Vitamin A Antagonizes Calcium Response to Vitamin D in Man

S. JOHANSSON and H. MELHUS

ABSTRACT

For unknown reasons, the highest incidence of osteoporosis is found in northern Europe. In these populations, the sunlight exposure is limited and the vitamin A intake is high. The interaction between vitamin A and D has been the subject of several in vitro and animal studies. We have studied the acute effects of vitamin A and D on calcium homeostasis in 9 healthy human subjects. We compared the effect of (i) 15 mg of retinyl palmitate, (ii) 2 μg of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], (iii) 15 mg of retinyl palmitate plus 2 μg of 1,25(OH)2D3, and (iv) placebo in a double-blind crossover study. The subjects took vitamin preparations at 10:00 p.m. and the following day blood samples were collected five times from 8:00 a.m. to 4:00 p.m. Serum levels of 1,25(OH)2D3 and retinyl esters increased (1.7-fold and 8.3-fold, respectively; \( p < 0.01 \)). As expected, serum calcium (S-calcium) increased (2.3%; \( p < 0.01 \)) and S-parathyroid hormone (PTH) decreased (\(-32\%\); \( p < 0.05 \)) after 1,25(OH)2D3 intake. In contrast, retinyl palmitate intake resulted in a significant decrease in S-calcium when taken alone (\(-1.0\%\); \( p < 0.05 \)) and diminished the calcium response to 1,25(OH)2D3 after the combined intake (1.4%; \( p < 0.01 \)). S-PTH was unaffected by retinyl palmitate. No significant changes in serum levels of the degradation product of C-telopeptide of type I collagen (CrossLaps), or U-calcium/creatinine levels were found. In conclusion, an intake of vitamin A corresponding to about one serving of liver antagonizes the rapid intestinal calcium response to physiological levels of vitamin D in man. (J Bone Miner Res 2001;16:1899–1905)

Key words: vitamin A, vitamin D, calcium, bone metabolism

INTRODUCTION

The incidence of osteoporosis, measured as the rate of the osteoporosis-related hip and distal forearm fractures, is remarkably high for both men and women in the Scandinavian countries.\(^1,2\) In this area the intake of retinol is high.\(^3\) Hypervitaminosis A is known to cause accelerated bone metabolism and spontaneous fractures in laboratory animals\(^4,5\) and a high dietary intake has been associated with the development of osteoporosis.\(^6\)

Vitamin A has been suggested to interfere with the action of vitamin D. The active metabolites of vitamin A and D, retinoic acid (RA) and 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], regulate gene expression through nuclear receptors in the steroid-thyroid hormone receptor family. The vitamin D receptor (VDR) and the RA receptors (RARs) bind to their target genes as heterodimers, consisting of VDR or RAR together with one of the retinoid X receptors (RXRs). Binding of 1,25(OH)2D3 to VDR-RXR activates the complex, resulting in initiation or suppression of gene transcription.\(^7\) The RXR ligand 9-cis-RA has been shown to be a modulator of VDR-RXR action in vitro, possibly by interacting with receptor dimer formation.\(^8\) In vitro studies have variously indicated antagonistic, additive, or synergistic interaction between the vitamins.\(^9\) In vivo, a high level of vitamin A intake is shown to reduce the toxicity associated with hypervitaminosis D in rats\(^10\) and turkey pouls\(^11\) and increase the need for dietary vitamin D.

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of 1,25(OH)\textsubscript{2}D\textsubscript{3}, (iii) 15 mg of retinyl palmitate plus 2 
mg of 1,25(OH)\textsubscript{2}D\textsubscript{3} (combined intake), or (iv) placebo. Vita-
mological vitamin D dose administered [2 
\mu g of 1,25(OH)\textsubscript{2}D\textsubscript{3}] was chosen because it has been reported 
previously to exert an acute effect on bone metabo-
lished order in which every subject had taken every vitamin prep-
the plasma half-life for retinyl palmitate 
is used as a control sample for the postvitamin samples. Serum and urine 
cer was obtained from all subjects volunteering for the 
mm and placebo were prepared as tablets and taken orally. 
and bone resorption in healthy human subjects.

**MATERIALS AND METHODS**

**Subjects**

People working in our department were invited to partic-
period from September to November 1999. On 
all subjects were studied on four different occasions 
and 9 healthy subjects, 4 men and 5 
and bone metabolism, any 
other chronic disease, or took any medication at the time of the study. The presence of any undiagnosed parathyroid 
disease was ruled out by the biochemical analyses of serum 
measured by a sandwich radioimmunometric method (intra-
interassay variation, 3.4%; Nichols Institute, San Juan Capist-
urine calcium (U-calcium) and U-creatinine. All samples 
from 1 individual were analyzed in the same assay to avoid 
interest in the study and 9 healthy subjects, 4 men and 5 
and bone resorption in healthy human subjects.

**Design**

All subjects were studied on four different occasions 
during the period from September to November 1999. On 
the meals. A morning urine sample was collected at 
the subjects had a history of 
any other chronic disease, or took any medication at the time of the study. The presence of any undiagnosed parathyroid 
disease was ruled out by the biochemical analyses of serum 
the first reference blood sample. Informed 
was done by AS Vitas (Oslo, Norway) on 
retinyl palmitate was used to quantify all retinyl esters. The 
retinyl palmitate because this corresponds to the amount of vitamin A in one serving of liver, that is, the 
maximum vitamin A intake from dietary sources. The phys-
ological vitamin D dose administered [2 
\mu g of 1,25(OH)\textsubscript{2}D\textsubscript{3}] was chosen because it has been reported 
previously to exert an acute effect on bone metabo-
Except for the vitamin intakes, the conditions were kept 
during the different study occasions and on every 
occasion the subjects went through the following protocol. 
On day 1, a blood sample was drawn through venous 
puncture at 8:00 a.m., after an overnight fast. This sample 
was used as a control sample for the postvitamin samples. 
The subjects were told not to perform any exhaustive exer-
cises and not to eat any food with a high content of fat-
soluble vitamins during day 1, but no other restrictions were 
asked for. At 10:00 p.m., the subjects had their randomized 

**Biochemical analysis**

Serum was obtained through centrifugation of the 
collected blood. Serum and urine were stored immediately in 
-70°C until assayed for serum 1,25(OH)\textsubscript{2}D\textsubscript{3} [S-1,25(OH)\textsubscript{2}D\textsubscript{3}], S-retinyl esters, S-calcium, S-albumin, 
protein C (S-PTH), and the degradation product of C-telopeptide of type I collagen (S-CrossLaps), and 
urine calcium (U-calcium) and U-creatinine. All samples 
were analyzed in duplicate. 

Analyses of S-1,25(OH)\textsubscript{2}D\textsubscript{3} and S-retinyl esters were 
performed for the 8:00 a.m. fasting samples only. 
S-1,25(OH)\textsubscript{2}D\textsubscript{3} was immunoextracted before radioimmuno-
assay quantification was performed. The kit used (IDS Ltd., 
and centrifuged at 4000 g, 
the supernatant was analyzed by liquid chromatography on a HP1100 high-
performance liquid chromatography (HPLC) system fur-
ished with a Supersphere 100 RP-18 column (Agilent 
Technologies, Palo Alto, CA, USA) and detected at 325 nm with a UV detector. The mobile phase consisted of 
2-propanol and methanol-dichloromethane and the injection volume was 100 \mu l. A three-point calibration curve constructed with 
albumin solutions enriched with different concentrations of 
retinyl palmitate was used to quantify all retinyl esters. The 
intrassay variation was 5.1%. 

S-calcium, S-albumin, S-PTH, and S-CrossLaps, were 
analyzed at all sampling points. S-calcium (intra-assay vari-
ation, 1.5%) and S-albumin (intra-assay variation, 4.5%) 
was determined by complexometric, colorimetric assays 
using the spectrophotometric Hitachi 717 autoanalyzer ac-
cording to the manufacturer’s instructions (Roche, Stock-
holm, Sweden). Briefly, for S-calcium, serum was alkalin-
ized and heated to 37°C before addition of the complex 
forming chromogen o-cresolphthalein and the calcium con-
centration was then determined at 546 nm. For S-albumin, 
serum was first acidified. The complex forming substrate 
broncresol green was then added and the albumin concen-
tration was determined at 570 nm. S-calcium levels were 
adjusted for the concomitant S-albumin. Intact S-PTH 
was measured by a sandwich radioimmunometric method (intra-
assay variation, 3.4%; Nichols Institute, San Juan Capist-
TABLE 1. AVERAGE SERUM LEVELS OF 1,25(OH)₂D₃ AND RETINYL ESTERS AT 8:00 A.M. BEFORE AND AFTER VITAMIN INTAKE

<table>
<thead>
<tr>
<th>Vitamin intake</th>
<th>1,25(OH)₂D₃ (pM) Before</th>
<th>1,25(OH)₂D₃ (pM) After</th>
<th>Retinylesters (µM) Before</th>
<th>Retinylesters (µM) After</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25(OH)₂D₃</td>
<td>127.09 ± 32.85</td>
<td>216.25 ± 17.80*</td>
<td>0.12 ± 0.14</td>
<td>0.09 ± 0.08</td>
</tr>
<tr>
<td>Retinyl palmitate</td>
<td>117.71 ± 34.02</td>
<td>111.95 ± 17.43</td>
<td>0.20 ± 0.28</td>
<td>1.24 ± 1.31*</td>
</tr>
<tr>
<td>Combined</td>
<td>114.70 ± 28.32</td>
<td>201.40 ± 27.47*</td>
<td>0.09 ± 0.08</td>
<td>1.10 ± 1.03*</td>
</tr>
<tr>
<td>Placebo</td>
<td>123.00 ± 35.68</td>
<td>130.38 ± 41.15</td>
<td>0.14 ± 0.13</td>
<td>0.14 ± 0.07</td>
</tr>
</tbody>
</table>

1,25(OH)₂D₃, 2 µg 1,25-(OH)₂D₃; retinyl palmitate, 15 mg retinyl palmitate; combined, 15 mg retinyl palmitate + 2 µg 1,25-(OH)₂D₃. Values are presented as mean ± SD.

*p < 0.01 versus before intake.

DISCUSSION

Here, we show that retinyl palmitate can antagonize the S-calcium response elicited by intake of 1,25(OH)₂D₃. The theoretical basis for a hypothesis of such an interaction is provided by the VDR-RXR heterodimer mediating the molecular action of 1,25(OH)₂D₃ and the allosteric receptor ligand interactions that have been shown in vitro.⁹ Previously, we have reported an antagonistic effect of RA on 1,25(OH)₂D₃-induced bone resorption in vitro.¹² In this study, we see no effect on the bone resorption as measured by the bone resorption marker S-CrossLaps (Fig. 2). Long-term excess of vitamin D leads to hypercalciuria and it is controversial whether this phenomenon is caused...
by the hypercalcemia secondary to increased intestinal calcium absorption or bone resorption or if it is caused by a direct effect on renal reabsorption of calcium. A few in vitro and animal studies show an acute effect of vitamin D on renal calcium reabsorption but it is not clear if the effect is direct or mediated via PTH. Little is known about this effect in humans. In our study, the average U-calcium after vitamin D intake apparently is increased but because of the large SD, which is not simply caused by an outlier, the difference is not statistically significant. Therefore, we cannot conclude that the vitamin intakes have affected renal calcium reabsorption.

Absorption competition between the vitamins is another possible mechanism for interaction. Both vitamin A and D are fat soluble and the important dietary sources of vitamin D, dairy products, fatty fish, and fortified foods also are rich in vitamin A. In our study, S-1,25(OH)2 D3 and S-retinyl esters increased significantly after intake of the corresponding vitamin. There was a large interindividual variation in retinyl esters, both regarding the absolute level and the degree of increase after vitamin intake. All individuals increased their vitamin levels, indicating that the vitamin absorption was efficient. Neither S-1,25(OH)2 D3 nor S-retinyl esters seemed to be affected by simultaneous intake of the other vitamin. However, because of the large variation among the subjects, we cannot with certainty exclude that the antagonistic effect was exerted at the level of vitamin absorption.

In summary, the antagonistic effect on S-calcium is not caused apparently by a direct effect on vitamin absorption or bone resorption and it is not mediated through renal calcium filtration or reabsorption. Instead, our data suggest that the antagonism may be exerted at the level of intestinal calcium absorption. Further studies are needed to confirm the mechanism of the antagonism.

Serum levels of calcium, vitamin D, and vitamin A can influence PTH secretion from the parathyroid glands. 1,25(OH)2 D3 exerts an immediate (seconds) suppressive effect on PTH secretion.
VITAMIN A ANTAGONIZES VITAMIN D IN MAN

TABLE 2. AVERAGE U-CALCIUM/CREATININE LEVELS THE MORNING AFTER VITAMIN INTAKE

<table>
<thead>
<tr>
<th>Vitamin intake</th>
<th>Retinyl palmitate</th>
<th>1,25(OH)2D3</th>
<th>Combined</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-calcium/creatinine</td>
<td>252 ± 145</td>
<td>669 ± 765</td>
<td>352 ± 200</td>
<td>370 ± 225</td>
</tr>
</tbody>
</table>

Retinyl palmitate, 15 mg retinyl palmitate; 1,25(OH)2D3, 2 µg 1,25(OH)2D3; combined, 15 mg retinyl palmitate + 2 µg 1,25(OH)2D3. Values are presented as mean ± SD.

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REFERENCES


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