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Chapter 5

Gender differences in vitamin D3 mediated effects on postprandial leukocyte activation and arterial stiffness

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Abstract

**Background:** The postprandial phase is considered pro-atherogenic, in part due to triglyceride-rich lipoprotein mediated inflammation. Vitamin D has been proposed to reduce inflammation and to improve arterial elasticity. We hypothesized that vitamin D3 could improve postprandial arterial elasticity by modulation of leukocyte activation.

**Methods:** Healthy volunteers underwent two oral fat loading tests (OFLT). Arterial elasticity by augmentation index (AIx) and flowcytometric quantification of leukocyte activation markers CD11b, CD66b, CD35 and CD36 were measured. After the first OFLT a dose of 100000 IU of vitamin D3 was administered and a second OFLT was carried out seven days later.

**Results:** Six men and 6 women were included. 25-Hydroxyvitamin D3 levels rose from 63.0 ± 28.8 nmol/l to 98.5 ± 26.8 nmol/l after vitamin D3 supplementation (P < 0.001). Baseline arterial elasticity did not change postprandially, but a significant favourable reduction in AIx was found after vitamin D3 supplementation (P = 0.042) in both men and women. After vitamin D3, exclusively in women a reduction in the area under the postprandial curve for monocyte CD11b and CD35 by 10.5% (P = 0.016) and 12.5% (P = 0.04) and neutrophil CD11b by 17.0% (P = 0.014) was observed.

**Conclusions:** Vitamin D3 probably increased postprandial arterial elasticity, but vitamin D3 reduced postprandial leukocyte activation exclusively in women.
Introduction

In the past 20 years, the diagnosis of vitamin D deficiency has increased to epidemic proportions, mainly due to obesity, lack of sun exposure and increased screening for vitamin D deficiency [1]. Vitamin D has not only been related to bone mineralization and the metabolism of calcium and phosphate, but also to numerous other physiological processes [1,2]. The vitamin D receptor has been identified on multiple cell types, including immunological and endothelial cells [3-5]. Several studies have shown that low vitamin D concentrations are associated with endothelial dysfunction, increased arterial stiffness, increased carotid intima media thickness, hypertension, the metabolic syndrome and an increased risk for cardiovascular disease [6-13]. Endothelial function and arterial elasticity can improve after vitamin D supplementation in vitamin D deficient subjects as shown in two different studies using cut-off points for vitamin D deficiency of 25 nmol/l and 75 nmol/l, respectively [14,15]. The exact mechanisms of the beneficial effects of vitamin D on cardiovascular disease and arterial stiffness are still unknown.

The immunological effects of vitamin D may be the key link between vitamin D and cardiovascular disease, since inflammation is highly involved in the development of impaired vascular function and atherosclerosis [16]. In vitro experiments have shown that the active metabolite of vitamin D, 1,25-dihydroxyvitamin D3, decreased the expression of CD11b on monocytes and their phagocytic capacity [17]. CD11b is an integrin expressed on monocytes and neutrophils, which improves the binding of monocytes and neutrophils to the endothelium, thereby facilitating subendothelial migration [18,19]. 1,25-Dihydroxyvitamin D3 reduces the uptake of cholesterol by macrophages with inhibition of macrophage differentiation into atherogenic foam cells [20]. Gender differences have been described concerning the activation of leukocytes mediated by sex steroids with a potential functional synergy between 1,25-dihydroxyvitamin D3 and estradiol [21,22]. Since acute inflammatory changes as well as vascular dysfunction occur in the postprandial state [18,19,23,24], the postprandial state can be considered an adequate physiological model to further investigate the relationships between vitamin D, postprandial leukocyte activation and arterial stiffness in vivo. Therefore, the aim of the present study was to investigate if vitamin D3 can reduce leukocyte activation and improve arterial elasticity postprandially in both genders.

Materials and methods

Subjects and study design

The study was designed as a non-randomized cross-over study of healthy males and females. Measurements were performed before and after the intervention i.e. supple-
mentation of vitamin D3; participants served as their own controls. Male and female volunteers were recruited by advertisement between March 2011 and October 2011. Included were healthy subjects between 18 and 50 years of age. Exclusion criterion was the use of any medication except oral contraceptives. At day one participants visited the hospital after a 10h overnight fast. Anthropometric characteristics (length, weight, body mass index, waist circumference) and blood pressure were measured. At baseline, arterial elasticity was determined by augmentation index (Alx), which was measured at the radial artery according to standard operating procedures (SphygmoCor, AtCor Medical, Sydney, Australia) in duplicate after a 5 minute rest in a supine position [25]. The Alx was corrected for a heart rate of 75/min. A fasting venous blood sample was drawn and subjects received an oral fat load using fresh cream (Albert Heijn, Zaandam, the Netherlands) in a dose of 50g of fat per square meter body surface. This method reflects the physiological diurnal increase in triglycerides. During the oral fat loading test participants were not allowed to eat or drink except water and they were asked to refrain from physical activity. Blood pressure, Alx and venous blood sampling were repeated at two hourly intervals until eight hours. At the end of the first oral fat loading test participants received 100000 IU of vitamin D3 dissolved in water (cholecalciferol, 50000 IU/ml). Since 25-hydroxyvitamin D3 reaches its highest serum concentration after 7 days [26,27], the second oral fat loading test was performed exactly seven days later. The procedure and measurements of the second oral fat loading test were identical to the first. All subjects gave written informed consent and the study was approved by the independent Regional Medical Ethical Committee Rotterdam, Maasstad Hospital, the Netherlands (NL3372110110). The study protocol was prospectively registered at EudraCT (2010-024182-44).

Laboratory measurements

All clinical chemistry and hematology measurements were carried out on freshly drawn blood at the Department of Clinical Chemistry, Sint Franciscus Gasthuis, Rotterdam. Baseline C-reactive protein, plasma cholesterol, HDL-C and triglycerides were measured using the LX20 or DxC analyzers (Beckman Coulter, Miami, USA). LDL-C values were calculated using the Friedewald formula. Apolipoprotein A-I and apo B were determined by nephelometry using an IMMAGE instrument with commercially available kits (Beckman Coulter). Blood cell counts and 5-part leucocyte differentiation were determined automatically using LH750 analyzers (Beckman Coulter). The vitamin D status was determined by measuring 25-hydroxyvitamin D3 on serum that had been stored at -70°C (Liaison, DiaSorin, Saluggia, Italy).
CD11b, CD66b, CD35 and CD36 as markers for in vivo leukocyte activation

Blood samples for the determination of leukocyte activation markers CD11b, CD66b, CD35 and CD36 were collected in tubes containing 5.4mg K2 EDTA (Becton Dickinson, Plymouth, United Kingdoms) at baseline and at four and eight hours postprandially. The method has been described in detail before [18,28]. Briefly, the staining procedure was started within 15 minutes after venipuncture. All measurements were carried out in triplicate to reduce variability. Two separate tubes were prepared: 1) a combination of fluorescein isothiocyanate (FITC) conjugated CD66b, phycoerythrin (PE) conjugated CD11b and phycoerythrin-Texas Red-X (ECD) conjugated CD45 and 2) a combination of FITC conjugated CD36, PE conjugated CD35 and ECD conjugated CD45. All antibodies were from Beckman Coulter, except for CD35-PE (BD Biosciences, Franklin Lakes, NJ, USA). A total of 20 µl of whole blood was added to each tube and incubated for 15 minutes in the dark at room temperature. Erythrocytes were lysed by addition of 500µl lysis solution (1.5 M ammonium chloride, 100 mM potassium hydrogen carbonate, 0.82 mM EDTA, pH 7.4) followed by 15 min of incubation in the dark. The samples were measured on a Navios flow cytometer (Beckman Coulter). Samples were measured for a maximum of five minutes or until at least 2000 monocytes were acquired. Lymphocytes, monocytes and neutrophils were identified in the side scatter versus CD45 dot plot. The fluorescence intensity of each cell type was expressed as the mean fluorescence intensity (MFI) of the triplicate measurements. Before each use, the optics and settings of the flow cytometer were checked with Flow-Check Pro and Flow-Set Pro beads (Beckman Coulter). Identical flow cytometric settings were used for the complete study.

Statistical analysis

Data are given as mean ± SD in the text and tables and as mean ± SEM in the figures. Differences between the first oral fat load and the second were tested using the paired Student’s t-test in case of normally distributed variables or by the non-parametric Wilcoxon matched pairs signed ranks test in case of skewed variables. Differences were tested for the total group and for men and women seperately. All statistical analyses were performed using PASW statistics version 18.0 (IBM SPSS Statistics, New York, United States). The total area under the postprandial curve (AUC) was calculated with PRISM version 5.0 (Graph Pad Software, San Diego). Statistical significance was set at P < 0.05 (two tailed).
Results

Cholesterol, apolipoproteins and postprandial lipemia after vitamin D3

A total of 6 men and 6 women were included. Oral contraceptives were used by three women. The baseline characteristics for the total group and for each gender are shown in Table 1. No significant differences were observed between genders. Seven days after the administration of 100000 IU of vitamin D3, serum 25-hydroxyvitamin D3 concentrations increased significantly from 63.1 ± 28.8 nmol/l to 98.5 ± 26.8 nmol/l for the total group (P < 0.001), from 49.9 ± 25.2 nmol/l to 88.2 ± 22.1 nmol/l for men (P < 0.001) and from 76.2 ± 27.9 nmol/l to 108.7 ± 28.9 nmol/l for women (P = 0.001). Only one male participant had an initial 25-hydroxyvitamin D3 serum concentration below 30.0 nmol/l in contrast

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total group (n = 12)</th>
<th>Men (n = 6)</th>
<th>Women (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.3 ± 12.7</td>
<td>34.5 ± 12.3</td>
<td>36.2 ± 14.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.9 ± 2.1</td>
<td>21.1 ± 2.6</td>
<td>22.7 ± 0.9</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110.5 ± 9.1</td>
<td>108.2 ± 10.6</td>
<td>108.7 ± 8.9</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>65.1 ± 8.8</td>
<td>65.8 ± 7.1</td>
<td>63.0 ± 5.8</td>
</tr>
<tr>
<td>Augmentation Index (%)</td>
<td>14.8 ± 11.7</td>
<td>14.0 ± 12.4</td>
<td>20.1 ± 11.9</td>
</tr>
<tr>
<td>25-hydroxyvitamin D3 (nmol/l)</td>
<td>63.1 ± 28.8</td>
<td>98.5 ± 26.8*</td>
<td>76.2 ± 27.9</td>
</tr>
<tr>
<td>Leukocyte count (*10⁹/l)</td>
<td>6.1 ± 1.1</td>
<td>5.8 ± 1.4</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>Absolute lymphocyte count (*10⁹/l)</td>
<td>1.68 ± 0.33</td>
<td>1.76 ± 0.38</td>
<td>1.70 ± 0.27</td>
</tr>
<tr>
<td>Absolute monocyte count (*10⁹/l)</td>
<td>0.46 ± 0.13</td>
<td>0.43 ± 0.12</td>
<td>0.40 ± 0.09</td>
</tr>
<tr>
<td>Absolute neutrophil count (*10⁹/l)</td>
<td>3.83 ± 0.87</td>
<td>3.49 ± 1.15</td>
<td>4.15 ± 0.88</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>1.42 ± 0.67</td>
<td>1.67 ± 1.72</td>
<td>1.67 ± 0.82</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.6 ± 0.6</td>
<td>4.6 ± 0.6</td>
<td>4.8 ± 0.7</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.7 ± 0.6</td>
<td>2.7 ± 0.6</td>
<td>2.7 ± 0.7</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.63 ± 0.39</td>
<td>1.58 ± 0.45</td>
<td>1.75 ± 0.46</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.71 ± 0.24</td>
<td>0.77 ± 0.18</td>
<td>0.78 ± 0.14</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>0.78 ± 0.14</td>
<td>0.76 ± 0.14</td>
<td>0.78 ± 0.20</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/l)</td>
<td>1.88 ± 0.35</td>
<td>1.75 ± 0.28</td>
<td>1.97 ± 0.38</td>
</tr>
</tbody>
</table>

* Significantly different from corresponding control value (P = 0.001)
** Significantly different from corresponding control value (P < 0.001)
to none in the females. No significant changes were observed in leukocyte counts, absolute lymphocyte, monocyte and neutrophil counts, lipids or arterial stiffness in the fasting state after vitamin D3 supplementation (Table 1). The postprandial triglyceride response was similar before and seven days after supplementation of vitamin D3 for the total group (Figure 1A) and for men and women separately (Figure 1B). As expected, women tended to have lower fasting and postprandial triglycerides when compared to men, but statistical significance was not reached. The AUC for triglycerides for the total group and for men and women separately remained unchanged after vitamin D3 (Table 2).

![Figure 1: Mean ± SEM postprandial changes in plasma triglycerides (TG) for the total group (n = 12) before (open diamonds, ◊) and seven days after the administration of 100000 IU of vitamin D3 (closed diamonds, *) (A). Postprandial changes in plasma TG were separated by gender (B). Men (n = 6) before (open square, □) and after vitamin D3 administration (closed square, ■) and women (n = 6) before (open circle, ○) and after vitamin D3 administration (closed circle, ●) are shown.](image)

**Postprandial leukocyte activation after vitamin D3 supplementation**

Postprandial leukocyte activation before and after supplementation of vitamin D3 is shown for men and women in Figure 2. In the fasting state, integrin expression on monocytes tended to be higher in women in contrast to the expression on neutrophils. Both in men and in women, the expression of integrins on monocytes and on neutrophils increased postprandially at T = 4h and returned to baseline at T = 8h.

In the fasting state, no changes in integrin expression for both monocytes and neutrophils in males and females were observed after vitamin D3 supplementation. After vitamin D3 supplementation the reduction of the AUC for CD11b expression on monocytes was 6.9% (P = 0.043) and on neutrophils 8.9% (P = 0.019) for the total group (Table 2). A similar trend was observed for the CD35 expression on neutrophils. In females, the AUC for CD11b expression on monocytes and neutrophils and for CD35 expression on monocytes was significantly reduced together with a similar trend for the
CD35 and CD66b expression on neutrophils and CD36 expression on monocytes, while no changes were observed in men (Table 2).

**Arterial stiffness after vitamin D3 supplementation**

Women tended to have a higher Alx when compared to men. The Alx did not change postprandially during the first oral fat load in contrast to the situation after supplementation of vitamin D3 showing a postprandial decrease. This pattern was observed in the

![Graphs showing postprandial changes in mean fluorescence intensity (MFI) of CD11b, CD35, and CD36 expression on monocytes and neutrophils before and after vitamin D3 supplementation.](image)

**Figure 2:** Mean ± SEM postprandial changes in mean fluorescence intensity (MFI) of CD11b expression on monocytes (MO) (A) and neutrophils (NE) (D), CD35 expression on monocytes (B) and neutrophils (E), CD36 expression on monocytes (E) and CD66b expression on neutrophils (F) before (open squares, □, for men and open bullets, ○, for women) and seven days after the administration of 100 000 IU of vitamin D3 (closed squares, ■, for men and closed bullets, ●, for women).
### Table 2: Postprandial changes in triglycerides, leukocyte activation markers and arterial stiffness expressed as the total area under the postprandial curve at baseline (Control) and seven days after the administration of 100000 IU of vitamin D3.

<table>
<thead>
<tr>
<th>Area under the curve</th>
<th>Total group (n = 12)</th>
<th>P-value</th>
<th>Men (n = 6)</th>
<th>P-value</th>
<th>Women (n = 6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vitamin D3</td>
<td>Control</td>
<td>Vitamin D3</td>
<td>Control</td>
<td>Vitamin D3</td>
</tr>
<tr>
<td>Triglycerides (mmol*h/l)</td>
<td>10.2±4.3</td>
<td>9.7±3.9</td>
<td>0.23</td>
<td>11.6±4.5</td>
<td>10.7±4.0</td>
<td>0.16</td>
</tr>
<tr>
<td>Monocyte CD11b (MFI*h)</td>
<td>123.8±14.2</td>
<td>115.2±16.3</td>
<td>0.043</td>
<td>115.4±15.2</td>
<td>112.0±18.9</td>
<td>0.60</td>
</tr>
<tr>
<td>Monocyte CD35 (MFI*h)</td>
<td>14.5±5.8</td>
<td>13.6±4.6</td>
<td>0.20</td>
<td>13.7±6.2</td>
<td>14.0±5.1</td>
<td>0.72</td>
</tr>
<tr>
<td>Monocyte CD36 (MFI*h)</td>
<td>92.6±24.9</td>
<td>88.9±25.8</td>
<td>0.33</td>
<td>87.7±18.2</td>
<td>89.3±9.8</td>
<td>0.78</td>
</tr>
<tr>
<td>Neutrophil CD11b (MFI*h)</td>
<td>180.6±39.4</td>
<td>164.5±42.9</td>
<td>0.019</td>
<td>197.6±33.0</td>
<td>193.0±28.3</td>
<td>0.51</td>
</tr>
<tr>
<td>Neutrophil CD35 (MFI*h)</td>
<td>22.6±8.0</td>
<td>20.2±7.0</td>
<td>0.069</td>
<td>25.2±9.4</td>
<td>24.2±7.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Neutrophil CD66b (MFI*h)</td>
<td>31.5±9.6</td>
<td>31.0±11.7</td>
<td>0.82</td>
<td>34.1±6.6</td>
<td>36.4±12.1</td>
<td>0.50</td>
</tr>
<tr>
<td>Augmentation index (%*h)</td>
<td>107.5±93.6</td>
<td>88.7±98.3</td>
<td>0.042</td>
<td>57.7±47.2</td>
<td>37.8±41.2</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Abbreviations: MFI = mean fluorescent intensity; CD = cluster of differentiation
total group (Figure 3A) and in both genders separately (Figure 3B). This resulted in a significant reduction of the AUC for Alx for the total group after vitamin D3 supplementation (107.5 ± 93.6 %*h vs 88.7 ± 98.3 %*h, P = 0.042), which was observed in both genders (Table 2).

**Figure 3:** Mean ± SEM postprandial changes in augmentation index (Alx) for the total group (n = 12) before (open diamonds, ◊) and seven days after the administration of 100000 IU of vitamin D3 (closed diamonds, ♦) (A). Postprandial changes in Alx were separated by gender (B). Men (n = 6) before (open square, □) and after vitamin D3 administration (closed square, ■) and women (n = 6) before (open circle, ○) and after vitamin D3 supplementation (closed circle, ●) are shown.

**Discussion**

Multiple studies have demonstrated the association between vitamin D deficiency and a subsequent increased cardiovascular risk [6,12,13], but the exact mechanism is not clearly understood. The atheroprotective effect of vitamin D may be attributed to beneficial modulation of the immune system [29]. The present study shows that a single high dose of vitamin D3 reduced postprandial leukocyte activation only in women, whereas the improvement in postprandial arterial elasticity was observed in both men and women. Serum 25-hydroxyvitamin D3 concentrations increased substantially in all our subjects seven days after a single dose of 100000 IU of vitamin D3. However, it should be noted that higher serum 25-hydroxyvitamin D3 concentrations do not necessarily translate into increased 1.25-dihydroxyvitamin D3 serum concentrations [30]. Nevertheless, it is generally accepted that 1.25-dihydroxyvitamin D3 will be higher when 25-hydroxyvitamin D3 serum concentrations rise.

The postprandial CD11b expression on monocytes and neutrophils was significantly reduced after vitamin D3 supplementation. However, this effect was mainly observed in women. Others have shown that 1,25-dihydroxyvitamin D3 reduced the CD11b expression on monocytes with a concomitant reduction in the phagocytic capacity *in vitro* [17]. On the contrary, 1,25-dihydroxyvitamin D3 upregulates the CD11b gene in
leukocytes [31]. Since in vitro 1,25-dihydroxyvitamin D3 reduced the CD11b expression on monocytes despite a contradictory upregulation of the CD11b gene, vitamin D may have differential effects on leukocyte CD11b regulation.

We hypothesize that sex steroids may have influenced the vitamin D3 effects on the expression of integrins like CD11b. It has been demonstrated that estradiol reduces the CD11b expression on monocytes in vitro [21], whereas testosterone potentiates neutrophil activation [32]. Animal studies showed that the CD11b expression on neutrophils is also regulated by estradiol, which limits the entry of calcium into cells, thereby blunting neutrophil activation [32,33]. This may explain the observed trend to lower fasting and postprandial CD11b, CD35 and CD66b expressions on neutrophils in women compared to men in the present study.

Another explanation for the gender differences observed here may be related to an interaction between estrogen and vitamin D. Others have shown that women have fewer CYP24A1 transcripts, encoding the 1,25-dihydroxyvitamin D3-inactivating enzyme, when compared to men [22]. Therefore, binding and cellular accumulation of 1,25-dihydroxyvitamin D3 is increased in women with a subsequent increased anti-inflammatory effect on T-lymphocytes and macrophages [22]. The ex vivo addition of estradiol to male T-lymphocytes and macrophages reproduced these effects, suggesting a synergistic effect between 1,25-dihydroxyvitamin D3 and estradiol [22]. Therefore, vitamin D induced reductions in postprandial leukocyte activation may be dependent on estrogen.

We observed that the relative change in 25-hydroxyvitamin D3 was greater in men compared to women, but all subjects reached levels that were well above generally accepted normal concentrations. In contrast, females showed higher absolute serum 25-hydroxyvitamin D3 concentrations after supplementation, probably because of higher initial levels. The results may have been influenced by the wide variation in 25-hydroxyvitamin D3 concentrations between subjects, especially in males, which may also have contributed to the observed gender differences. In vitro a clear dose response relationship between a reduction in CD11b expression on monocytes with increasing concentrations of 1,25-dihydroxyvitamin D3 has been demonstrated [17]. Future studies including primarily vitamin D deficient subjects are needed to evaluate whether vitamin D3 supplementation in vitamin D deficiency is beneficial in reducing postprandial leukocyte activation.

The postprandial CD36 expression on monocytes was reduced by 9.3% in women after vitamin D3 supplementation. CD36 is a scavenger receptor facilitating the uptake of free fatty acids and oxidized LDL by macrophages with subsequent risk of foam cell formation. In vitro, macrophages showed less cholesterol uptake and foam cell formation, with suppression of the CD36 expression when 1,25-dihydroxyvitamin D3 was present in the culture medium [20,34]. Recently, comparable results were obtained in a vitamin D-deficient mouse model [35]. However, it should be noted that this mouse
model included both male and female animals. Therefore, our results showing a blunted postprandial response in leukocyte activation by vitamin D3 in females are partly in line with previous publications.

We did not observe any effect of vitamin D3 on postprandial lipemia and these results are comparable to previous reports [36,37]. Therefore, the observed effects on leukocyte activation and arterial stiffness we found could not be attributed to postprandial lipid changes. Although it should be noted that triglycerides reflect total postprandial response of both very low density lipoproteins and chylomicrons and that we did not differentiate between them using specific antibodies against apo B-100 and apo B-48.

Low 25-hydroxyvitamin D3 levels have been associated with increased arterial stiffness [38] and vitamin D supplementation improved endothelial function, which was determined with flow mediated vasodilatation, in asymptomatic subjects [14] and in patients with type 2 diabetes mellitus [39]. In addition, vitamin D3 supplementation can reduce arterial stiffness during childhood (14-18 years of age) [15]. All these interventional studies were performed in both genders and the beneficial effects of vitamin D3 supplementation on arterial elasticity were observed in both [14,15,39]. In addition, the association between 25-hydroxyvitamin D3 and arterial stiffness was independent from gender status [38]. Our data extend those observations showing similar effects in both genders. Since we observed a reduction in postprandial leukocyte activation solely in women, the improved postprandial arterial elasticity after vitamin D3 in both genders can not be explained by the reduction in postprandial leukocyte activation after vitamin D3 supplementation. Therefore, a direct effect of vitamin D on the arterial wall may have played a role. In vitro 1,25-dihydroxyvitamin D3 inhibited vascular cellular adhesion molecule-1 and interleukin-8 production in human arterial endothelial cells [40]. However, a placebo controlled randomized trial was unable to show any changes in circulating vascular cell adhesion molecules after vitamin D2 supplementation [41].

In our study women tended to have a higher AIx, which is in line with a recent community-based cohort study with 983 participants showing that women have a higher degree of arterial stiffness and concomitant AIx compared to men [42]. We did not observe any changes in AIx postprandially during the first oral fat load, whereas the AIx was temporarily reduced in the postprandial state after vitamin D3 supplementation. A postprandial improvement in arterial elasticity has been observed by others during the first 120 minutes after the ingestion of a mixed meal [43]. These authors suggested that postprandial changes in autonomic function could be responsible for temporary postprandial improvements in arterial elasticity. We did not measure parameters like heart rate variability to investigate changes in autonomic function in the postprandial state or after vitamin D3 supplementation. It has been reported that vitamin D3 may alter the autonomic nervous system [44]. In vitro 1,25-dihydroxyvitamin D3 stimulates myocytes
in a similar way as the beta-adrenergic agonist isoproterenol [45]. Moreover, vitamin D3 maintained the expression of cholinergic receptors in rats with diabetes mellitus [46].

In conclusion, a single dose of 100000 IU of vitamin D3 increased postprandial arterial elasticity in healthy men and women probably by a direct effect on the arterial wall. Vitamin D3 reduced postprandial leukocyte activation exclusively in women and therefore, could not fully explain the increased postprandial arterial elasticity after vitamin D supplementation.
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