Effect of alcohol consumption on serum concentration of 25-hydroxyvitamin D₃, retinol, and retinol-binding protein¹⁻³

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ABSTRACT The effect of chronic alcohol consumption on the concentration of 25-hydroxyvitamin D₃, total retinol, and retinol-binding protein in serum was studied in chronic alcoholics (n = 12) and controls (n = 19). Ethanol intake during the last year was 178 ± 116 and 3.7 ± 4.5 g/day, respectively (p < 0.002). Of the alcoholics, 58% had a concentration of 25-hydroxyvitamin D₃ below lower limit of reference (20 ng/ml). Estimated dietary intake of vitamin D last year was not significantly different for the alcoholics and controls. Concentration of calcium in serum was significantly lower in alcoholics than in controls (p < 0.05). The serum concentration of retinol and retinol-binding protein was similar in the two groups. These observations may be of relevance for some of the clinical findings related to bone disease among heavy alcohol consumers. Am J Clin Nutr 1986;44:678–82.

KEY WORDS Alcohol, 25-hydroxyvitamin D₃, retinol, retinol-binding protein

Introduction

Disturbed immunoregulation, reduced night vision, abnormal gonadal function, and osteomalacia are frequently observed in alcoholics (1). These symptoms may partly be due to inadequate tissue levels of the fat-soluble vitamins A and D (2–5).

Vitamin D has an endocrine function in calcium homeostasis (6). It has been shown that chronic alcoholics may have decreased bone density and increased susceptibility to fractures (7, 8). Measurements of serum levels of 25-hydroxyvitamin D₃ in alcoholics have demonstrated both normal (9) and low values (10).

In mammals, vitamin A is essential for vision, reproduction, and for the regulation of proliferation and differentiation of various cell types (11). Alterations in metabolism of vitamin A have been reported in chronic alcoholics (12). Concentrations of retinol and retinol-binding protein (13) were within normal ranges among alcoholics with fatty liver but were markedly reduced after development of alcoholic hepatitis or cirrhosis.

Because of the earlier conflicting results, the objective of the current study was to evaluate possible effects of heavy alcohol consumption on serum concentrations of 25-hydroxyvitamin D₃, calcium, phosphate, total retinol, and retinol-binding protein. In contrast to other studies, we elicited detailed information about drinking patterns, alcohol consumption, and an estimate of dietary intake of vitamin

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D3. Furthermore, the measurement of 25-hydroxyvitamin D3 was performed by high-performance liquid chromatography (HPLC) and UV detection.

**Methods**

**Subjects**

We studied 12 alcoholics (25–65 yr of age) admitted to a treatment unit and 19 control subjects (22–59 yr of age). Participants were given detailed information about the study design and signed a written consent. None of the subjects had known diseases of the liver, gastrointestinal tract, or kidneys.

**Study design**

All participants were personally interviewed to determine drinking history and patterns, alcohol consumption, and dietary intake of vitamin D3 in different drinking periods. They also responded to the Short Michigan Alcoholism Screening Test (SMAST) questionnaire (14) and Severity of Alcoholism Dependence Questionnaire (SADQ) (15). Dietary intake of vitamin D3 was estimated from consumption of butter, margarine (fortified with 250 IU of vitamin D3/100 g), eggs, and milk, which contributes at least 50% of the total intake of this vitamin (16). Furthermore, a frequency analysis of fish consumption was performed because fish normally contributes about 25% of the vitamin D intake (16).

All participants underwent a physical examination and blood samples were taken for analysis of 25-hydroxyvitamin D3, retinol, and retinol-binding protein.

Measurement of the concentration of 25-hydroxyvitamin D3 was carried out by a modified HPLC method combined with UV photometry (17). Equipment included a Waters 6000 A pump (Waters Associated, Milford, PA), a Rheodyne injector 7125 (Berkeley, CA), and a Waters absorbance detector, model 400. All determinations were performed on duplicate 1-ml serum extracts. After extraction with 10 ml of chloroform and methanol (2:1, v/v), the extracts were prepurified on Sep-Pak® silica cartridges (Waters). The 25-hydroxyvitamin D3 was eluted with 10 ml of hexan and isopropanol (95:5, v/v). After further purification on a LC-18 column (250 × 4.6 mm, Supelco, Bellefonte, PA), final determination was performed by separation on a LiChrosorb Si-60 column (250 × 4 mm, E Merck, Darmstadt, FRG) and UV detection device (17).

Total retinol was determined by HPLC and UV photometry (18) and retinol-binding protein was measured by a double immunodiffusion (19) on agar gel plates (LC-parpinger, Behringwerke, AG, Marburg, FRG).

Standard biochemical methods at Dr V Furst Medical Laboratory in Oslo were used for determining the concentrations of calcium, phosphate, albumin, and creatinine and for measuring the activities of the liver enzymes—γ-glutamyl-transpeptidase (GGT), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and alkaline phosphatase (ALP).

**Statistics**

The significance of differences between groups were calculated by the Mann-Whitney test, the correlation coefficients by Spearman’s test. Probabilities of differences at the level of p < 0.05 were regarded as statistically significant.

**Results**

Group values are reported as means ± SD. Ethanol consumption (in grams per day) for the year and month previous to the study were 178 ± 116 and 227 ± 103 for the alcoholics and 3.7 ± 4.5 and 3.7 ± 4.4 for the controls (p < 0.002). All alcoholics scored more than 5 points on SMAST (9.9 ± 2.2) (14), and eight had more than 35 points (a high degree of dependency) on SADQ (59 ± 20.6).

The period of problem drinking ranged from 2 to 40 yr (17 ± 13.3) among the alcoholics and were characterized by mostly hard drinking (24.7 ± 16) and moderate-drinking (18.8 ± 16) weeks, whereas the controls were drinking during 26.8 ± 19.2 wk and abstinent during 25.2 ± 19.2 wk. Control subjects consumed less alcohol when drinking (4.9 ± 4.8 g/day) as compared to the alcoholics in moderate-drinking (53 ± 52 g/day) and hard-drinking (271 ± 116 g/day) periods.

The mean value of 25-hydroxyvitamin D3 in serum was 22.4 ± 10.5 ng/ml among the alcoholics and 31.2 ± 8.8 ng/ml among the controls (p < 0.002) (Table 1). Of 12 patients, 7 had a 25-hydroxyvitamin D3 concentration below the lower limit of the reference value (20 ng/ml).

Dietary intake of vitamin D3 (estimated from consumption of margarine, butter, eggs, and milk and corresponding to about 50% of total) differed significantly during different drinking periods, with marked reduction during hard-drinking weeks (0.7 ± 0.6 μg/day) as compared to moderate-drinking weeks (1.8 ± 1.1 μg/day) (p < 0.002). However, no significant difference was registered in intake of vitamin D3 (from the same sources) during the previous year or month between the alcoholics (419 ± 237, 23 ± 16 μg) and controls (363 ± 297, 30 ± 25 μg). Furthermore, the frequency analysis of fish consumption showed no significant difference between the groups.

No significant correlation was observed among the alcoholics with regard to intake of ethanol (grams per day) and serum concentration of 25-hydroxyvitamin D3. A signifi-
TABLE 1
Serum concentration of 25-hydroxyvitamin D₃, total retinol, retinol-binding protein, calcium, phosphate, alkaline phosphatase, and albumin

<table>
<thead>
<tr>
<th>Alcoholic patients</th>
<th>25-hydroxyvitamin D₃ ng/ml</th>
<th>Total retinol μmol/L</th>
<th>Retinol-binding protein mg/L</th>
<th>Calcium mmol/L</th>
<th>Phosphate mmol/L</th>
<th>Alkaline phosphatase U/L</th>
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Alcoholics (n = 12)  
\( \bar{x} \pm SD \) 22.4 ± 10.5* 1.99 ± 0.51 52.2 ± 16.1 2.39 ± 0.11† 1.14 ± 0.12 206.8 ± 88.70† 37.3 ± 3.1

Controls (n = 19)  
\( \bar{x} \pm SD \) 31.2 ± 8.8 1.74 ± 0.37 43.0 ± 13.6 2.48 ± 0.10 1.17 ± 0.12 141.4 ± 43.4 39.7 ± 2.9

* p < 0.002; † p < 0.05 (Mann-Whitney).

Significantly reduced concentration of calcium was found among the alcoholics as compared to the controls (p < 0.05, Table 1), whereas no such difference was observed with respect to the level of phosphate. However, only one patient had a serum-calcium concentration below the lower level of the reference value. Even though all activities of ALP were within normal range in the two groups, the alcoholics had a significantly higher level than the control subjects (p < 0.05).

We did not find any correlation between serum concentration of calcium or phosphate and 25-hydroxyvitamin D₃, but a significant negative correlation (r = -0.72, p < 0.002) was observed between serum activity of ALP and level of 25-hydroxyvitamin D₃.

Mean values of serum activities of GGT, ASAT, and ALAT were markedly enhanced among the alcoholics compared to the control subjects (p < 0.002), and only 2 of 12 alcoholics had all these enzyme activities within normal ranges. No significant correlation was estimated between serum activities of GGT and ALP. However, a significant positive correlation was found between activity of GGT and 25-hydroxyvitamin D₃ (r = 0.65, p < 0.002) among the alcoholics.

Concentrations of albumin and creatinine were within normal ranges in both groups and no significant differences were calculated. However, a significant positive correlation coefficient was estimated with respect to albumin and 25-hydroxyvitamin D₃ (r = 0.62, p < 0.05) among the alcoholics.

Neither the concentration of total retinol, retinol-binding protein, nor the ratio of total retinol to retinol-binding protein showed any significant differences between the groups (Table 1). A significant positive correlation was found between the serum concentrations of total retinol and retinol-binding protein (r = 0.75, p < 0.002) among the alcoholics.

No significant correlation was found between SMAST or SADQ scores and serum levels of the fat-soluble vitamins studied.

Discussion

Alcohol consumption may reduce appetite and displace several nutrients from the diet (20). Our study revealed no difference in the dietary intake of foods containing vitamin D between the groups. However, mean serum concentration of 25-hydroxyvitamin D₃ was reduced by 28% among the alcoholics compared to the control subjects. Reduced levels of 25-hydroxyvitamin D₃ have previously been reported in alcoholics both with (21, 22) and
without (23) cirrhosis of the liver, although normal levels have also been reported (5).

Seasonal variations in the concentration of 25-hydroxyvitamin D₃ have been reported in normal control populations (21, 24). Thus, a diminished exposure to UV light might be one factor partially explaining why 7 out of 12 alcoholics had serum values of 25-hydroxyvitamin D₃ below the lower limit of the reference value, although we do not have any evidence that alcoholics are exposed to less UV light than controls.

Furthermore, ethanol may interact with the absorption of fat-soluble vitamins and their metabolism in the liver. Ten of the patients had increased serum activities of liver enzymes, which may indicate subclinical liver disease. Reduced activity of the liver enzyme, 25-hydroxylation, might be another contributing factor to the diminished serum levels of 25-hydroxyvitamin D₃, even though previous studies failed to demonstrate reduced conversion of vitamin D₃ or vitamin D₂ to the corresponding 25-hydroxyvitamin D₃ in cirrhosis (21, 25).

Only a mitochondrial vitamin D 25-hydroxylase has been detected in human liver (17, 26), and the synthesis of this protein might possibly be reduced by ethanol, as described for cytochrome-c oxidase (27). Another postulated mechanism is that ethanol might increase degradation of vitamin D metabolites in the liver by induction of the cytochrome P 450 system in alcoholics (28).

Any disturbance of calcium and phosphate metabolism may contribute to the high incidence of bone fractures, osteoporis, osteonecrosis, and osteomalacia seen in alcoholics (29–31). Increased urinary loss of calcium induced by ethanol is reported (10) and a reduced level of 25-hydroxyvitamin D₃ may further potentiate an altered calcium homeostasis.

In accordance with previous studies, our data did not reveal any significant differences between the two groups with regard to the serum concentration of retinol or retinol-binding protein (32, 33).

Further research is required to examine the influence of heavy ethanol consumption upon the pharmacokinetics of the fat-soluble vitamins. At the present time, it appears that alcohol affects the status of vitamins A, D, and E in different ways (unpublished observations).

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References

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