Vitamin D absorption in healthy subjects and in patients with intestinal malabsorption syndromes[1-3]

Clifford W Lo, MD, MPH, Philippe W Paris, MD, Thomas L Clemens, PhD, Joyce Nolan, AB, and Michael F Holick, PhD, MD

ABSTRACT We developed a test procedure for the clinical evaluation of the absorption of vitamin D. Serum vitamin D concentrations were evaluated in seven patients with intestinal fat malabsorption syndromes and in seven healthy, normal subjects, after being given a single oral dose of 50,000 IU (1.25 mg) vitamin D_2. In the normal subjects, serum vitamin D concentrations rose from a baseline of less than 5 ng/ml to a peak of over 50 ng/ml by 12 h, gradually falling to baseline levels by 3 days. In five of the seven patients with intestinal fat malabsorption, oral administration of 50,000 IU vitamin D_2 did not raise serum vitamin D concentrations above 10 ng/ml. Two patients with severe inflammatory bowel disease had a normal absorption pattern, however. These findings suggest that an oral vitamin D absorption test may be of value for determination of patients at risk for development of vitamin D deficiency. They also raise questions about the efficacy of oral vitamin D preparations in patients with intestinal fat malabsorption. *Am J Clin Nutr* 1985;42:644–649.

KEY WORDS Vitamin D, 25-hydroxyvitamin D, ergocalciferol, cholecalciferol, vitamin D absorption, malabsorption, inflammatory bowel disease, cystic fibrosis

Introduction

In the early years of this century, long before the identification of vitamin D, Pavlov and Wisner and Whipple had noticed that fat malabsorption secondary to biliary obstruction led to osteoporosis (1, 2). Investigations in the 1930s by Heymann (3–6) demonstrated that the serum of dogs with biliary fistulas lacked antirachitic activity when injected into rats. Kodicek (7) traced ^{14}C-vitamin D_2 from its absorption in the small intestine to its localization in the liver, kidney, and bone. Similar studies by Norman and DeLuca (8) using tritiated vitamin D_2 and D_3 laid the foundation for our current understanding of vitamin D metabolism. In particular, studies in rats (9) demonstrated that vitamin D_3 was absorbed via the lymphatics, supporting previous work on the necessity of bile secretions for optimal absorption. Thompson et al (10) showed that orally administered tritiated vitamin D_3 was malabsorbed in patients with celiac disease, biliary obstruction, or pancreatic dysfunction (11, 12). In five patients with celiac disease, less than 50% of radioactivity was absorbed after administration of 1 mg of ^{3}H-vitamin D_3. In three patients with chronic pancreatitis, absorption was less than 18%, and in two patients with biliary obstruction, absorption was 0 and 28%. In contrast, vitamin D absorption was greater than 60% in 9 control subjects.

Despite this evidence of vitamin D malabsorption in patients with gastrointestinal or liver disease, the vitamin status of these pa-

[1] From the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA; and the Vitamin D Laboratory, Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

[2] Supported by US Department of Agriculture Grant 82-CRCR-1-1070 and US Public Health Service Grants PO1-AG04390, 5-T32-8M-07070, and General Clinical Research Center Grant RR-00088 from the National Institutes of Health.

[3] Address reprint requests to: Michael F Holick, PhD, MD, USDA Human Nutrition Research Center at Tufts University, 711 Washington St, Boston, MA 02111.

Received February 11, 1985.
Accepted for publication March 26, 1985.

tients is often neglected. Although vitamin D supplements are often prescribed, adequate absorption of these pharmacological doses has not been documented.

Patients and methods

After approval from the Human Studies Subcommittee of the Massachusetts General Hospital and with informed consent, seven patients with intestinal fat malabsorption and seven normal control subjects were each given a single 50,000 IU (1.25 mg or 3250 nmol) oral dose of vitamin D_2 in capsule form (Eli Lilly, Inc). Initially, doses of 10,000 IU (650 nmol), 25,000 IU (1625 nmol), and 50,000 IU of vitamin D_2, dissolved in 0.5 ml of 100% ethanol and evaporated on bread, were given to one of the subjects to determine a dose–response curve. All subjects were instructed to avoid excessive exposure to direct sunlight for 1 wk before and during the study. None of the subjects were taking anticonvulsant medications. Six of the seven patients required total parenteral nutrition and were receiving nothing by mouth; the other patient and the seven control subjects were given vitamin D_2 after an overnight fast.

Patients (Table 1)

Patient A, a 19-yr-old man, had a 3-yr history of Crohn's ileocolitis, presenting as diarrhea and a 20-pound weight loss. Barium enema and upper gastrointestinal series showed a narrowed cecum and severe involvement of the terminal ileum. The patient's course was complicated by a perianal fistula and a persistent inflammatory abscess in the right lower quadrant. In March 1983, a severe exacerbation of the inflammatory bowel disease was unresponsive to high doses of steroids and total parenteral nutrition. A vitamin D challenge test was performed just before he underwent an ileocolectomy. Baseline serum vitamin D concentration was 8 ng/ml and initial serum 25-hydroxyvitamin D concentration was 30 ng/ml.

Patient B, a 21-yr-old man with cystic fibrosis, had had a relatively mild course, with only three hospital admissions for pulmonary infections. A vitamin D challenge test was performed while he was taking his regular pancreatic enzyme supplements for steatorrhea. Baseline serum vitamin D concentration was less than 1 ng/ml and initial serum 25-hydroxyvitamin D concentration was 20 ng/ml.

Patient C, a 24-yr-old man, had a 5-yr history of Crohn's ileocolitis, with biopsy demonstration of crypt abscesses and focal inflammation of the sigmoid colon. An upper gastrointestinal series showed extensive ileal disease. The patient did very well until he was admitted with abdominal cramps and bloody diarrhea in August 1982. He received intravenous steroids and was placed on total parenteral nutrition, and a vitamin D challenge test was performed. Baseline serum vitamin D concentration was 4 ng/ml and initial serum 25-hydroxyvitamin D concentration was 10 ng/ml. He responded rapidly to therapy, was allowed to eat, and was discharged after 6 days.

Patient D, a 49-yr-old woman, had a 10-yr history of diarrhea and weight loss. Extensive investigation indicated small intestinal villous atrophy and chronic mucosal inflammation without a specific diagnosis. She absorbed only 70% of a 100 g daily fat diet. While receiving total parenteral nutrition, a vitamin D challenge test was performed. Baseline serum vitamin D concentration was undetectable (less than 1 ng/ml) and initial serum 25-hydroxyvitamin D concentration was 34 ng/ml.

Patient E, a 58-yr-old male, had a history of severe intestinal malabsorption secondary to scleroderma. While he was receiving total parenteral nutrition, a vitamin D challenge test was performed. Baseline serum vitamin D concentration was 1 ng/ml.

Patient F, a 24-yr-old woman, had a 7-yr history of inflammatory bowel disease, initially diagnosed as Crohn's disease. However, when she was readmitted for abdominal pain and diarrhea, review of the colonoscopy findings led to a change of diagnosis to ulcerative colitis. While she was receiving total parenteral nutrition, a vitamin D challenge test was performed. Baseline serum vitamin D concentration was undetectable and initial serum 25-hydroxyvitamin D concentration was 21 ng/ml. A colectomy was performed soon afterward, and she has been asymptomatic since that time.

Patient G, a 22-yr-old woman, had a 5-yr history of Crohn's disease involving the small intestine from the second portion of the duodenum to the ileocecal valve and also the colon, for which a right colectomy had been performed. Subsequently, a recto-jejunal fistula developed, and she was admitted for a 3-wk course of total parenteral nutrition. A vitamin D challenge test was performed just before surgical resection of the fistula. Baseline serum vitamin D concentration was 2 ng/ml and initial serum 25-hydroxyvitamin D concentration was 37 ng/ml.

Methods

Serum samples were obtained at 0, 4, 8, 12, 24, 48, and 72 h afterward. In two of the control subjects, serum sam-

TABLE 1
Baseline serum vitamin D and 25-hydroxyvitamin D concentrations in patients with intestinal fat malabsorption syndromes

Pt	Age	Sex	Disease	Duration	TPN	Vit D	25-OH-D
A	19	M	Crohn's	3 yr	Yes	8 ng/ml	30 ng/ml
B	21	M	Cystic fibrosis		No	1	20
C	24	M	Crohn's	5 yr	Yes	4	10
D	49	F	Villous atrophy	10 yr	Yes	1	34
E	58	M	Scleroderma		Yes	1	
F	24	F	Ulcerative colitis	7 yr	Yes	1	21
G	22	G	Crohn's	5 yr	Yes	2	37

ples were followed for up to 2 wk after the dose. These samples were stored at −20°C in glass vials under argon gas. All samples from each patient were extracted and chromatographed in the same batch by the method of Clemens et al (13). To each 3-ml serum sample, 1000 cpm of tritiated vitamin D_3 was added for subsequent determination of recovery during extraction and chromatography. After equilibration for 30 min, lipids were extracted with methylene chloride and methanol. The lipid extract was redissolved in 7% ethylacetate in n-hexane and applied to a silica Sep-Pak cartridge (Waters Associates, Milford, MA) for preparative chromatography (14). The vitamin D fraction was eluted in 30 ml of 7% ethylacetate in hexane.

This fraction was then further purified by initial reverse-phase, high-performance liquid chromatography (HPLC) on a Radial-Pak-A column (Waters Associates) in 2% water in methanol. Reverse-phase HPLC allowed differentiation between vitamins D_2 and D_3, but both fractions were collected. A second normal-phase HPLC on a Zorbax-SIL column (Dupont Instruments, Wilmington, DE) with 2% isopropanol in n-hexane allowed quantitation of total vitamin D_2 and D_3 by ultraviolet absorbance at 254 nm. Peak areas were compared with a standard curve of known concentrations of vitamin D_2, and corrections for recovery were made by counting the remaining tritiated vitamin D_3 in a beta liquid scintillation counter.

Determinations of 25-hydroxyvitamin D concentrations in serum samples were performed by a modified competitive protein-binding assay (15).

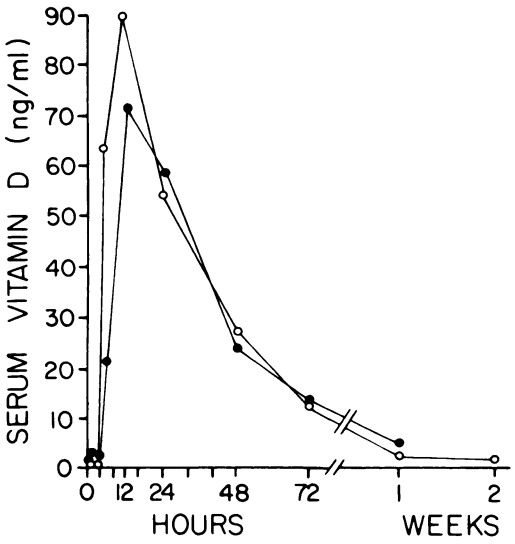

FIG 2. Serum vitamin D concentrations in two normal control subjects after ingestion of a single capsule of 50,000 IU (1.25 mg) vitamin D_2. Peak concentrations of vitamin D increased to over 50 ng/ml by 12 h and declined to baseline by 1 to 2 wk. Note that peak serum concentrations were lower when equivalent doses of vitamin D were given in capsule form instead of in ethanol.

Results

Initially, one normal volunteer was given various doses of vitamin D in ethanol to determine a dose–response curve (**Fig 1**). Peak serum vitamin D concentrations at 12 h after 10,000 IU, 25,000 IU, and 50,000 IU of vitamin D_2 were 11 ng/ml, 43 ng/ml, and 141 ng/ml respectively. To be certain that administration in a capsule form did not alter the kinetics, we next determined the time course for the appearance of vitamin D in the serum after a single capsule of 50,000 IU of vitamin D_2 in two normal volunteers. The initial serum vitamin D concentrations were below 10 ng/ml and began to rise in the blood within 4 h. By 12 h, the peak vitamin D concentrations reached 70 and 80 ng/ml, respectively, before gradually declining to baseline levels by 7 days (see **Fig 2**). Five additional subjects showed similar absorption curves, tested over 3 days. In all seven normal subjects, the serum levels of vitamin D rose to a 12-h maximum of over 50 ng/ml, more than 10 times their baseline values. This was in marked contrast to results in five patients with clinical fat malabsorption secondary to inflammatory bowel disease (patients A and C), cystic fibrosis (patient B), scleroderma (patient E), and mucosal villous atrophy (patient D) who showed essentially no rise from their basal serum levels of vitamin D (see **Fig 3**). All values at 4, 8, 12, 24, 48,

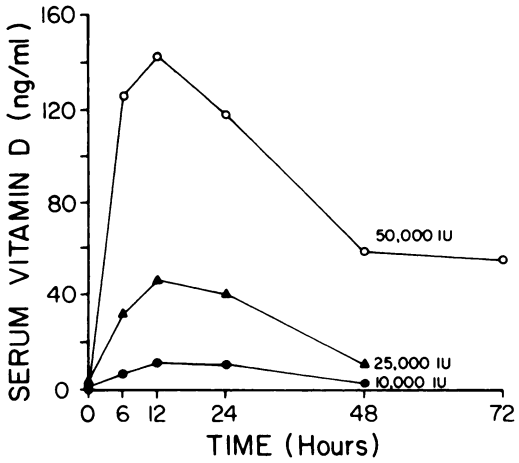

FIG 1. Serum vitamin D concentrations in a normal control subject after 50.000 IU (— ○ —), 25,000 IU (— ▲ —), and 10,000 IU (— ● —) of vitamin D_2 in 100% ethanol, evaporated on toast.

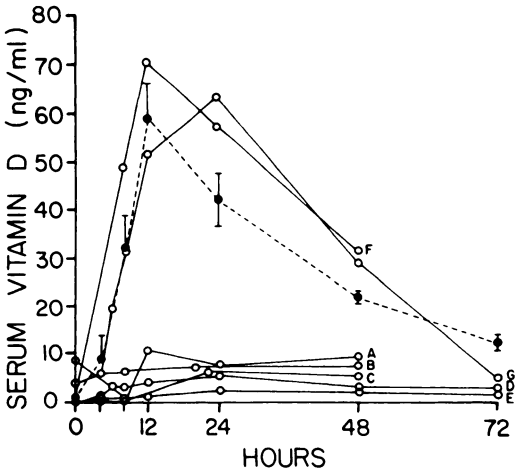

FIG 3. Serum vitamin D concentrations in seven patients with intestinal fat malabsorption syndromes after a single oral dose of 50,000 IU (1.25 mg) of vitamin D_2. For comparison, the means and standard errors of vitamin D concentrations measured in seven normal control subjects after a similar dose are indicated by the closed circles and dotted lines (- - ● - -). Note that two patients, one with Crohn's ileocolitis (patient F) and one with ulcerative colitis (patient G), had essentially normal absorption curves. Five patients, however, showed a dramatic lack of response, with no values above 10 ng/ml.

and 72 h after a single oral dose of 50,000 IU of vitamin D_2 were below 7.2 ng/ml.

Two patients with severe inflammatory bowel disease and diarrhea, one with ulcerative colitis (Patient F), and one with Crohn's ileocolitis (Patient G), both of whom required total parenteral nutrition and subsequent ileocolectomy, showed a normal rise in serum vitamin D concentrations to 60 ng/ml after oral challenge. Serum concentrations of 25-hydroxyvitamin D were all within normal limits in both patients and control subjects and were not significantly affected by the single oral dose of vitamin D_2.

Discussion

There is no direct method currently available to quantitate vitamin D malabsorption in patients with gastrointestinal diseases, other than using radiolabeled vitamin D_3. Decisions to supplement fat-soluble vitamins are usually made without a determination of whether oral doses are adequately absorbed. Previous studies of vitamin D absorption in humans have been hampered by the lack of an adequate clinical assay for this fat-soluble prohormone, which is present in only trace quantities in the blood. Indirect evidence of vitamin D malabsorption (osteomalacia, rickets, hypocalcemia, hypophosphatemia, or reduced circulating concentrations of 25-hydroxyvitamin D) persists despite routine vitamin D supplementation in cystic fibrosis (16, 17), Crohn's disease (18), intestinal resection (19–22), liver disease (23–26), and other malabsorption syndromes (27).

Many factors are involved in the absorption of vitamin D, including gastric, pancreatic, and biliary secretions, micellar formation, diffusion through the unstirred-water layer, brush-border-membrane uptake, and transport out of the intestinal cell (28–30). Because vitamin D is a relatively nonpolar sterol, it must be solubilized by incorporation into bile-salt micellar solutions in order to be absorbed in the aqueous phase (31). This process is severely inhibited if there is any interruption of normal pancreatic or biliary secretions. Because the fat-soluble vitamins are fairly sensitive to disturbances in lipid absorption, vitamin D malabsorption may occur even in the absence of clinical steatorrhea.

One of the major reasons that little is known about the absorption of vitamin D in humans is the difficulty of measuring the circulating concentrations of vitamin D in the blood. Although a competitive protein-binding assay exists for 25-hydroxyvitamin D, this metabolite is produced only after hydroxylation in the liver. Serum concentrations of 25-hydroxyvitamin D are good indicators of long-term vitamin D stores in the body but are insensitive to single doses of vitamin D and do not rise out of the normal range unless large doses of vitamin D are chronically administered (32).

Indeed, an oral vitamin D challenge test may be an excellent test for malabsorption of the fat soluble vitamin D, because it directly measures serum concentrations of this secosterol. A single serum sample 12 h after oral ingestion of a single 50,000 IU capsule of vitamin D will readily discriminate between normal absorption (serum level greater than 50 ng/ml) and malabsorption (serum level less than 10 ng/ml). In contrast, serum 25-hydroxyvitamin D concentrations are much less

responsive to oral vitamin D challenges. They are useful for determining whether a vitamin D deficiency state exists, but do not establish whether vitamin D is actually malabsorbed. Tolerance tests measuring serum 25-hydroxyvitamin D after an oral challenge dose of 25-hydroxyvitamin D may not be a sensitive or valid test of vitamin D absorption because 25-hydroxyvitamin D is more polar than vitamin D is and may be absorbed into the portal blood even in the absence of bile salts or micelles (33–35). In rats, up to 78% of enterally administered 25-hydroxyvitamin D was absorbed in the absence of bile acids, whereas less than 30% of a vitamin D dose was absorbed under similar conditions (36).

It is believed that vitamin D is absorbed in the proximal segments of the intestine; therefore it is not surprising that malabsorption of vitamin D was not shown in two patients whose inflammatory bowel disease was limited to the distal segments of the bowel. However, both patients had severe diarrhea and underwent subsequent surgical resection within 1 wk of the vitamin D challenge. In contrast, other patients with Crohn's disease and cystic fibrosis did not show any absorption of vitamin D, even though their disease activities were relatively mild, not requiring more than a few days' hospitalization. These examples highlight the unpredictability of vitamin D absorption in patients with intestinal disorders, which does not seem to be correlated with the extent of inflammatory involvement or other clinical parameters. Unfortunately, no correlation with quantitative fat absorption could be made for these patients, as most were receiving total parenteral nutrition.

The peak serum vitamin D concentration in every normal subject was not reached until 12 h after ingestion of the capsule. Although this may have been caused partly by the delayed release of vitamin D from the gelatin capsule, peak concentrations at 12 h were also seen when vitamin D_2 was administered in ethanol. This delay may represent some processing of the vitamin D in the liver before it is released into the systemic circulation (37).

The lack of frank vitamin D deficiency in our patients with malabsorption syndromes, as reflected by normal serum 25-hydroxyvitamin D concentrations, may be the result of adequate exposure to sunlight or of the vitamin D supplements provided by total parenteral nutrition solutions. Patients on total parenteral nutrition routinely receive 500 IU (12.5 μg) of vitamin D_2 per l of solution (usually 1500 IU for an adult) on a weekly basis, which is sufficient to maintain their serum 25-hydroxyvitamin D in the normal range, but would not raise their serum vitamin D concentration by more than 2 ng/ml. Although some vitamin D may have been absorbed even in those subjects who did not show any rise in their serum baseline levels, our assay is sensitive down to less than 1 ng/ml, and therefore the magnitude of any rise not detected by this assay was less than 2% of a normal response.

In summary, we have developed a provocative oral vitamin D challenge test, by measurement of serum vitamin D levels 12 to 24 h after a single oral dose of 50,000 IU of vitamin D_2. We have used this test to evaluate vitamin D absorption in patients with intestinal fat malabsorption syndromes, and have found that while patients often do not absorb vitamin D, this cannot be predicted by the severity of their clinical symptoms. Hence, it may be particularly important to test vitamin D absorption in patients with intestinal fat malabsorption to ensure adequate vitamin D nutrition. This test may be a simple indirect measure of fat malabsorption, and clinical studies under controlled conditions are underway to compare vitamin D absorption and fat absorption.

The authors thank Dr JM Richter and the Gastrointestinal Unit of the Massachusetts General Hospital for their help in clinical studies.

References

1. Cited in Greaves JD, Schmidt CLA. The role played by bile in the absorption of vitamin D in the rat. J Biol Chem 1933;102:101–12.
2. Wisner FP, Whipple GH. Variations in the output of bile salts and pigments during 24-hour periods. Am J Physiol 1922;60:119–33.
3. Heymann W. Metabolism and mode of action of vitamin D. II. Storage of vitamin D in different tissues in vivo. J Biol Chem 1937;118:371–6.
4. Heymann W. Metabolism and mode of action of vitamin D. IV. Importance of bile in the absorption and excretion of vitamin D. J Biol Chem 1937;122:249–56.
5. Heymann W. Metabolism and mode of action of vi-

tamin D. V. Intestinal excretion of vitamin D. J Biol Chem 1937;122:257–62.
6. Heymann W. Further studies on the absorption, excretion and mode of action of vitamin D. J Biol Chem 1937;119:xlviii.
7. Kodicek E. The fate of ^{14}C-labeled vitamin D_2 in rats and infants. In: Garratini S, Paoletti R, eds. Drugs affecting lipid metabolism. Amsterdam: Elsevier, 1961: 515–9.
8. Norman AW, DeLuca HF. The preparation of ^{3}H-vitamins D_2 and D_3 and their localization in the rat. Biochemistry 1963;2:1160–8.
9. Schachter D, Finkelstein JD, Kowarski S. Metabolism of vitamin D. I. Preparation of radioactive vitamin D and its intestinal absorption in the rat. J Clin Invest 1964;43:787–95.
10. Thompson GR, Lewis B, Booth CC. Absorption of Vitamin D_3-^{3}H in control subjects and patients with intestinal malabsorption. J Clin Invest 1966;45:94–102.
11. Avioli LV. Absorption and metabolism of vitamin D3 in man. Am J Clin Nutr 1969;22:437–46.
12. Avioli LV, Lee SW, McDonald JE, Lund J, DeLuca HF. Metabolism of vitamin D_3-^{3}H in human subjects: distribution in blood, bile, feces and urine. J Clin Invest 1967;46:983–92.
13. Clemens TL, Adams JS, Nolan JM, Holick MF. Measurement of circulating vitamin D in man. Clin Chim Acta 1982;121:301–8.
14. Adams J, Clemens T, Holick M. Silica Sep-Pak preparative chromatography for vitamin D and its metabolites. J Chromatogr 1981;226:198–201.
15. Preece MA, O'Riordan JLH, Lawson DEM, Kodicek E. A competitive protein-binding assay for 25-hydroxycholecalciferol and 25-hydroxyergocalciferol in serum. Clin Chim Acta 1974;54:235–42.
16. Serum vitamin D and related mineral metabolism in cystic fibrosis. Nutr Rev 1979;37:247–9.
17. Hahn TJ, Squires AE, Halstead LR, Stroninger DB. Reduced serum 25-hydroxyvitamin D concentration and disordered mineral metabolism in patients with cystic fibrosis. J Pediatr 1979;94:38–42.
18. Driscoll RH, Meredith SC, Sitrin M, Rosenberg IH. Vitamin D deficiency and bone disease in patients with Crohn's disease. Gastroenterology 1982;83: 1252–8.
19. Markestad T, Aksnes L, Finee PH, Aarskog D. Decreased vitamin D absorption after limited jejunal resection in a premature infant. J Pediatr 1982;101: 1001–3.
20. Compston JE, Creamer B. Plasma levels and intestinal absorption of 25-hydroxyvitamin D in patients with small bowel resection. Gut 1977;18:171–5.
21. Compston JE, Horton LWL, Ayers AB, Tighe JR, Creamer B. Osteomalacia after small intestinal resection. Lancet 1978;1:9–12.
22. Compston JE, Merrett AL, Ledger JE, Creamer B. Fecal tritium excretion after intravenous administration of ^{3}H-25-hydroxyvitamin D_3 in control subjects and in patients with malabsorption. Gut 1982;23: 310–5.
23. Wagonfield JB, Bolt M, Boyer JL, et al. Comparison of vitamin D and 25-hydroxy-vitamin-D in the therapy of primary biliary cirrhosis. Lancet 1976;2: 391–4.
24. Kaplan MM, Goldberg MJ, Matloff DS, Neer RM, Goodman DBP. Effect of 25-hydroxyvitamin D3 on vitamin D metabolites in primary biliary cirrhosis. Gastroenterology 1981;81:681–5.
25. Matloff DS, Kaplan MM, Neer RM, Goldberg MJ, Bitman W, Wolfe HJ. Osteoporosis in primary biliary cirrhosis: effects of 25-hydroxyvitamin D_3 treatment. Gastroenterology 1982;83:97–102.
26. Danielsson A, Lorentzon R, Larsson S-E. Intestinal absorption and 25-hydroxylation of vitamin D in patients with primary biliary cirrhosis. Scand J Gastroenterol 1982;17:349–55.
27. Schoen MS, Lindenbaum J, Roginsky MS, Holt PR. Significance of serum level of 25-hydroxycholecalciferol in gastrointestinal disease. Digestive Dis Sci 1978;23:137–42.
28. Thompson GR. Absorption of fat soluble vitamins and sterols. J Clin Pathol 1971;24(suppl 5):85–9.
29. Hines C. Vitamins, absorption and malabsorption. Arch Intern Med 1978;138:619–21.
30. Hollander D. Intestinal absorption of vitamins A, E, D, and K. J Lab Clin Med 1981;97:449–62.
31. Maislos M, Silver J, Fainaru MI. Intestinal absorption of vitamin D sterols: differential absorption into lymph and portal blood in the rat. Gastroenterology 1980;80: 1528–34.
32. Davies M, Mawer EB, Klass HJ, Lumb GA, Berry JL, Warnes TW. Vitamin D deficiency, osteomalacia, and primary biliary cirrhosis. Digestive Dis Sci 1983;28:145–52.
33. Nechama H, Hoff D, Harell A, Edelstein S. The intestinal absorption of vitamin D and its metabolites. J Molec Med 1977;2:413–22.
34. Krawitt EL, Chastenay BF. 25-Hydroxy vitamin D absorption test in patients with gastrointestinal disorders. Calcif Tissue Int 1980;32:183–7.
35. Sokol RJ, Farrell MK, Heubi JE, Tsang RC, Balistreri WF. Comparison of vitamin E and 25-hydroxyvitamin D absorption during childhood cholestasis. J Pediatr 1983;103:712–7.
36. Sitrin MD, Pollack KL, Bolt MJG, Rosenberg IH. Comparison of vitamin D and 25-hydroxyvitamin D absorption in the rat. Am J Physiol 1982;242:G326.
37. Ponchon G, DeLuca H. The role of the liver in the metabolism of vitamin D. J Clin Invest 1969;48: 1273–9.