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VITAMIN D METABOLISM IN HYPERTHYROIDISM

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SUMMARY

The serum concentrations of 25-hydroxycholecalciferol (25 OH D_3), 24,25-dihydroxycholecalciferol [24,25$(OH)_2D_3$] and 1,25-dihydroxycholecalciferol [1,25$(OH)_2D_3$] were measured in twenty-one patients with untreated hyperthyroidism. Compared with control subjects, 25 OH D_3 concentrations were not altered, 24,25$(OH)_2D_3$ concentrations were increased, although not significantly and 1,25$(OH)_2D_3$ concentrations were decreased ($P=0{\cdot}01$). Following oral carbimazole therapy, 24,25$(OH)_2D_3$ concentrations fell ($P<0{\cdot}01$), 1,25$(OH)_2D_3$ concentrations increased ($P<0{\cdot}01$) and 25 OH D_3 concentrations were unchanged. The altered 1,25$(OH)_2D_3$ and 24,25$(OH)_2D_3$ concentrations found in hyperthyroidism are probably due to the effects of thyroid hormone on bone and mineral metabolism. Increased serum calcium and phosphate concentrations with secondary hypoparathyroidism result in stimulation of the renal 24-hydroxylase and suppression of the 1-hydroxylase enzymes. In addition, serum 24,25$(OH)_2D_3$ concentrations were significantly correlated with serum triiodothyronine levels (T3) ($r=0{\cdot}66$, $P<0{\cdot}002$) before treatment. This may indicate a direct stimulatory effect of T3 on 24-hydroxylase activity. No relationship was found between serum 1,25$(OH)_2D_3$ concentrations before therapy and serum T3.

The metabolism of vitamin D is of great importance in the control of bone and mineral metabolism (Haussler & McCain, 1977a and b). In hyperthyroidism abnormalities of bone and mineral metabolism are well recognized (Smith *et al.*, 1973). It seemed likely, therefore, that thyroid hormone excess would be associated with alterations in vitamin D metabolism. Bouillon *et al.* (1980) reported that serum levels of the vitamin D metabolite 1,25-dihydroxycholecalciferol [1,25$(OH)_2D_3$] were reduced in hyperthyroidism and increased in hypothyroidism. These changes in serum 1,25$(OH)_2D_3$ were thought to be secondary to a direct effect of thyroid hormones on bone turnover. In hyperthyroidism there is an increase in net bone resorption associated with an increase in bone turnover. Serum calcium and phosphate concentrations rise and parathyroid hormone secretion is then reduced (Mosekilde & Christensen, 1977). Alterations in calcium and phosphate concentrations and the resultant changes in parathyroid function influence the renal

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1-hydroxylase enzyme (DeLuca, 1980). Therefore 1-hydroxylase activity would be suppressed in hyperthyroidism.

In the present study we confirm that serum $1,25(OH)_2D_3$ concentrations are reduced in hyperthyroidism. Following oral carbimazole therapy, to render the patients euthyroid, $1,25(OH)_2D_3$ levels increased into the normal range. We now report a relationship between serum thyroid hormone concentrations and serum 24,25-dihydroxycholecalciferol [$24,25(OH)_2D_3$] concentrations in hyperthyroidism. Carbimazole therapy was associated with a reduction in $24,25(OH)_2D_3$ concentrations.

PATIENTS

Twenty-one consecutive hyperthyroid patients, less than 50 years old, were studied. There were eighteen women and three men, median age 33 years (range 21–49). All had a normal serum creatinine concentration, none had received previous antithyroid drugs or thyroid surgery and none had a nodular goitre. Patients were admitted to the study over an 18-month period. Carbimazole 30 mg was prescribed as one dose each morning and propranolol 40 mg TID was also given for the first 3 weeks.

Patients were reviewed every 3 weeks and the following variables measured in the non-fasting state: serum calcium, phosphate, creatinine, total alkaline phosphatase, albumin, T3 and T4 concentrations; the 20-min TSH response to 200 μg of intravenous TRH and the clinical score, according to the Crookes-Wayne Index (Crookes *et al.*, 1960). Measurements of serum vitamin D metabolites were carried out on three occasions: before treatment – occasion 1 (twenty-one patients); when the patients were clinically euthyroid – occasion 2 (nineteen patients) and when the TSH response to TRH became positive – occasion 3 (sixteen patients). In twelve patients serial measurements of serum immunoreactive parathyroid hormone (iPTH) were also carried out. Blood samples were allowed to clot, separated and serum was stored at $-20°C$. All samples from individual patients were measured in the same assay for vitamin D metabolites or for iPTH. The number of weeks of carbimazole therapy were (median and range): occasion 1–2, 12 weeks (6–18); occasion 1–3, 21 weeks (9–39).

Controls for the vitamin D metabolite assays

There were twenty-six controls, twenty women and six men, median age 42 years (range 20–64). These were healthy hospital personnel and patients admitted for minor surgical procedures seen during the same 18-month period as the patients. One blood sample was taken from each control subject and sera were stored at $-20°C$ for a similar length of time as the patients' sera, before assay.

METHODS

The serum calcium, phosphate, creatinine, total alkaline phosphatase and albumin concentrations were measured by autoanalyser (Technicon). The serum calcium concentrations were corrected for changes in the serum albumin (Leading Article, 1977). The serum iPTH, TSH, T3 and T4 were measured by radioimmunoassay. The MRC antiserum 211/32 was used in the PTH assay (Mawer *et al.*, 1975). The serum 25-hydroxycholecalciferol [25 OH D_3] and $24,25(OH)_2D_3$ concentrations were measured

as previously described (Taylor *et al.*, 1979). The serum $1,25(OH)_2D_3$ was measured by radioimmunoassay (Clemens *et al.*, 1979).

Statistical analysis

For unpaired data between patients and controls, Wilcoxon's rank sum test was used. For paired data from the patients, Wilcoxon's signed rank test and Pearson's correlation were used.

RESULTS

The data are shown in Tables 1 and 2. Thyrotoxicosis was confirmed by increased serum T3 and T4 levels, an elevated 2-h ^{132}I uptake and an undetectable serum TSH level with no TSH response to TRH. Before treatment, serum calcium and phosphate concentrations were within the normal range in the majority of patients. Three patients had mild hypercalcaemia (2·62, 2·67 and 2·72 mmol/l) and three had mild hyperphosphataemia (1·5, 1·52 and 1·6 mmol/l). Serum alkaline phosphatase was increased in eleven of the twenty-one patients.

Following treatment there was a progressive and significant fall in the serum calcium concentration [occasion (1) v. (3), $P<0{\cdot}01$]. The serum phosphate declined initially but had increased slightly by occasion 3. These changes in serum phosphate were not statistically significant. There was a small increase ($P<0{\cdot}05$) in the serum alkaline phosphatase level at occasion (2) but by occasion (3) it had fallen.

Serum iPTH concentration was undetectable (<0·1 ng/ml) in seven of the twelve

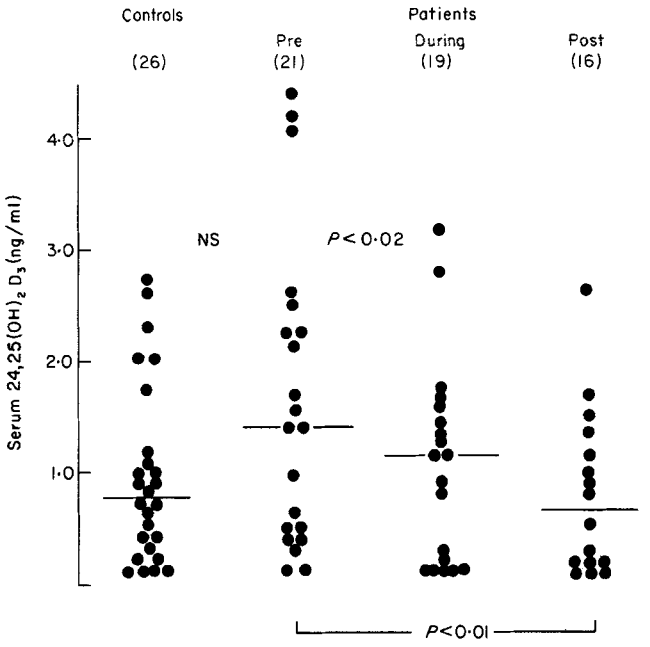

Fig. 1. Serum $24,25(OH)_2D_3$ concentrations in controls and hyperthyroid patients before and following carbimazole therapy (with median).

Table 1. Serum concentrations of thyroid hormones, calcium, phosphate, alkaline phosphatase (total) and iPTH in hyperthyroid patients before and following oral carbimazole therapy, see text for definition of occasions

	Normal values (2 × SD above and below mean)	Patients (median and range)			Occasion 1 v. 2 (P value)	Occasion 1 v. 3 (P value)
		Occasion 1	Occasion 2	Occasion 3		
T3 (nmol/l)	1·2–2·8	7 (3·3–12) ($n=21$)	2·6 (1·5–3·6) ($n=19$)	1·6 (1·1–2·8) ($n=16$)	<0·01	<0·01
T4 (nmol/l)	50–150	224 (130–320)	112 (35–195)	55 (30–112)	<0·01	<0·01
Calcium (mmol/l, corrected for albumin)	2·2–2·6	2·41 (2·3–2·72)	2·32 (2·16–2·6)	2·27 (2·08–2·42)	<0·01	<0·01
Phosphate (mmol/l)	0·7–1·4	1·21 (0·8–1·6)	1·04 (0·78–1·4)	1·1 (0·84–1·4)	NS	NS
Alkaline phosphatase (iu/l)	<10–90	94 (50–136)	102 (51–213)	67 (37–160)	<0·05	NS
iPTH (ng/ml)	<0·1–0·8	<0·1 (<0·1–0·5) ($n=12$)	<0·1 (<0·1–0·5) ($n=12$)	0·2 (<0·1–1·8) ($n=10$)	NS	NS

Table 2. Serum concentrations of vitamin D metabolites in hyperthyroid patients before and following carbimazole therapy (median and ranges)

	Control subjects	Patients Occasion 1 ($n=21$)	Patients Occasion 2 ($n=19$)	Patients Occasion 3 ($n=16$)	Controls v. patients (P values) Occasion 1	Controls v. patients (P values) Occasion 3	Patients (P values) Occasion 1 v. 2	Patients (P values) Occasion 1 v. 3
25 OH D_3 (ng/ml)	11 (4–33) ($n=26$)	12 (5–51)	12 (5–50)	9 (4–27)	NS	NS	NS	NS
$24,25(OH)_2D_3$ (ng/ml)	0·7 (<0·1–2·7) ($n=26$)	1·4 (<0·1–4·3)	1·1 (<0·1–3·2)	0·6 (<0·1–2·6)	NS	NS	<0·02	<0·01
$1,25(OH)_2D_3$ (pg/ml)	32 (13–68) ($n=23$)	22 (<2–45)	36 (12–96)	34 (13–121)	0·01	NS	<0·01	<0·01

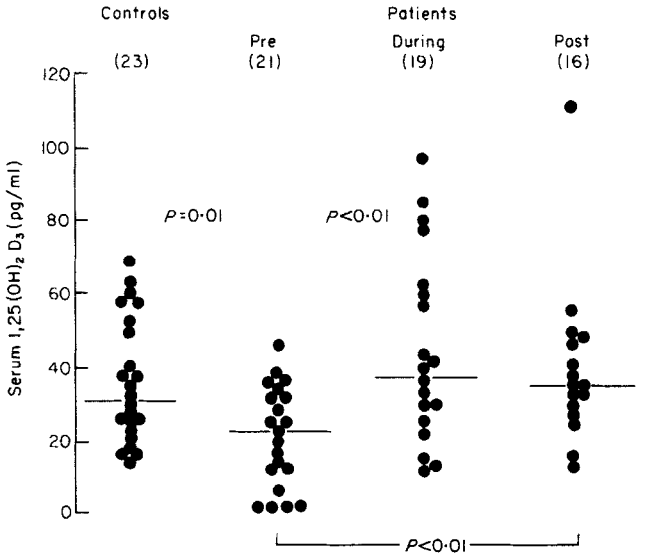

Fig. 2. Serum $1,25(OH)_2D_3$ concentrations in controls and hyperthyroid patients before and following carbimazole therapy (with median).

patients studied before treatment. Following treatment the serum iPTH concentration of the group as a whole did not increase significantly although three patients did show definite increases (iPTH levels of 0·8, 0·8 and 1·8 ng/ml at occasion 3).

The serum 25 OH D concentration was similar in the controls and the patients on the three occasions. The serum $24,25(OH)_2D_3$ concentration was higher in the patients, before treatment, compared to the controls (median 1·4 ng/ml v. median 0·7 ng/ml) although this was not statistically significant. Following treatment $24,25(OH)_2D_3$ levels fell significantly [occasion (1) v. (3), $P<0·01$], (Fig. 1).

The serum $1,25(OH)_2D_3$ concentration was lower in the patients before treatment compared with controls ($P=0·01$) (Fig. 2). Following treatment there was a marked

Table 3. Correlations between the serum vitamin D metabolites and the indices of mineral metabolism and thyroid function (twenty-one thyrotoxic patients, before treatment)

Serum concentrations	$1,25(OH)_2D_3$ (r)	$24,25(OH)_2D_3$ (r)
T3	0·07	0·66**
T4	0·12	0·55*
Calcium (corrected for albumin)	0·05	0·26
Phosphate	−0·32	0·33
Alkaline phosphatase	0·07	0·04

* $P=0·01$; ** $P<0·002$.

increase in serum $1,25(OH)_2D_3$ [occasion (1) v. (2), $P<0{\cdot}01$]. By occasion (3), serum $1,25(OH)_2D_3$ concentrations had fallen slightly. Maximum increases of serum $1,25(OH)_2D_3$ were seen in blood samples taken 12–15 weeks after starting therapy.

Table 3 shows the relationship between serum T3, T4, calcium and phosphate concentrations and the concentrations of serum $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ before treatment. Serum $24,25(OH)_2D_3$ was significantly correlated with serum T3 ($r=0{\cdot}66$, $P<0{\cdot}002$) and serum T4 ($r=0{\cdot}55$, $P=0{\cdot}01$). However, there was no correlation between serum $1,25(OH)_2D_3$ and serum T3 or T4. Of the parameters of calcium–phosphate metabolism the best correlation with these metabolites was found with serum phosphate: $1,25(OH)_2D_3$ ($r=-0{\cdot}32$) and $24,25(OH)_2D_3$ ($r=0{\cdot}33$). However, these negative and positive correlations did not reach significant levels ($P>0{\cdot}1$).

DISCUSSION

The effects of hyperthyroidism on bone and mineral metabolism have been extensively studied in man (Adams *et al.*, 1967; Smith *et al.*, 1973; Mosekilde & Christensen, 1977; Mosekilde *et al.*, 1977; Mosekilde *et al.*, 1978; Mosekilde & Melsen, 1978; Hendricks & Smeenk, 1979). Slight increases in serum calcium, phosphate and alkaline phosphatase concentrations, hypercalciuria and increased urinary excretion of hydroxyproline have been found, suggesting increased bone turnover. In the rat, direct stimulation of bone resorption by thyroid hormones has been demonstrated (Mundy *et al.*, 1976). The renal fractional reabsorption of phosphate is increased (Parsons & Anderson, 1964) and serum iPTH concentrations are reduced in hyperthyroidism (Mosekilde & Christensen, 1977). This tendency towards hypoparathyroidism is probably secondary to the mildly increased serum calcium concentrations. In the present study the changes in serum calcium, phosphate, alkaline phosphatase and iPTH concentrations, before and after antithyroid drug treatment, were very similar to the findings of previous studies.

We found no difference in serum 25 OH D_3 concentrations in the patients before treatment compared with the controls and there was no significant change after carbimazole therapy. Other studies have also shown similar serum 25 OH D_3 concentrations in hyperthyroid patients and control subjects matched for the time of the year that samples were taken (Cleeve & Brown, 1978; Bouillon *et al.*, 1980).

Serum $24,25(OH)_2D_3$ concentrations were increased in our patients before therapy, though not significantly. There was, however, a significant fall of $24,25(OH)_2D_3$ with carbimazole treatment. Serum $1,25(OH)_2D_3$ concentrations were significantly reduced in the patients before therapy and increased significantly after carbimazole. This finding may explain the reduced intestinal calcium absorption found in hyperthyroidism (Schafer & Gregory, 1972; Haldiman *et al.*, 1980). Bouillon *et al.* (1980) also reported reduced serum $1,25(OH)_2D_3$ levels in hyperthyroidism. In the same study serum vitamin D-binding protein concentrations were not significantly different from those of euthyroid controls, indication that the reduced $1,25(OH)_2D_3$ levels were not due to alterations in serum binding.

These abnormal concentrations of vitamin D metabolites in hyperthyroid patients could be due either to changes in the rate of production or rate of clearance of the individual metabolites. It is not possible to exclude the influence of altered clearance without additional data on the metabolic fate of these metabolites in hyperthyroidism. However, it is possible to speculate on mechanisms by which the production rates of the

metabolites may be altered. Direct stimulation of renal 24-hydroxylase by thyroid hormones may explain the highly significant positive correlation between pre-treatment $24,25(OH)_2D_3$ concentrations and thyroid hormone levels. There was, however, no association between thyroid hormone levels and pre-treatment $1,25(OH)_2D_3$ concentrations. The reduced levels of this metabolite were probably secondary to the action of thyroid hormone on bone. Increased intra and extracellular calcium and phosphate concentrations and reduced iPTH concentrations all lead to reduced 1-hydroxylase activity (DeLuca, 1980). It is of interest in our study that a stronger association with pre-treatment $1,25(OH)_2D_3$ levels was obtained with serum phosphate concentration than with serum calcium. This finding is supported by in-vitro data which suggest that modulations in serum phosphate may be the most powerful moderator of 1-hydroxylase (DeLuca, 1979).

The potent, bone-resorbing effect of $1,25(OH)_2D_3$ is well known (DeLuca, 1980). Claims that $24,25(OH)_2D_3$ exerts an action on mineralization of bone remain controversial (Kanis *et al.*, 1978, 1979). Certainly, at physiological concentrations, $1,25(OH)_2D_3$ exerts a considerably greater influence on bone metabolism than $24,25(OH)_2D_3$ (Raisz, 1980). The altered vitamin D metabolism seen in hyperthyroidism may be seen as part of the homeostatic response to the disturbances of mineral metabolism brought about by thyroid hormones. Thus the increased concentrations of calcium and phosphate mobilized from bone may of themselves or by the resultant secondary hypoparathyroidism stimulate the 24-hydroxylase and suppress the 1-hydroxylase enzymes. Decreased production of $1,25(OH)_2D_3$ may be beneficial both by minimizing the excessive bone turnover induced by thyroid hormones and by reducing intestinal calcium absorption.

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