LIVER AND BILIARY TRACT

Low Serum 25-Hydroxyvitamin D in Hereditary Hemochromatosis: Relation to Iron Status

L. H. CHOW, J. V. FREI, A. B. HODSMAN, and L. S. VALBERG
Departments of Medicine and Pathology, University Hospital and St. Joseph's Hospital, and the University of Western Ontario, London, Ontario, Canada

Under normal conditions, vitamin D absorbed from the diet or synthesized in the skin is transported to the liver where it undergoes hydroxylation. The purpose of this study was to determine whether excess hepatic iron affects this process and the subsequent production of 1,25-dihydroxyvitamin D (1,25-[OH]$_2$D) in the kidney. Mean serum 25-hydroxyvitamin D (25-OHD) concentrations in untreated hereditary hemochromatosis were 13 ± 6 (SD) in 9 patients with cirrhosis, 13 ± 6 in 5 patients with hepatic fibrosis, and 22 ± 6 in 10 patients with normal hepatic architecture aside from siderosis and were significantly lower than the levels found in 24 controls matched for age, sex, and season, $p < 0.05$. The mean serum 25-OHD levels in the two groups with hemochromatosis and hepatic damage were significantly lower than the value in the group with normal hepatic architecture, $p < 0.05$. Serum 25-OHD levels in individual patients were inversely related to the size of body iron stores as measured by exchangeable body iron, $r = -0.64$, or serum ferritin, $r = -0.47$, $p < 0.05$. In 15 patients removal of excess body iron by venesection therapy produced a significant increase in the mean serum 25-OHD from 20 ng/ml to 30 ng/ml, $p < 0.05$. In contrast, mean serum 1,25-[OH]$_2$D levels were similar in iron-loaded and control subjects, indicating that the regulation of this metabolite was intact in patients with hemochromatosis. The results reveal that the low serum 25-OHD concentration in patients with hemochromatosis is directly related to the extent of iron loading and it is improved by venesection therapy.

Deficiencies of vitamin A, C, and E have been reported in patients with iron overload, but abnormality in vitamin D metabolism is not a well-recognized complication of hemochromatosis (1–3). Low serum 25-hydroxyvitamin D (25-OHD) concentration has been found in patients with iron overload associated with thalassemia major and hereditary hemochromatosis (4–7). Under normal conditions, plasma 25-OHD is formed in the liver from vitamin D absorbed from the diet or synthesized in the skin under the influence of ultraviolet light (8). It is then further hydroxylated in the kidney to 1,25-dihydroxyvitamin D (1,25-[OH]$_2$D) (8). It is not clear whether the low serum 25-OHD in iron overload is a primary effect of excess iron or secondary to chronic liver damage and if it is accompanied by a concomitant decrease in 1,25-[OH]$_2$D. The purpose of this study was to determine serum 25-OHD and 1,25-[OH]$_2$D concentrations in patients with hereditary hemochromatosis before and after venesection therapy and to consider the mechanism of the low 25-OHD.

Methods and Materials

Selection of Subjects

A total of 24 patients (21 men and 3 women) with untreated hereditary hemochromatosis were investigated and 15 of these patients were studied again after venesection therapy. Approximately 500 ml of blood was removed at weekly intervals and the duration of treatment ranged from 2 to 22 mo. Exchangeable body iron stores were calculated from the amount of iron removed. Correction

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Address requests for reprints to: Dr. L. S. Valberg, University Hospital, Room 5-OF5, London, Ontario, Canada N6A 5C1.

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Abbreviations used in this paper: 25-OHD, 25-hydroxyvitamin D; 1,25-[OH]$_2$D, 1,25-dihydroxyvitamin D.

was made for iron absorbed during the treatment period, based on the assumption that the daily absorption of iron from the diet was 5 mg. In 11 of 15 patients, venesection therapy began and ended in the same season; 3 patients began therapy in winter and ended in summer and; 1 patient began therapy in summer and ended in winter.

The diagnosis of hemochromatosis in 19 probands and in 5 patients subsequently discovered during screening of family members was established by the presence of stainable iron in more than 50% of cells in a liver biopsy specimen and by the subsequent removal of at least 5 g of iron by phlebotomy before the development of iron deficiency. The discovered cases of hemochromatosis had identical HLA A and B haplotypes to the respective probands. None of the patients had poor nutritional status. The histopathology of the percutaneous liver biopsy specimen was also classified by one of us (J. F.) into three categories: normal (except for siderosis), fibrosis, or cirrhosis without knowledge of the vitamin D status of the patients.

The study was in part retrospective in that frozen serum was available from 15 patients who had completed venesection therapy. These 15 patients and 9 patients studied prospectively were matched for age and sex with 24 control subjects having normal iron status. The controls were selected from hospitalized patients with cardiac disorders. None of the controls had clinical or biochemical evidence of gastrointestinal or hepatic disease and biopsy specimens were not taken from them. Blood samples from controls were obtained during the same season of the year as the samples from the iron-loaded patients and were assayed for vitamin D metabolites.

All of the subjects who participated gave written informed consent and the study was approved by the University of Western Ontario Health Sciences Committee on Human Research.

Techniques

Serum iron concentration and iron binding capacity were measured with a diagnostic kit (Hoffman LaRoche Inc., Vaudreuil, Quebec, Canada). The normal range of values given by the manufacturer for these tests was 12–31 μmol/L and 45–74 μmol/L, respectively. The normal range for percent serum transferrin saturation was 20%–55%. The method described by Luxton et al. (9) was used for the analysis of serum ferritin. The normal range was 15–350 ng/ml for men and 15–200 ng/ml for women. Serum glutamic oxaloacetic transaminase (SGOT) concentration was determined on an LKB 8600 autoanalyzer using Plus Chem SGOT reagent (Smith Kline Instruments, Inc., Sunnyvale, Calif.). The normal range was 10–30 U/L. Serum 25-OHD concentration was assayed by a competitive protein binding method as described by Haddad and Chyu (10). Serum 1,25-$[OH]_2$D concentration was assayed by the competitive binding protein method of Eisman et al. (11) as modified by Mallon et al. (12). The established normal range was 12–40 ng/ml for 25-OHD and 15–55 pg/ml for 1,25-$[OH]_2$D. Both interassay and intraassay variation were 15%.

The statistical significance between pairs of observations was determined by Student's *t*-test (13). To determine the relationship between the serum vitamin D metabolites and the amounts of exchangeable iron or serum ferritin, the least-square criterion of best fit was adopted (14,15).

Results

In the individual controls, serum 25-OHD levels were above the lower limit of normal, 12 ng/ml, in 23 of 24 subjects. Similarly, serum 1,25-$[OH]_2$D in these individuals was $\geq$15 pg/ml in 22 of them. No significant difference was found in the mean serum 25-OHD between the summer, 30 $\pm$ 11 ng/ml, and winter, 27 $\pm$ 15 ng/ml, seasons. The mean serum 1,25-$[OH]_2$D was higher in the samples taken in the summer, 38 $\pm$ 16 pg/ml, than in the winter, 29 $\pm$ 11 pg/ml, but this did not achieve statistical significance ($0.05 < p < 0.10$, one-tailed test).

Compared with normal controls, the mean transferrin saturation level and serum ferritin concentration were markedly increased in the three groups of patients with hemochromatosis (Table 1). The average SGOT was significantly increased in the group of patients with cirrhosis, $p < 0.05$. The SGOT results in individual patients were directly correlated with size of body iron stores as measured by exchangeable iron, $r = 0.62$, $p < 0.01$.

The respective serum 25-OHD levels in groups of untreated patients with hemochromatosis classified as to normal hepatic architecture, hepatic fibrosis, or cirrhosis were significantly lower than the corresponding values in the matched controls, $p < 0.05$, $p < 0.01$, and $p < 0.01$, respectively (Table 1). The mean serum 25-OHD levels in the patients with cirrhosis were lower than the level in patients from the other groups combined (Table 1) and 4 of 9 patients were below the lower limit of normal. In contrast to the results for serum 25-OHD, the values for 1,25-$[OH]_2$D were similar to the controls.

However, the mean value of serum 1,25-$[OH]_2$D in patients with cirrhosis was significantly lower than the level in the two other groups combined and 3 of 9 patients fell below the lower limit of normal.

The serum 25-OHD levels in individual patients with hemochromatosis before treatment were correlated inversely with the size of body iron stores, $r = -0.64$, $p < 0.01$ (Figure 1), and with the logarithm of the serum ferritin concentration, $r = -0.47$, $p < 0.05$. There was also a significant inverse correlation between serum 25-OHD levels and SGOT, $r = -0.420$, $p < 0.05$. The serum 1,25-$[OH]_2$D values were not correlated with any of these parameters.

For the 15 patients who completed a course of phlebotomy therapy, the mean serum ferritin fell from 851 ng/ml to 32 ng/ml and the SGOT from 45

Table 1. Serum Vitamin D Metabolites in Controls and Untreated Hemochromatosis

Group	Number of subjects	Hepatic histopathology	Exchangeable body iron (g)	Transferrin saturation (%)	Serum ferritin (ng/ml)	SGOT (U/L)	Serum vitamin D: 25-OHD (ng/ml)	Serum vitamin D: 1,25-$[OH]_2D$ (pg/ml)
Hemochromatosis	10	Siderosis only	5 ± 2	83 ± 12[b]	616[b] (295–1288)	37 ± 10	22 ± 6[a]	46 ± 23
	5	Siderosis and fibrosis	8 ± 1	79 ± 7[b]	1621[b] (977–2691)	57 ± 14	13 ± 6[b,d]	37 ±23
	9	Siderosis and cirrhosis	13 ± 4[d,f]	79 ± 8[b]	2570[b,c,f] (1318–5011)	61 ± 23[b,c,e]	13 ± 6[b,d,e]	24 ± 19[e]
Controls	24	—	—	24 ± 11	91 (59–141)	25 ± 12	29 ± 11	34 ± 17

SGOT, serum glutamic oxaloacetic transaminase. [a,b] Mean value significantly different from mean of control group, paired *t*-test: [a] $p < 0.05$, [b] $p < 0.01$. [c,d] Mean value significantly different from mean of siderosis only subgroup, paired *t*-test: [c] $p < 0.05$, [d] $p < 0.01$. [e,f] Mean value significantly different from mean of siderosis only and siderosis and fibrosis subgroups combined, paired *t*-test: [e] $p < 0.05$, [f] $p < 0.01$.

U/L to 28 U/L, $p < 0.01$ (Table 2). After treatment, the mean serum 25-OHD values increased from 20 ng/ml to 30 ng/ml, paired *t*-test, $p < 0.05$ (Table 2). The rise in serum 25-OHD levels, however, was not universal. Levels rose in 11 of 15 patients, in 6 of 9 subjects with normal histopathologic architecture, in 2 of 2 subjects with fibrosis, and in 3 of 4 subjects with cirrhosis (Figure 2). Three of the patients, all with cirrhosis, had prephlebotomy levels of serum 25-OHD <12 ng/ml (Figure 2). Of these 3 patients, one showed a pronounced increase in serum 25-OHD level after venesection therapy, one displayed a small increase, and the level actually fell in the third patient (Figure 2). In contrast to the first 2 patients, the latter subject drank alcohol to excess before and after treatment. For the group as a whole the post-treatment values for serum 25-OHD were not significantly different from values in matched controls, $p > 0.10$. In contrast to serum 25-OHD, the serum levels of 1,25-$[OH]_2D$ were not significantly affected by venesection therapy, $p > 0.10$ (Figure 2).

Discussion

Our finding that the serum 25-OHD level was low in patients with untreated hemochromatosis is in agreement with those of Monnier et al. (6) and of Pawlotsky et al. (7). In addition, our results show that the degree of abnormality is related to the extent of liver injury as reflected by the SGOT level and by hepatic histologic abnormalities. Although the lowest levels of serum 25-OHD were found in patients with hepatic fibrosis or cirrhosis, the values in iron-loaded patients without architectural hepatic abnormality were also significantly lower than those of the controls (Table 1). Liver function was well preserved in patients with normal hepatic architecture and the only abnormality observed in some of these patients was a minor increase in SGOT. The inverse relationship between serum 25-OHD levels in individual patients and the size of body iron stores, and the increase in serum 25-OHD levels to the average level found in controls after venesection therapy implicates iron as the main etiologic factor. That the response to venesection therapy was observed in noncirrhotic patients also suggests a biochemical abnormality in vitamin D metabolism due to iron overload rather than to liver disease per se.

A nonspecific effect of repeated phlebotomy therapy on serum 25-OHD levels seems unlikely because Aloia et al. (5) found that removal of iron by chelation therapy increased serum 25-OHD levels in patients with thalassemia major. The increase in serum 25-OHD levels in patients with hemochromatosis could not be attributed to a seasonal effect because control blood samples were taken at the same time of year as those from the iron-loaded subjects. Improvement in the general function of the liver after removal of excess iron may have contributed to the increase in serum 25-OHD levels in patients with fibrosis or cirrhosis, but it was unlikely to be a factor in the patients with normal hepatic architecture who had well-preserved liver function.

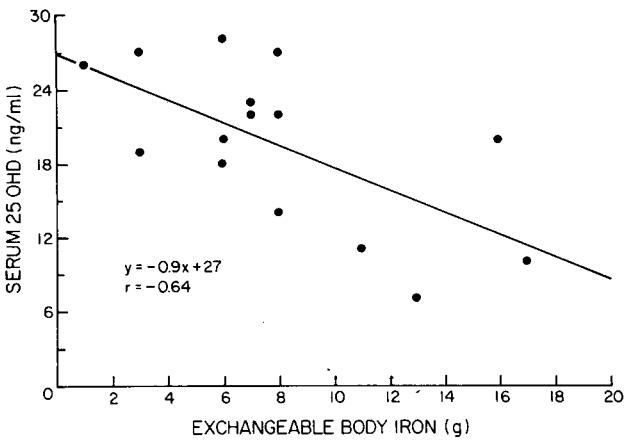

Figure 1. Relationship between serum 25-OHD concentration and exchangeable body iron.

Table 2. Effect of Phlebotomy Therapy on Serum Vitamin D in 15 Patients With Hemochromatosis

Measurement	Before treatment	After treatment
Serum ferritin (ng/ml)	851[a] (457–1584)	32[d] (2–56)
SGOT (U/L)	45 ± 15[b]	28 ± 15[d]
25-OHD (ng/ml)	20 ± 7	30 ± 26[c]
1,25-$[OH]_2D$ (pg/ml)	46 ± 26	40 ± 23

SGOT, serum glutamic oxaloacetic transaminase. [a] Mean and 95% confidence interval. [b] Mean ± SD. [c,d] Mean value significantly different from pretreatment mean value, paired *t*-test: [c] $p < 0.05$; [d] $p < 0.01$.

This suggests that iron may have had a direct effect on serum 25-OHD levels. Several investigators have found that 25-hydroxylation of vitamin D is well preserved in patients with cirrhosis (16–18), although others have reported it is impaired (19,20).

Serum 25-OHD is normally formed in the liver by a microsomal mixed-function monooxygenase that is dependent on cytochrome P_{450} (21). Both ferritin and ferric iron are capable of lowering cytochrome P_{450} content in rats (22,23). These observations in experimental animals suggest that the low serum 25-OHD levels in patients with hereditary hemochromatosis may be due to iron inhibition of hepatic microsomal hydroxylase activity. Another possibility is that the excess hepatic iron initiates the formation of free radicals and lipid peroxidation with resultant oxidation of 25-OHD (3,24). A further possibility is that excess iron may alter the structure or impair the binding capacity of the plasma transport

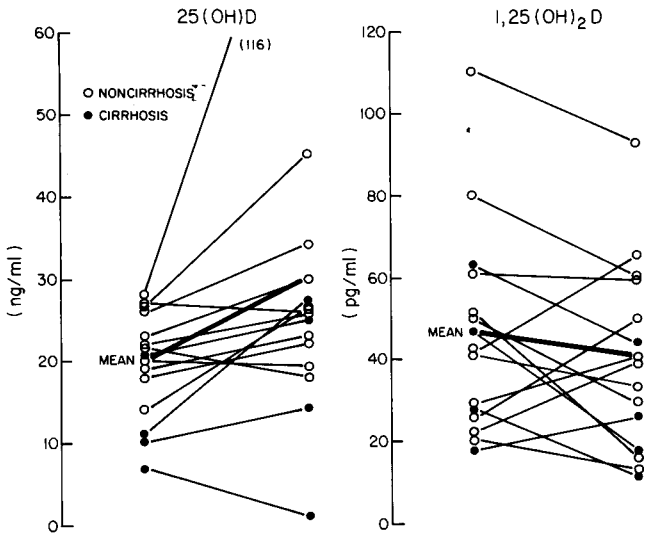

Figure 2. Effect of phlebotomy therapy on serum 25-OHD and 1,25-$[OH]_2D$. The "noncirrhosis" group comprised 9 subjects with normal hepatic histopathology except for siderosis and 2 subjects with siderosis and fibrosis.

binding protein for 25-OHD and that venesection therapy may reverse this. Because of the large number of binding sites on the protein, however, this is probably unlikely. Other potential explanations for the effect include changes in enterohepatic circulation or the urinary excretion of a small unbound portion of serum 25-OHD. The dietary intake of vitamin D was not measured, but all patients were well nourished, even though 6 of them drank alcoholic beverages to excess. There were no clinical features of malabsorption. Epidermal synthesis of vitamin D_3 may be diminished in black subjects, but none of our patients was deeply pigmented (25).

The deficiency in 25-OHD produced by iron loading was not associated with a significant reduction in the average 1,25-$[OH]_2D$ level compared with controls and removal of excess iron had no effect on the serum levels of this metabolite. This indicates that serum concentration of 1,25-$[OH]_2D$ remains carefully regulated in iron overload and that the kidney is able to compensate for the low plasma 25-OHD. This also applies in overt nutritional vitamin D deficiency (26,27).

It is unlikely that the minor abnormality in the average serum 25-OHD in noncirrhotic iron-loaded patients would be sufficient to produce clinically important bone disease. However, it may be that in iron overload complicated by cirrhosis, the degree of abnormality in serum 25-OHD is sufficient to contribute to the development of bone disease. Alternatively, other factors unrelated to 25-OHD metabolism may lead to the development of clinical bone disease. It was not within the scope of this study to assess calcium or bone metabolism. Nonetheless, Monnier et al. (6) reported diminished calcium absorption and reduced bone mineral content in 10 patients with advanced hemochromatosis, and de Vernejoul et al. (28) detected low 25-OHD levels associated with mild hypocalcemia during the winter months in iron-loaded patients with thalassemia major. Moreover, Pawlotsky et al. (7) found normocalcemic hyperparathyroidism in 14 of 28 patients with hereditary hemochromatosis. Further investigation of bone metabolism in patients with advanced iron overload is warranted.

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