<0.02$) and even more in the fasting state ($P<0.001$) in all seven patients (Fig. 2) as compared to the pretreatment values.
Ethinyloestradiol therapy in hyperparathyroidism

Fasting urine calcium was also related to the fasting plasma calcium in order to assess tubular reabsorption of calcium by the kidney. This was done by estimating urine calcium excretion/100 ml of glomerular filtrate \( (\text{Ca}_E) \) \[ \text{Ca}_E = \frac{\text{urine calcium}}{\text{urine creatinine}} \times \text{plasma creatinine} \]. The normal relationship between these two parameters has been defined by Peacock et al. (1969) and the hyperparathyroid patients are shown in relation to this normal range in Fig. 3. Values for all eight hyperparathyroid patients lie below the mean line, five of them being outside the normal range, thus indicating increased tubular reabsorption of calcium by the kidney. Administration of ethinyloestradiol was associated with a fall in plasma and urine calcium down a line generally parallel to the normal range, suggesting that tubular reabsorption had not been significantly altered.

**Plasma and urine phosphate**

Administration of ethinyloestradiol caused a fall in fasting plasma phosphate in six patients; the mean control value was 2.27 mg/100 ml which fell to a mean value of 1.88 mg/100 ml \((P<0.05)\).

The urine phosphorus/creatinine ratio \((\text{P/Cr})\) was derived for the day, night and fasting states as described previously for urine \(\text{Ca/Cr}\) (Fig. 2). In the control balances the day and the night \(\text{P/Cr}\) were similar, but the fasting \(\text{P/Cr}\) was slightly lower. During ethinyloestradiol therapy there was a significant fall in the day \((P<0.01)\) and the night \((P<0.05)\) \(\text{P/Cr}\) in six of the patients, and the \(\text{P/Cr}\) fell in the fasting state in all seven patients \((P<0.05)\) compared to the untreated values.

The relationship between the control plasma and urine phosphorus excretion (expressed as the \(P_E\) in mg/100 ml of glomerular filtrate) is shown in Fig. 4. All patients lie outside the normal range.
range as defined by Nordin & Bulusu (1968) indicating a decreased tubular reabsorption of phosphorus by the kidney. During ethinyloestradiol therapy the fasting urine phosphorus excretion expressed as the $P_E$ fell in six patients and increased in two patients ($P < 0.1$). However, in only one of these patients was there an increase in $P_E$ relative to plasma phosphate, and this was due to a temporary rise in plasma creatinine. Thus ethinyloestradiol does not generally appear to affect the tubular reabsorption of phosphorus (Fig. 4).
Plasma calcium (mg/100 mL)

FIG. 3. Effect of ethinyloestradiol on the relationship between the fasting plasma calcium and urine calcium excretion ($C_a$) expressed as mg/100 ml of glomerular filtrate (G.F.) in eight post-menopausal patients with primary hyperparathyroidism. The initial results are shown as (○) and those after treatment by the arrows. The solid lines represent the mean ± 2 SD for normal subjects as defined by Peacock et al. (1969). The hatched area represents the normal range.

Plasma phosphate (mg/100 mL)

FIG. 4. Effect of ethinyloestradiol on the relationship between the fasting plasma phosphate and urine phosphorus excretion ($P_e$) expressed as mg/100 ml of glomerular filtrate (G.F.) in eight post-menopausal patients with primary hyperparathyroidism. The initial results are shown as (○) and those after treatment by the arrows. The solid lines represent the mean ± 2 SD for normal subjects as defined by Nordin & Bulusu (1968).
Plasma proteins

Total plasma protein and albumin were measured before and during ethinyloestradiol therapy. No consistent change was noted in total protein during therapy but plasma albumin decreased slightly in five patients, increased in two patients and was unchanged in the other patient. The mean fall in plasma albumin of 0.24 g/100 ml on ethinyloestradiol therapy was not statistically significant ($P < 0.3$).

Urine creatinine

The mean 24 h urine creatinine for the eight patients during the control balances was 1012 mg ($SD = 189$ mg), the mean value on ethinyloestradiol treatment was 1005 mg ($SD = 220$ mg), thus no significant change occurred in urine creatinine excretion ($P < 0.9$).

Alkaline phosphatase

Plasma alkaline phosphatase was elevated above the upper limit of the normal range in three patients in the control period. During ethinyloestradiol therapy plasma alkaline phosphatase fell in seven of the eight patients ($P < 0.05$).

Balance studies

Calcium. The results of the calcium balance studies on each patient are summarized in Table 2. The control calcium balances were negative in seven of the eight patients. The results obtained in the balance studies on the hyperparathyroid patients are shown in Fig. 5 in relation to the normal range for calcium intake against calcium output (urine plus faeces) which has been calculated from ninety-two normal balances by Nordin (1960). During the control balances values for five of the hyperparathyroid patients were outside the normal range and in seven of the eight patients calcium output exceeded calcium intake. Ethinyloestradiol therapy produced a decrease in calcium output relative to intake in seven balances so that the treated values for all eight patients are within the normal range. Calcium output decreased mainly because of the decrease in urine calcium in all cases. The mean control 24 h urine calcium was 6.14 mg kg$^{-1}$ day$^{-1}$ and this fell on ethinyloestradiol treatment to 2.82 mg kg$^{-1}$ day$^{-1}$ ($P < 0.02$) (Table 2). Fig. 6 shows the normal range of urine calcium in relation to dietary calcium in the ninety-two normal balances (Nordin, 1960). Values for six of the hyperparathyroid patients initially were outside this normal range because urine calcium was high relative to calcium intake, and during ethinyloestradiol therapy the values moved into or towards the normal range in all cases.

In all the control balances except one, faecal calcium, in relation to calcium intake, fell within the calculated normal range (Nordin, 1960) and during treatment with ethinyloestradiol the change in faecal calcium relative to intake was variable, decreasing in two patients, increasing in three and remaining unchanged in three patients. Thus there was no consistent effect of ethinyloestradiol on net absorption of calcium (Table 2). In none of the cases could the fall in urine calcium be explained by a fall in net absorption of calcium.

Ethinyloestradiol did not produce any consistent change in endogenous faecal calcium nor in digestive-juice calcium (Table 2).

An example of the typical effect of ethinyloestradiol therapy on calcium and phosphorus balance in a hyperparathyroid patient is shown in Fig. 7.
Phosphorus. The results of the phosphorus balances carried out before and during ethinyl-oestradiol therapy are summarized in Table 3. In six patients phosphorus output (urine plus faeces) was greater than the phosphorus intake, resulting in negative phosphorus balance. The increased phosphorus output was due to the urine phosphorus which was high in relation to intake, all control values being above the mean line as shown in Fig. 8. [The mean line and 95% limits have been calculated for twenty-seven normal subjects (J. C. Gallagher & R. Wilkinson, unpublished observations).] Ethinyl-oestradiol caused a fall in urine phosphorus in relation to intake in seven of the eight patients. The mean control 24 h urine phosphorus in eight patients was 12.40 mg kg\(^{-1}\) day\(^{-1}\) which fell on ethinyl-oestradiol therapy to 10.65 mg kg\(^{-1}\) day\(^{-1}\) (P<0.1). In seven patients before therapy, faecal phosphorus relative to intake was below the normal mean line for the twenty-seven normal subjects indicating high absorption of phosphorus. During ethinyl-oestradiol therapy faecal phosphorus relative to intake increased in three patients, decreased in one patient and was unchanged in four patients and thus there was no consistent change in net phosphorus absorption. Phosphorus balance improved in five patients and became more negative in three patients (Table 3).

Bone turnover studies

The untreated values for bone mineralization rate were high in six patients and normal in
the other patient in relation to the normal range established by Bullamore et al. (1971). During ethinyloestradiol therapy the mineralization rate fell in every patient, two of them falling to very low levels (Table 2). This fall was highly significant \( (P < 0.001) \).

In the control balances the bone resorption rate was very high in two patients. There was a fall in the bone resorption rate in all seven cases on ethinyloestradiol therapy and this fall was highly significant \( (P < 0.001) \).

![Fig. 6. Effect of ethinyloestradiol on urine calcium in relation to dietary calcium intake in eight post-menopausal patients with primary hyperparathyroidism. All values are expressed as mg of Ca kg\(^{-1}\) day\(^{-1}\). The initial results are shown as (●) and those after treatment by the arrows. The solid lines represent the mean ± 2 SD for ninety-two normal balances given by the equation \( y = 0.056x + 2.09 ± 1.95 \) mg kg\(^{-1}\) day\(^{-1}\) (Nordin, 1960).](image)

**Urine hydroxyproline**

The mean 24 h urine hydroxyproline before therapy was 0.696 mg kg\(^{-1}\) day\(^{-1}\); however, this includes one very high result (Table 2). On ethinyloestradiol therapy urine hydroxyproline fell to a mean value of 0.354 mg kg\(^{-1}\) day\(^{-1}\). Although urine hydroxyproline fell on treatment in every case the fall was not significant \( (P < 0.1) \). The relationship of urine hydroxyproline, which is an independent measure of bone resorption, to the calculated bone resorption rate, and the effect of ethinyloestradiol therapy on these two variables is shown in Fig. 9.

**DISCUSSION**

It is apparent that ethinyloestradiol lowers fasting plasma and urine calcium and phosphorus in post-menopausal women with primary hyperparathyroidism. The large fall in plasma calcium
Ethinyloestradiol therapy in hyperparathyroidism cannot be explained by the small fall in serum albumin and neither can the latter account for the fall in urine calcium. These changes are associated with an improvement in calcium and phosphorus balance produced predominantly by the fall in urine calcium and phosphorus without any significant changes in absorption. The decrease in 24 h urine calcium and phosphorus can only be accounted for by a decrease in bone resorption. The fall in 24 h urine phosphorus is smaller (1.75 mg kg⁻¹ day⁻¹) than the fall in urine calcium (3.32 mg kg⁻¹ day⁻¹) and this is compatible with the concept of inhibition of bone resorption since the mineral calcium/phosphorus ratio in bone is approximately 2:1.

Fig. 7. Example of the effect of ethinyloestradiol on daily calcium and phosphorus balance in a post-menopausal patient with primary hyperparathyroidism (case 5). All values are expressed as mg kg⁻¹ day⁻¹. The mean data for each balance period are shown for that patient in Table 2. Ethinyloestradiol had been given for 4 months before the repeat balance was performed. The days when no faecal values are shown are the days on which no faecal samples were obtained. Hatched columns, urine; solid columns, faeces.

The fall in bone resorption previously postulated by Gallagher & Nordin (1972) is now confirmed by the kinetic studies which show that ethinyloestradiol produces a fall in both the bone mineralization rate and the resorption rate in every patient. Further evidence that ethinyloestradiol inhibits bone resorption is shown by the fall in urine hydroxyproline excretion in every patient on therapy. Oestrogens have previously been shown to decrease bone
TABLE 3. Phosphorus balance. Values are expressed in mg kg\(^{-1}\) day\(^{-1}\)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Intake</th>
<th>Faeces</th>
<th>Urine</th>
<th>Balance</th>
<th>± SEM (n)</th>
<th>Net absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>18.28</td>
<td>4.75</td>
<td>14.90</td>
<td>-1.37</td>
<td>0.97 (7)</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>17.67</td>
<td>10.11</td>
<td>10.81</td>
<td>-3.25</td>
<td>0.627 (5)</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>19.20</td>
<td>3.66</td>
<td>14.72</td>
<td>+0.82</td>
<td>0.221 (5)</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>24.44</td>
<td>6.49</td>
<td>18.08</td>
<td>-0.13</td>
<td>1.14 (4)</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>15.61</td>
<td>5.72</td>
<td>14.51</td>
<td>-4.62</td>
<td>0.402 (4)</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>15.47</td>
<td>2.69</td>
<td>12.34</td>
<td>+0.43</td>
<td>0.460 (4)</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>9.74</td>
<td>4.06</td>
<td>7.49</td>
<td>-1.81</td>
<td>0.260 (3)</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>11.16</td>
<td>6.89</td>
<td>5.20</td>
<td>-0.93</td>
<td>0.343 (3)</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>21.37</td>
<td>7.20</td>
<td>13.30</td>
<td>+0.87</td>
<td>0.465 (6)</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>20.57</td>
<td>6.31</td>
<td>10.32</td>
<td>+3.94</td>
<td>0.182 (5)</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>17.69</td>
<td>7.04</td>
<td>12.83</td>
<td>-2.18</td>
<td>0.352 (6)</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>15.39</td>
<td>6.50</td>
<td>9.19</td>
<td>+0.70</td>
<td>0.530 (7)</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>13.78</td>
<td>3.64</td>
<td>11.43</td>
<td>-1.29</td>
<td>0.550 (5)</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>12.64</td>
<td>4.42</td>
<td>10.34</td>
<td>-2.12</td>
<td>0.258 (6)</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>12.73</td>
<td>4.63</td>
<td>10.02</td>
<td>-1.92</td>
<td>0.221 (5)</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>14.38</td>
<td>5.16</td>
<td>8.95</td>
<td>+0.27</td>
<td>0.185 (7)</td>
</tr>
</tbody>
</table>

FIG. 8. Effect of ethinylestradiol on urine phosphorus in relation to dietary phosphorus intake in eight post-menopausal patients with primary hyperparathyroidism. All values are expressed as mg kg\(^{-1}\) day\(^{-1}\). The initial results are shown as (○) and those after treatment by the arrows. The solid lines represent the mean ± 2 SD for twenty-seven normal balances given by the equation \(y = 0.60x + 1.0 ± 0.2\).
Ethinyloestradiol therapy in hyperparathyroidism

resorption as measured by histological techniques on osteoporotic patients (Riggs, Jowsey, Kelly, Jones & Maher, 1969).

Primary hyperparathyroidism in females presents most commonly after the menopause (Muller, 1969) and post-menopausal women constitute the majority of cases of primary hyperparathyroidism who have bone disease (McGeown, 1969). It has also been shown that post-menopausal hyperparathyroid women have a lower metacarpal cortical area to metacarpal total area ratio (MCA/MTA) than normal subjects, while hypoparathyroid women have a greater metacarpal cortical area than normal subjects (Hossain, Smith & Nordin, 1970).

![Fig. 9. Effect of ethinyloestradiol on the relation between urine hydroxyproline and the calculated bone resorption rate in seven post-menopausal patients with primary hyperparathyroidism. Bone resorption rate is calculated as the difference between the mineralization rate and the balance. All values are expressed as mg kg\(^{-1}\) day\(^{-1}\). The initial results are shown as (●) and those after treatment by the arrows.](image)

Histological bone involvement in hyperparathyroidism, even without X-ray changes, is extremely common as demonstrated by quantitative measurements on iliac crest biopsies. Although bone formation is increased, the increase is usually within the normal range whereas bone resorption is commonly increased above the normal range (Riggs, Kelly, Jowsey & Keating, 1965; Byers & Smith, 1971).

In this study there is no significant variation in the day, night or fasting urine Ca/Cr and P/Cr in the hyperparathyroid group before treatment. Normally there is a fall in the fasting urine Ca/Cr to less than 0.15 in pre-menopausal women (Gallagher, Bulusu & Nordin, 1972). During ethinyloestradiol therapy, however, there is a significant fall in urine Ca/Cr and P/Cr in all these periods. This suggests that in the untreated state bone resorption is an active
continuous process throughout the day and night and is independent of calcium and phosphorus intake and calcium and phosphorus absorption. It appears from the present study that parathyroid hormone-induced bone resorption has been inhibited by ethinyloestradiol and that the main site of antagonism is bone, for no convincing change in intestinal absorption nor renal tubular resorption of calcium has been demonstrated. This is compatible with observations made by other workers. Atkins, Zanelli, Peacock & Nordin (1972) have demonstrated *in vitro* that different oestrogen compounds directly inhibit parathyroid hormone-induced bone resorption, and this occurs as a dose-related response. It has also been shown that stilboestrol can lower plasma calcium and urine hydroxyproline in a man with a parathyroid carcinoma presumably by inhibition of bone resorption (Sigurdsson, Woodhouse, Taylor & Joplin, 1973).

The commonest clinical presentation of hyperparathyroid patients is renal stone disease (Lloyd, 1968). Four of the patients studied have renal stones and in all eight patients ethinyloestradiol produced a fall in 24 h urine calcium. The decrease in 24 h urine calcium during ethinyloestradiol therapy was between 40 and 50% of that in the untreated state, and implies that bone resorption makes a very significant contribution to the hypercalciuria of primary hyperparathyroidism. This is quite apparent in those patients who have had relatively low intakes of calcium in the balance study. In primary hyperparathyroidism there is also usually an increased absorption of calcium to account for the hypercalciuria (Stanbury, 1968) and it has been postulated by this author that this represents an increased demand for calcium by bone. If this is so then clearly in the balances on ethinyloestradiol the 'demand' for increased absorption of calcium in the hyperparathyroid patients will have been greatly decreased by the decrease in bone turnover, and one might expect absorption to decrease if the 'signal' is from bone and not parathyroid hormone itself.

The effect of ethinyloestradiol is clearly reversible. After discontinuing therapy in three patients, the plasma and urine calcium rise over a 3 month period and the calcium balances become more negative. That this is due to bone resorption is confirmed by the rise in urine hydroxyproline excretion, and the rise in the bone mineralization rate.

These studies may simulate the physiological change that occurs at the menopause. During the decline in oestrogenic activity parathyroid hormone exerts its full effect on bone causing increased bone resorption. The increased bone resorption produces a further rise in plasma calcium sufficient to double urine calcium and thereby increase the likelihood of renal stones. This may explain why female patients present with this disease predominantly after the menopause.

Although serious side effects have been reported with higher doses of ethinyloestradiol in pre-menopausal women (Inman, Vessey, Westerholm & Engelund, 1970), there is no evidence that the dose of ethinyloestradiol used in the present study (0-05 mg daily) is associated with such side effects. In the present study there have been no serious side effects during the treatment period which has now continued for up to 2 years in some of the patients; withdrawal bleeding occurred in seven patients but did not always occur during the week off therapy.

The main use for ethinyloestradiol would seem to be as a medical treatment in cases of mild primary hyperparathyroidism, in cases of unsuccessful parathyroidectomy or in situations where the patient may be too ill for operation. It should effectively lower urine calcium and decrease the development of renal stones, and by suppressing bone resorption it should prevent the onset of osteoporosis in hyperparathyroid patients.
Ethinyloestradiol therapy in hyperparathyroidism

ACKNOWLEDGMENTS

The authors wish to acknowledge the interest and advice of Professor B. E. C. Nordin and to thank him for permission to publish details of studies on patients under his care. Our thanks go to the nursing and dietetic staff of the metabolic units at Leeds General Infirmary, to Dr Munro Peacock for the parathyroid hormone assays, to Dr H. Marshall for the isotope calculations, to Miss W. Oldfield, Miss L. Wade, Mrs V. Fearnley, Mrs A. Williams, P. D. Bower and M. Fearnley for skilled technical assistance, and to Mrs S. Rutter for secretarial help.

REFERENCES


HEANEY, R.P. & SKILLMAN, T.G. (1964) Secretion and excretion of calcium by the human gastrointestinal tract. Journal of Laboratory and Clinical Medicine, 64, 29-41.


