Comparative absorption of vitamin D₃ and 25-hydroxyvitamin D₃ in intestinal disease

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SUMMARY The absorption of simultaneously administered equimolar doses of ¹⁴C vitamin D₃ and ³H 25 hydroxyvitamin D₃ (25-OH-D₃) has been studied in controls and patients with a variety of gastrointestinal disorders. As assessed from peak radioactivity in the serum and from faecal excretion of radioactivity, malabsorption of both vitamin D₃ and 25-OH-D₃ occurred in patients with steatorrhoea. Malabsorption of vitamin D₃ was greater than 25-OH-D₃. The magnitude of malabsorption of these compounds was related to the severity of the steatorrhoea but was moderate enough to suggest that replacement therapy in patients with intestinal malabsorption should be accomplished with relatively small doses of vitamin D. The more potent vitamin D metabolites are probably unnecessary in this situation.

Osteomalacia occurs in patients with a variety of disorders affecting the stomach and small bowel, especially when associated with steatorrhoea. The pathogenesis of this osteomalacia has in part been explained by malabsorption of vitamin D,¹ but other factors, including diminished intake of foods containing vitamin D, too little sunlight, and interruption of the enterohepatic circulation of 25-hydroxyvitamin D₃ (25-OH-D₃) have been suggested.²

Because it is widely assumed that vitamin D is poorly absorbed in malabsorptive states, parenteral therapy is often given to patients both for treatment and prophylaxis of vitamin D deficiency and it has recently been suggested that the use of vitamin D metabolites and their analogues may offer certain advantages over the parent vitamin.³ The present investigation was undertaken to study the simultaneous absorption of orally administered ³H 25-OH-D₃ and ¹⁴C vitamin D₃ in control subjects and patients with various disorders of the upper gastrointestinal tract.

Methods

PATIENTS

Clinical details are given in Table 1. Five patients with no detectable gastrointestinal disease acted as

controls. Patient 1 had osteomalacia but all tests of gastrointestinal function including jejunal biopsy were normal. In two of the three patients with coeliac disease osteomalacia was present despite normal or only marginally raised faecal fat excretion. Of five patients with gastrectomy (of the polya type except when stated), four had osteomalacia and in two this was associated with steatorrhoea. Five patients had had extensive small bowel resection resulting in steatorrhoea, two of these had clinical osteomalacia (patients 14 and 15). Informed consent was obtained from all patients.

MATERIALS

25-[26, 27-³H] 25 hydroxyvitamin D₃ (12.2 Ci/mmol) and [4-¹⁴C] vitamin D₃ (32 Ci/mmol) were obtained from the Radiochemical Centre Amersham. Unlabelled vitamin D₃ was obtained from Koch-Light Laboratories Limited and 25-OH-D₃ from Roussel.

EXPERIMENTAL DESIGN

Each patient received by mouth 25 µg (2 uCi) [4-¹⁴C] vitamin D₃ and 26 µg (8 uCi) 25-[26,27-³H] 25-OH-D₃ dissolved in 1.0 ml ethanol and mixed with 200 ml of milk after a light breakfast. Blood samples were drawn two hourly for eight hours and then at regular intervals for 13 days after the administration of the tracers. Faeces were collected for the four days and urine for the three days after the ingestion of the vitamin preparations. All patients received a standard 90 g fat diet with the

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Table 1 Clinical details

Patient no.	Clinical diagnosis	Age (yr)	Sex	Weight (kg)	Faecal fat (mmol/d)	Serum		P	Alkaline phosphatase (IU/l)	Bone histology
						25 OH-D	Ca			
						(ng/ml)	(mmol/L)			
Controls										
1	Nutritional osteomalacia	40	F	30	7	3.5	1.95	0.6	320	—
2	Primary hyperparathyroidism	50	F	71	3.5	11.7	2.9	0.8	70	—
3	Obesity	64	F	81	10.5	13	2.4	1	40	—
4	Osteoporosis	66	F	54	7	7	2.45	1.3	60	Osteoporosis
5	Renal calculus	41	M	71	17.5	7	2.5	1	80	—
Coeliac disease										
6	Osteomalacia	56	M	48	11	3.1	1.52	1.12	168	Osteomalacia
7	No clinical bone disease	66	M	55	70	7.2	2.1	1.4	185	No calcification defect
8	Osteomalacia	56	F	48	21	2.5	1.3	1.3	150	Osteomalacia
Partial gastrectomy										
9	Osteoporosis	60	F	50	7	8.3	2.4	1.3	80	Osteoporosis
10	Osteomalacia and steatorrhoea	73	M	51	45.5	2.7	2.1	0.8	256	Osteomalacia
11	Osteoporosis and osteomalacia	66	F	35	7	3.8	2.3	0.8	110	Osteoporosis and osteomalacia
12	Steatorrhoea treated									
	osteomalacia	82	F	46	28	17	2.1	1	154	Healing osteomalacia
13	Total gastrectomy osteomalacia	60	M	48	7	4.8	2.4	0.7	300	Osteomalacia
Short bowel										
14	Small bowel infarct ileum and distal jejunum resected	61	F	54	137	2.5	1.7	1.1	337	Osteomalacia
15	Crohn's disease. Distal ileum and colon resected	63	F	43	140	3.3	2.1	0.9	235	—
16	Crohn's disease. Colon and ileum resected	40	F	48	196	3.6	2.4	1.1	80	—
17	Crohn's disease. Colon and ileum resected	40	M	61	126	11.4	2.4	0.8	76	—
18	TB 140 cm of distal ileum resected	47	F	34	130	60	2.4	1.4	40	—

exception of patient 14 (70 g) and patients 15–18 who took diets in excess of 90 g/day. These diets were started at least five days before the study.

ANALYTICAL PROCEDURES

Serum samples were extracted and chromatographed on Sephadex LH 20 using 50:50 chloroform/hexane as solvent, to separate vitamin D₃ from 25-OH-D₃ as previously described.⁴

Urinary radioactivity was measured by counting triplicate 1 ml samples from each 24-hour collection. Faecal homogenates were extracted and the radioactivity determined using the methods described by Krawitt *et al.*⁴ Quenching of pigmented samples was usually corrected for by the use of an external standard, severely quenched, samples were corrected additionally by adding an internal standard.

Serum levels of 25 hydroxyvitamin D (25-OH-D) were measured by a competitive protein binding assay modified from Haddad and Chyu.⁵ Normal values in Manchester 9–60 ng/ml in summer, 6–28 ng/ml in winter. Statistical difference between groups was assessed by the Mann-Whitney U test,⁶ and paired *t* test was used when applicable.

Results

ASSESSMENT OF ABSORPTION OF VITAMIN D₃ AND 25-OH-D₃

SERUM RADIOACTIVITY

The appearance of radioactivity in the serum at varying time intervals after ingestion of the vitamin preparations is shown in Figs 1 and 2. Radioactivity appearing in the serum from the absorption of vitamin D₃ was assessed by measuring unchanged ¹⁴C vitamin D₃ and ¹⁴C-labelled 25-OH-D₃ formed from it; radioactivity in other metabolite fractions was quantitatively insignificant. ³H radioactivity was measured in the form of unchanged 25-OH-D₃, polar metabolites forming an insignificant proportion of the total radioactivity. The range of activity found in the five control subjects and the mean values $\pm$ SEM for the patient groups have been plotted. All serum data have been corrected to a body weight of 70 kg in view of the discrepancies in the weights of the subjects. This correction tended to exaggerate differences between the serum data of control patients and the other groups by the very nature of the greater body mass of the control group.

Fig. 1 Serum radioactivity mean $\pm$ SEM following ^{14}C vitamin D₃ ingestion. ● Partial gastrectomy; ○ coeliacs; + short bowel patients.

Vitamin D₃ (Fig. 1)

Apart from a significantly higher level of ^{14}C radioactivity in the serum of the partial gastrectomy group at two hours ($P < 0.05$), there was no difference between the pattern of serum radioactivity in this group of patients and controls. The mean concentration of radioactivity in the coeliac patients was lower than the lower limit of the control group but the difference was not significant ($0.05 < P < 0.1$), while in patients with small intestinal resection serum radioactivity was always significantly less than in the control subjects ($P < 0.02$).

The concentration of ^{14}C vitamin D₃ reached a maximum between six and 24 hours after ingestion and differences in the time course between various groups of patients were insignificant (Table 2).

25-hydroxyvitamin D₃ (Fig. 2)

No significant difference existed in the pattern of serum ^3H activity between the control group and patients with coeliac disease. There was significantly lower ^3H activity ($P < 0.05$) at eight and 24 hours in the partial gastrectomy group and throughout the study in the short bowel patients ($P < 0.05$).

The maximum level of radioactivity occurred between four and 12 hours after ingestion of ^3H 25-OH-D₃ and in the partial gastrectomy patients this peak occurred significantly earlier ($P < 0.05$) than in controls (Table 2).

The maximum concentration of ^3H 25-OH-D₃ was always higher than that of ^{14}C vitamin D₃ and was reached significantly earlier ($P < 0.01$).

FAECAL RADIOACTIVITY

The net intestinal absorption of ^{14}C vitamin D₃ and ^3H 25-OH-D₃ is shown in Table 2; it can be seen that only the short bowel group of patients differed from the controls. The relationship between absorption of vitamin D₃ and ^3H 25-OH-D₃ and faecal fat excretion in the individual patients was examined. A strong and significant negative relationship exists in those patients with steatorrhoea, faecal fat excretion > 20 mmol/day (Fig. 3) but not in patients with normal faecal fat excretion.

In individual patients with malabsorption the net intestinal absorption of ^3H 25-OH-D₃ was significantly greater than that of ^{14}C vitamin D₃ ($P < 0.01$). Although there was a tendency for ^3H 25-OH-D₃ absorption to be greater than ^{14}C vitamin D₃ where the faecal fat excretion was normal, the differences were insignificant ($P > 0.1$).

In three patients (7, 8, 13) with apparent normal net intestinal absorption of ^{14}C vitamin D₃, the peak serum radioactivity was subnormal; conversely, patient 16 had a normal peak serum radioactivity but a net intestinal absorption of ^{14}C vitamin D₃ of 49.9%. Similar variations occurred with ^3H 25-OH-D₃ (Table 2).

Table 2 $^{14}\text{C D}_3$ and $^3\text{H 25-OH-D}_3$ levels in patients and controls

Patient no.	$^{14}\text{C D}_3$				$^3\text{H 25-OH-D}_3$			
	Net absorption (% dose)	Peak level ^{14}C in serum (% dose/l)	Time (h) of peak radioactivity	^{14}C in urine 0-3 day (% dose)	Net absorption (% dose)	Peak level ^3H in serum (% dose/l)	Time (h) of peak radioactivity	^3H in urine 0-3 day (% dose)
Controls								
1	99.1	3.68	12	3.4	97.4	6.1	6	1.9
2	96.9	3.02	8	2.0	97.0	12.26	8	2.1
3	69.8	3.18	24	3.3	81.1	9.02	8	1.8
4	80.3	4.8	12	3.3	91.0	11.89	6	1.7
5	83.0	5.39	24	3.0	100.0	12.3	8	1.4
Mean	85.8	4.01	16	3.0	93.3	10.30	7.2	1.8
SEM	5.5	0.47	3.35	0.3	3.4	1.36	0.5	0.1
Celiacs								
6	80.9	4.3	8	—	93.8	11.9	8	—
7	68.4	1.28	12	2.58	87.6	7.56	6	3.04
8	86.2	2.35	12	2.35	95.4	5.67	12	2.81
Mean	78.5	2.64	10.67	—	92.3	8.38	8.67	—
SEM	5.3	0.88	1.33	—	2.4	1.84	1.77	—
P	NS	NS	NS	—	NS	NS	NS	—
PGX								
9	94.5	5.03	12	—	76.4	7.71	6	—
10	81.4	4.3	12	3.5	97.2	8.43	4	1.7
11	85.1	4.68	6	2.8	97.4	9.1	6	2.7
12	80.5	3.2	12	1.3	80.7	4.87	6	5.7
13	100	2.2	8	2.6	100.0	5.52	4	1.94
Mean	88.3	3.88	10	2.6	90.3	7.13	5.2	3.0
SEM	3.8	0.45	1.27	0.45	4.9	0.82	0.5	0.9
P	NS	NS	NS	NS	NS	<0.05	<0.05	NS
Short gut								
14	56.0	0.93	24	0.7	71.6	5.0	6	2.53
15	56.2	2.64	12	2.1	76.3	5.68	6	1.5
16	49.9	3.13	12	1.5	62.5	6.13	6	0.6
17	60.5	2.63	24	2.2	62.9	7.43	6	1.6
18	41.1	1.83	12	1.4	68.4	3.86	12	0.9
Mean	52.7	2.23	16.8	1.6	68.3	5.62	7.2	1.4
SEM	3.4	0.39	2.9	0.3	2.6	0.6	1.2	0.3
P	<0.005	<0.02	NS	<0.02	<0.005	<0.02	NS	NS

Fig. 2 Serum radioactivity mean $\pm$ SEM following $^3\text{H 25-OH-D}_3$ ingestion. ● Partial gastrectomy; ○ celiacs; + short bowel patients.

Fig. 3 Relationship between faecal fat excretion and net intestinal absorption of vitamin D₃ (+—+ $r=-0.9$) and 25-OH-D₃ (o—o $r=-0.85$) in those patients with steatorrhoea.

URINARY RADIOACTIVITY

Urinary radioactivity was all water soluble and much more polar than vitamin D₃ or its hydroxylated metabolites. The patients with short bowel excreted significantly less radioactivity in the urine compared with the other groups of patients (Table 2).

Discussion

The concentration of radioactivity from administered vitamin D₃ or 25-OH-D₃ that is measured in serum (Table 2) is the resultant of several processes, absorption, distribution between body tissues, metabolism, and excretion.⁷ The measured concentration of serum radioactivity may reflect many processes in addition to intestinal absorption and the influence of such processes may account for the discrepancy observed in some patients between faecal and serum measurements of radioactivity. This has been noted previously during studies of absorption of ³H-labelled vitamin D₃,¹⁻⁸ but assessment of ³H 25-OH-D₃ absorption using both faecal and serum analysis has not been previously reported.

Urinary excretion is not normally quantitatively important for vitamin D₃ and its metabolites. Except in the case of the nephrotic syndrome urinary excretion is in the form of polar metabolites probably glucuronides or sulphates.⁹ In the present study urinary excretion was normal except in the short bowel group where it was low, reflecting diminished intestinal absorption.

Since the main metabolic pathway for vitamin D₃ is its conversion to 25-OH-D₃ it follows that ad-

ministered 25-OH-D₃ is metabolised to far less an extent than is vitamin D₃. The serum is considered to constitute the major pool of 25-OH-D₃ in the body, and has a binding capacity far in excess of physiological requirements.¹⁰ The more polar structure of 25-OH-D₃ by comparison with the native vitamin probably makes it less likely to be taken up by fatty tissues.⁷ This, together with the much greater affinity of the serum binding protein for 25-OH-D₃,¹⁰ and the longer serum half life,⁷ probably ensures that proportionately more of the 25-hydroxylated derivative remains in serum at any given time than does vitamin D₃.

Differences in polarity between vitamin D₃ and 25-OH-D₃ may also affect the manner of absorption of the two forms. In the rat, 25-OH-D₃ is absorbed more proximally in the small bowel and is not as dependent upon bile and micelle formation for its absorption as is vitamin D₃.¹¹ Our data support this experimental evidence. In view of the differences we have shown between the absorption of ¹⁴C vitamin D₃ and ³H 25-OH-D₃ in certain of the patients with steatorrhoea, we do not believe that a normal 25-OH-D₃ absorption test¹² necessarily excludes an impairment in the absorption of vitamin D₃. It is theoretically possible to have malabsorption of vitamin D₃ and normal absorption of 25-OH-D₃.

Thompson and colleagues,¹⁻⁸ having given 0.5–1.0 mg doses of ³H-labelled vitamin D₃ to seven control subjects, showed that the net intestinal absorption of this compound varied from 62.4% to 91.3%. Serum radioactivity reached a peak between six and 12 hours. Malabsorption was found in coeliac disease with varying degrees of steatorrhoea and four of five patients with post-gastrectomy osteomalacia and steatorrhoea. Our control data using smaller doses of vitamin D₃ agree with those of Thompson *et al.*¹ In the present study, however, the two patients with post-gastrectomy steatorrhoea and three patients with coeliac disease had normal net intestinal absorption of vitamin D₃, only patient 7 having a reduced serum peak radioactivity. As four of these five patients had osteomalacia, it is difficult to believe that malabsorption of vitamin D was of prime importance in its aetiology. Other factors, including poor dietary intake of vitamin D and lack of solar exposure, may have been more relevant. It is of interest that in a recent paper by Poskitt *et al.*¹³ the serum 25-OH-D values in 11 healthy adults were found to be determined more by exposure to sunlight (25-OH-D₃ 9.8 (±9.2) ng/ml) than by dietary intake (25 hydroxy-vitamin D₂ 2.4 (±1.0) ng/ml) even in mid-winter. It would seem reasonable to suggest, therefore, that patients with gastro-intestinal disease who develop osteomalacia do so because of poor solar exposure, perhaps consequent

upon chronic ill health, and that malabsorption of vitamin D, if present, is an aggravating factor.

Even in the presence of the gross steatorrhoea found in the short bowel group of patients, at least 40% of the ^{14}C vitamin D_3 and 60% of ^3H 25-OH- D_3 was absorbed and, while in this situation malabsorption of vitamin D may contribute to the development of vitamin D deficiency and osteomalacia, the provision of vitamin D in slightly larger doses than are used in simple vitamin D deficiency should suffice in the management of these patients. Since 450 units of vitamin D_2 are more than enough to correct simple vitamin D deficiency, 900 units/day were considered sufficient for patients with malabsorption.

An excellent clinical and biochemical response followed treatment with 900 units of vitamin D_2 in patient 15, achieving serum 25-OH-D concentrations of 20–30 ng/ml and, although patient 14 initially received 50 000 units daily of vitamin D_2 , the serum 25-OH-D concentration remains above 20 ng/ml on prolonged treatment with 900 units of vitamin D_2 daily.

The present data show that malabsorption of vitamin D is not a universal problem in patients with intestinal disease, and that vitamin D deficiency can be cured or prevented by relatively small oral doses of vitamin D_2 . Such treatment avoids the necessity of regular injections and the hazards of hypercalcaemia from large oral doses of vitamin D or the newer more potent vitamin D metabolites.

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