Vitamin D Metabolism and Chronic Liver Disease*

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ABSTRACT

In recent years, considerable developments have taken place in the understanding of the vitamin D compounds which allows them, or at least their metabolites, to be classified as hormones. The liver has, for many years, entered into any consideration of the normal control of vitamin D status and metabolism in a number of roles. The main importance of the liver in vitamin D metabolism is now recognized to be the critical role it plays in the hydroxylation pathway and consequent formation of biologically active metabolites. Because the liver plays a critical central role in vitamin D metabolism, and possibly determines the overall efficiency of utilization, it may be expected that disorders of vitamin D metabolism, with associated clinical sequelae in the form of metabolic bone disease and hypocalcemia, and clinical features of patients with chronic disorders of liver function. The serum concentration 25-hydroxyvitamin D (25-OHD) is generally accepted as reflecting total body vitamin D status in patients with normal renal function. Low serum 25-OHD concentrations have been reported in patients with a variety of hepatic disorders including: symptomatic primary biliary cirrhosis, alcoholic liver disease, chronic active liver disease, and large bile-duct obstruction owing to carcinoma or stones. The mechanisms for the low circulating serum 25-OHD concentrations in patients with chronic liver disease are complex and multifactorial. The disturbances in vitamin D metabolism in patients with chronic liver disease and biliary disease are associated with disturbances in calcium homeostasis and together they present clinically as hepatic osteodystrophy. The latter consists of osteomalacia, possibly sometimes complicated by secondary hyperparathyroidism, osteoporosis, and periosteal new bone formation.

Introduction

Vitamin D is the name given to a group of steroid compounds which possess antirachitic properties. The two major forms of vitamin D are vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol); within the body these two compounds have identical metabolic pathways. Cholecalciferol is the naturally occurring compound in man. Ergocalciferol is the synthetic form of vitamin D
prepared by ultraviolet irradiation of ergosterol, a plant sterol. It is the exogenous, dietary or "added" form of vitamin D which is used in foods that are artificially fortified. When vitamin D was initially identified, it was classified as one of the trace nutrients or food factors that were considered to be 'vital amines'. The natural diet of man is, however, a poor source of vitamin D. The main natural dietary sources of vitamin D are egg yolk, fatty fish, and to some extent milk fat; the latter has a variable content because of seasonal variations.

In recent years, considerable developments have taken place in the understanding of the vitamin D compounds which allows them, or at least their metabolites, to be classified as hormones. The main development in the understanding of vitamin D has been the recognition of the fact that cholecalciferol and ergocalciferol are metabolized to more polar compounds which account for their actions on the gastrointestinal tract, kidney and bone. Vitamin D has been recognized for many years to have an important role in normal calcium homeostasis. In regard to the latter, it has been established that either hypo- or hypercalcemia are associated with clinical situations in which there is either a deficiency or excess of vitamin D, and that metabolic bone disease manifested as either rickets in childhood or osteomalacia in an adult is a consequence of vitamin D deficiency.

The liver has, for many years, entered into any consideration of the normal control of vitamin D status and metabolism in a number of roles. By analogy with the fat soluble vitamins A, E, and K, which are stored in the liver, and because livers from some species of fish contained high concentrations of vitamin D, the mammalian liver was for many years erroneously considered to be a storage site for vitamin D. It has now been well established that the mammalian liver contains only trace quantities of vitamin D. The normal secretion of bile and its overall role in fat absorption is of importance in the absorption of exogenous vitamin D from dietary sources of either natural or "added" origin. The main importance of the liver in vitamin D metabolism is now recognized to be the critical role it plays in the hydroxylation pathway and consequent formation of the biologically active metabolites. The liver also plays a role in the metabolic inactivation of the vitamin D molecule and excretion of the products in bile. Fraser has recently proposed that the balance between these anabolic and catabolic processes determines the efficiency with which available vitamin D is used,—a potentially important role of the liver which has not been previously recognized.

**Vitamin D Synthesis and Metabolism**

**Skin Synthesis**

Cholecalciferol (vitamin D₃) (figure 1) is the naturally occurring compound in man; it is formed in the skin. The initial step in the formation of cholecalciferol involves the photochemical formation of a pre-compound from 7-dehydrocholesterol (figure 1) with cleavage of the B-ring carbon-carbon bond between C-9 and C-10 of the steroid structure. The cleavage is achieved by irradiation with ultraviolet light in the wavelengths 280 to 305 nm. Pre-vitamin D₃ is the primary product of the irradiation process and forms a temperature-dependent equilibrium mixture with cholecalciferol. There is some evidence for the existence of a regulatory control mechanism for cholecalciferol production at this initial level, which is in part based on a slow release-rate of cholecalciferol from the skin into the circulation. In the latter compartment, cholecalciferol is transported by vitamin D-binding protein, an
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alpha-globulin, which has a high affinity for cholecalciferol and is synthesized in liver hepatocytes.

The rate of endogenous synthesis of cholecalciferol in the skin is related to the amount of biologically effective ultraviolet light in solar and sky radiation and to the duration of exposure to that ultraviolet light. Seasonal variations in total solar and ultraviolet radiation are associated with variations in endogenous cholecalciferol synthesis and account for the seasonal fluctuations in the serum concentrations of vitamin D metabolites in normal subjects. It is recognized that in normal adults sufficient cholecalciferol can be derived from endogenous synthesis to meet body requirements if there is adequate exposure to ultraviolet light.

Liver Metabolism

The first step in the conversion of cholecalciferol to its ultimate biologically active form is hydroxylation in the liver at the C-25 position with the formation of 25-hydroxycholecalciferol [25-OHD₃] (figure 1). The liver is the major site of formation of 25-OHD₃, it also 25-hydroxylates ergocalciferol. Cholecalciferol and ergocalciferol are transported to the liver by vitamin D-binding protein. Although the liver is the main site of 25-hydroxylation, it is not the exclusive site; significant amounts of the 25-hydroxylase enzyme are also present in kidney and intestine.

It is now generally agreed that 25-OHD₃ represents the major circulating metabolite of cholecalciferol in plasma and that more polar metabolites accumulate in the target tissues which account for the characteristic actions following the administration of cholecalciferol. 25-Hydroxycholecalciferol is the form of cholecalciferol that undergoes either passive or facilitative transfer across the placenta and determines the cholecalciferol status of infants at birth. The

Figure 1. The major interconversion forms of cholecalciferol (vitamin D₃).
rate of hepatic 25-hydroxylation does not seem to be strictly controlled. There is some control of 25-hydroxylation by substrate concentration; however, other than this mechanism, there appears to be minimal, if any, regulation by product formation. The evidence that is available supports the view that the rate of 25-hydroxylation is not influenced by variations in the circulating concentrations of either calcium or phosphorus. Variations in the serum concentration of 25-OHD₃ are primarily reflections of changes in seasonal exposure of ultraviolet light, with some variation from dietary intake, rather than being due to variations in 25-hydroxylase activity.

**Renal Metabolism**

The kidney appears to be the only organ involved in the final C-1 hydroxylation of 25-OHD₃ and the formation of 1,25-dihydroxycholecalciferol[1,25(OH)₂D₃] (figure 1) which is the major biologically active metabolite of cholecalciferol. The dominant action of 1,25-(OH)₂D₃ is to stimulate the active absorption of calcium from the gastrointestinal tract; it is also active in the mobilization of calcium from bone and increases the renal tubular reabsorption of calcium. The 1α-hydroxylase with substrate specificity for 25-OHD₃ is located in the proximal convoluted tubules and the cortical thick loops. In plasma 1,25(OH)₂D₃ is transported, from the kidney to its target organs bound to the vitamin D-binding protein; there is no evidence for the existence of a specific transport protein. There is, however, some evidence that a small fraction in circulation may be in the “free” unbound form; it is not known if this is the metabolically active fraction as is the case with other steroid hormones.

On its target tissues 1,25(OH)₂D₃ acts in an analogous fashion to the classical steroid hormones in directing the de novo synthesis of a protein. The kidney may in this respect be regarded as an endocrine organ which secretes a highly active hormone that plays a central role in calcium homeostasis through its action on the intestinal absorption, bone mobilization, and renal tubular reabsorption of calcium. There was some doubt initially as to whether cholecalciferol induced protein synthesis directly, via ribonucleic acid (RNA), or indirectly by altering the permeability of the nuclear membrane to calcium. It is now generally agreed that the active cholecalciferol metabolites act directly, via RNA, in the induction of the specific transport protein; a mechanism comparable to that for other steroid hormones.

The modulators of the rate of formation of the renal steroid hormone 1,25(OH)₂D₃ are probably the state of vitamin D nutrition as well as a number of other closely interrelated factors, which include their serum concentrations of calcium ion, phosphorus and parathyroid hormone. All of these modulators may be regarded as part of one system that regulates 1,25(OH)₂D₃ production, to meet the short-term needs involved in calcium homeostasis, and is under negative feedback control. A second system of modulators appears to exist which are involved in long-term calcium homeostasis. The modulators in this second system include a number of hormones foremost amongst which are growth hormone, the sex steroids, and prolactin.

**1,25(OH)₂D₃ and Intestinal Calcium Absorption**

The majority of foods in the human diet contain calcium. The calcium content of foods is varied and the main source of dietary calcium is from milk and dairy products. Calcium is absorbed throughout the length of the small intestine and absorption is greater in the duo-
denum and proximal jejunum than in the ileum. The mechanisms involved in the absorption of calcium across the intestinal wall are (1) simple passive ionic diffusion, (2) facilitated diffusion, and (3) active transport. The rate of absorption of calcium from the intestine is dependent on a number of factors. Among the latter are: age and body requirements, previous dietary calcium intake, the absolute amount of calcium in the gut, the availability of calcium in the gut and the effects of other substances on the availability of calcium for absorption, bile secretion and fatty acids, parathyroid hormone and the biologically active metabolites of cholecalciferol.26 It is now undisputed that the dominant factor regulating calcium absorption from the intestinal tract is the renal steroid hormone 1,25(OH)2D3 through its action on the active transport mechanism.

Because the liver plays a critical central role in vitamin D metabolism, and possibly determines the overall efficiency of utilization, it may be expected that disorders of vitamin D metabolism, with associated clinical sequelae in the form of metabolic bone disease and hypocalcaemia are clinical features of patients with chronic disorders of liver function.

Disorders of Vitamin D Metabolism in Chronic Liver Diseases

**Serum 25-hydroxy-vitamin D**

Until relatively recently the only laboratory determinations available were for vitamin D “activity” and these involved either in vivo or in vitro biological assays. The assays responses were based on the ability to either induce the healing of rickets in experimental animals, to mobilize calcium from bone, or to increase calcium transport in everted duodenal sac preparations in vitro. All of these biological techniques were research procedures; they were also insensitive and lacked specificity. In the past decade a number of assay techniques have been developed, and are still being developed, which have made the estimation of vitamin D metabolites available as routine laboratory procedures. A competitive protein binding assay is the most commonly used method for the determination of 25-hydroxyvitamin D concentration and is now available in many laboratories as a routine procedure.21 It is important to recognize the fact that the serum or tissue binding protein used in the assay also binds 25-OH-D2, 25-OH-D3, and the dihydroxy metabolites of these two compounds [24,25(OH)2D2 or D3 and 25,26(OH)2D3] with equal affinity. The results obtained using a competitive protein binding technique should therefore be correctly expressed as 25-hydroxyvitamin [25-OHD] concentration.

The serum concentration of 25-OHD is generally accepted as reflecting total body vitamin D status in patients with normal renal function. Low serum 25-OHD concentrations have been reported in patients with a variety of hepatic disorders including: symptomatic primary biliary cirrhosis (P.B.C.), alcoholic liver disease, chronic active liver disease and large bile-duct obstruction due to carcinoma or stones.4,5,10,14,16,24 The only vitamin D-untreated patients with chronic liver disease who usually have normal serum 25-OHD concentrations are patients with asymptomatic P.B.C. and anicteric patients who have spent prolonged periods of time in bright sunshine.14 Treatment with vitamin D supplements usually results in normal serum 25-OHD concentrations in patients with symptomatic PBC20 and alcoholic liver disease.16

The mechanisms for the low circulating serum 25-OHD concentrations in patients with chronic liver disease are complex and multifactorial. As the serum
25-OHD concentration can usually be corrected to reference range values with vitamin D supplements, a major failure of either hepatic 25-hydroxylation or synthesis of the vitamin D binding globulin is likely to be relatively unimportant. The main defect appears to be a lack of vitamin D substrate. In this respect, a poor diet, lack of exposure to sunlight, and malabsorption of endogenous and exogenous vitamin D owing to a lack of intraluminal bile acids together with a reduction in hepatic 25-hydroxylation are all potential contributory factors. Malabsorption of endogenous vitamin D and the hydroxylated metabolites may occur during an enterohepatic circulation. Alcohohies have a diet which is low in most vitamins and patients with cryptogenic cirrhosis, and chronic aggressive hepatitis may be deficient in vitamins A, E, and carotene. Some patients rarely go out into the sun and it is also possible that jaundice may interfere with the skin’s absorption of ultraviolet light. As bilirubin absorbs light at the wavelength of 280 nm which is also that required for the conversion of 7-dehydrocholesterol to pre-vitamin D₃, interference in the cutaneous synthesis of cholecalciferol by bilirubin may be of importance in jaundiced patients. A lack of intraluminal bile acids causing malabsorption of fat-soluble vitamins is well recognized. The absorption of exogenous vitamin D from the gut is dependent on the presence of intraluminal bile-acids; the availability of the latter may be further reduced in patients with hepatic disorders by the use of the anion binding agent cholestyramine in the treatment of pruritus. It has been proposed that the therapeutic administration of cholestyramine to patients with P.B.C. may be an important contributory factor in the pathogenesis of the associated osteomalacia by reducing the absorption of exogenous, (dietary) vitamin D and the 25-hydroxylated metabolites; the latter during their enterohepatic circulation.

An enterohepatic circulation of vitamin D metabolites has been reported both in man and in experimental animals. The precise nature of these metabolites is ill defined; some are conjugated with glucuronic acid and can be released by treating bile with β-glucuronidase. Arnaud et al reported that in normal subjects following the intravenous injection of tritiated 25-OHD 17 per cent of the dose injected appeared in the duodenum within six hours of administration and approximately 36 percent of the dose appeared after 24 hours; it was concluded from data based on fecal measurements that more than 85 percent of this “bile” secreted 25-OHD was reabsorbed from the small intestine. Wiesner et al reported that in normal subjects within six hours following the intravenous administration of radio-labelled 1,25(OH)₂D₃ 16 percent of the dose appeared in the bile as polar metabolites of 1,25(OH)₂D₃. The findings of Arnaud et al and Wiesner et al are consistent with the concept of an enterohepatic circulation for vitamin D metabolites, analogous to that for bile salts. Malabsorption of vitamin D metabolites during their enterohepatic circulation, especially in these chronic hepatic disorders associated with steatorrhea, could potentially play a role in the mechanism for the low serum 25-OHD concentrations in these disorders. Fraser has, however, recently stated that “there is no evidence for a conservative enterohepatic circulation of the vitamin D molecule in any form.” He concluded that although an enterohepatic circulation of vitamin D catabolites does exist, it is no significance with regard to either vitamin D status or the hepatic metabolism of vitamin D.

**Hydroxylation of Vitamin D**

25-Hydroxylase. Depression or loss of hepatic 25-hydroxylase activity could be a contributory factor in the pathogenesis of the low serum 25-OHD concentrations
found in patients with chronic liver disease. Skinner et al. reported that in eight patients with symptomatic P.B.C. there was no significant increase in mean serum 25-OHD concentrations, from basal value, at 12 days following a single intramuscular injection of vitamin D (100,000 units D₂); in contrast there was a significant increase in mean values in eight normal subjects and in seven patients with nutritional osteomalacia. In patients with P.B.C. who had been previously treated with parenteral vitamin D₂ their serum 25-OHD concentrations were in the reference range, while those who had been receiving no treatment had subnormal values. Skinner et al. reported that the patients in the latter group were able to 25-hydroxylate vitamin D over the long term as manifested by the fact that their serum 25-OHD concentrations increased following regular monthly injections of vitamin D₂.

In the P.B.C. patients studied by Skinner et al. the initial low serum 25-OHD concentrations suggested that they were vitamin-D depleted. In the short-term 12-day study the reduced response in the P.B.C. patients compared with the osteomalacic control group suggested that there was either a diminished production of 25-OHD by the liver (which may be accounted for by impaired uptake of vitamin D, reduced 25-hydroxylase activity, or impaired release of 25-OHD from the liver) or possibly an increased turnover of 25-OHD to more polar metabolites. In conflict with the report of Skinner et al. were the findings of Hepner et al. who reported that short-term 25-hydroxylation was normal after the intravenous injection of vitamin D₃ in three patients with P.B.C. The disparity between the results obtained by these two groups of workers could be due to the differences in their methods of administration of vitamin D. Hepner et al. used the intravenous route which presents a large bolus of vitamin D to the liver over a short time. The rate of release of vitamin D from the depot site after intramuscular injection, as used by Skinner et al., would be slow and, therefore, the circulating concentrations of vitamin D available as substrate would be likely to be low. The different 25-OHD increments observed in these short-term studies may therefore have been due to defective removal of vitamin D from the blood in patients with P.B.C. and not necessarily caused by a gross defect in vitamin D-25-hydroxylase activity. Hepatic 25-hydroxylase activity may possibly be impaired in some patients with P.B.C. and the ability to form 25-OHD from vitamin D may decrease as the disease progresses. However, the regular long-term administration of vitamin D appears to usually correct low serum-25-OHD to within the reference range.

**Formation of Di-hydroxy metabolites.** One of the major forms of metabolic bone disease in patients with chronic hepatic and biliary disorders is osteomalacia. The latter, in the final analysis, is a failure in the mineralization process of bone matrix. A number of mechanisms can account for a failure in mineralization and amongst these is a lack of the dihydroxy metabolites of vitamin D. There is, however, evidence that patients with liver disease can form dihydroxy vitamin D metabolites in an appropriate manner. The eight patients with hepatic diseases studied by Long et al. had either P.B.C. or cirrhosis due to active chronic hepatitis, they were judged to be either vitamin D repleted or depleted on the basis of their serum 25-OHD concentration. All of the patients studied formed 25-OHD following the injection of radiolabelled precursor; this finding is consistent with the proposal that the hepatic rate of 25-hydroxylation is not finely controlled. Three of the four vitamin D depleted patients formed 1,25(OH)₂D₃; all of the four vitamin D repleted patients formed 24,25-dihydroxycholecalciferol and one also formed 1,25(OH)₂D₃. The findings of Long et al. in patients with
hepatic disorders were similar to those obtained, using the same technique, in patients with normal liver function.\textsuperscript{18} It would appear, therefore, that if sufficient substrate is available, patients with chronic liver diseases can form both the monohydroxy- and dihydroxy-metabolites of vitamin D both appropriately and in normal quantities.

Clinical Manifestations

The disturbances in vitamin D metabolism in patients with chronic liver disease and biliary disease are associated with disturbances in calcium homeostasis and may present clinically with hepatic osteodystrophy. The latter consists of osteomalacia, possibly sometimes complicated by secondary hyperparathyroidism, osteoporosis, and periosteal new bone formation. In these patients, as in those with chronic renal failure, the etiology of the osteodystrophy is undoubtedly multifactorial. Osteomalacia is commoner with cholestasis but is also found in hepatocellular disease. The etiology may be in part due to a lack of vitamin D substrate rather than to a failure in either the mono- or di-hydroxylating enzyme systems. There are a number of possible causes for a lack of vitamin D substrate. Among the major possible causes are: diminished skin synthesis; poor nutrition; malabsorption of either of endogenous or exogenous vitamin D and their metabolites during an entero-hepatic circulation. It is probable that these together with other factors play varying roles in patients with different disease states. The variation between different disease states is reflected in the observations that the intestinal absorption of cholecalciferol is usually normal in alcoholic liver disease but is impaired in P.B.C., while hepatic 25-hydroxylation is normal in alcoholic liver disease but may be defective in primary biliary cirrhosis.\textsuperscript{2} The major problem in hepatic osteodystrophy is probably the osteomalacic component. The long-term administration of vitamin D supplements may improve intestinal calcium absorption but does not prevent either osteomalacia, or an associated proximal myopathy, even in the presence of normal serum 25-OHD concentrations.\textsuperscript{13} Hepatic osteomalacia can be cured by adequate treatment with various metabolites of vitamin D including D\textsubscript{2}, D\textsubscript{3}, 25-OHD\textsubscript{3}, 1-alpha-hydroxyvitamin D\textsubscript{3}, and 1,25(OH)\textsubscript{2}D\textsubscript{3}.\textsuperscript{12}

References