# Type of Dietary Fat Is Associated with the 25-Hydroxyvitamin $D_3$ Increment in Response to Vitamin D Supplementation

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**Context:** Mono- and polyunsaturated fats may have opposing effects on vitamin D absorption.

**Objective:** The purpose of this study was to determine whether intakes of different dietary fats are associated with the increase in serum 25-hydroxyvitamin D (25OHD) after supplementation with vitamin $D_3$.

**Design, Setting, and Participants:** This analysis was conducted in the active treatment arm of a randomized, double-blind, placebo-controlled trial of vitamin D and calcium supplementation to prevent bone loss and fracture. Subjects included 152 healthy men and women age 65 and older who were assigned to 700 IU/d vitamin $D_3$ and 500 mg/d calcium. Intakes of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and saturated fatty acids (SFA) were estimated by food frequency questionnaire.

**Main Outcome Measure:** The change in plasma 25OHD during 2 yr vitamin D and calcium supplementation was assessed.

**Results:** The change in plasma 25OHD (nanograms per milliliter) during vitamin D supplementation was positively associated with MUFA, ($\beta = 0.94$; $P = 0.016$), negatively associated with PUFA, ($\beta = -0.93$; $P = 0.038$), and positively associated with the MUFA/PUFA ratio ($\beta = 6.46$; $P = 0.014$).

**Conclusion:** The fat composition of the diet may influence the 25OHD response to supplemental vitamin $D_3$. Diets rich in MUFA may improve and those rich in PUFA may reduce the effectiveness of vitamin $D_3$ supplements in healthy older adults. More studies are needed to confirm these findings. **(*J Clin Endocrinol Metab* 96: 3170–3174, 2011)**

The 25-hydroxyvitamin D (25OHD) responses to supplementation with vitamin D vary widely among individuals. As a result, it is difficult to predict the dose that an individual needs to reach a specified target 25OHD level. Several sources of variability have been identified, including body mass index (BMI), which is inversely associated with change in 25OHD. The starting level of 25OHD is also important. The increment in 25OHD in response to a given dose of vitamin $D_3$ is inversely related to the starting 25OHD level (1). Genetic factors influence not only ambient 25OHD levels but also the increment in response to supplemental vitamin $D_3$ (2). Fu and colleagues (2) found that people with different vitamin D-binding protein genotypes have different serum 25OHD responses to a given dose of vitamin D. Together, the known sources account for no more than one third of the variability in 25OHD increment in response to supplemental vitamin $D_3$; the remainder is unexplained.

Some of the variability may be related to the presence or absence of a meal and to the fat content and composition of the meal or the diet. *In vivo* studies in the rat have shown that greater fatty acid chain length and degree of

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Abbreviations: BMI, Body mass index; MUFA, monounsaturated fatty acid; 25OHD, 25-hydroxyvitamin D; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

**TABLE 1.** Baseline characteristics of the 152 subjects by tertiles of the dietary MUFA/PUFA ratio

| | MUFA/PUFA | | | |
|---|---|---|---|---|
| | **<1.77** | **1.77–2.10** | **>2.10** | ***P*** |
| n | 50 | 51 | 51 | |
| Age (yr) | 71.0 ± 4.5 | 70.2 ± 4.2 | 70.0 ± 4.0 | 0.404 |
| Female (%) | 62.0 | 43.1 | 52.9 | 0.165 |
| Caucasian (%) | 96.0 | 98.0 | 98.0 | 0.762 |
| Weight (kg) | 72.0 ± 14.0 | 76.4 ± 13.0 | 74.6 ± 12.8 | 0.249 |
| BMI (kg/m$^2$) | 26.8 ± 4.4 | 26.7 ± 3.6 | 26.8 ± 3.2 | 0.977 |
| Body fat (kg) | 25.0 ± 8.7 | 25.1 ± 8.0 | 26.1 ± 6.7 | 0.751 |
| Baseline 25OHD (ng/ml) | 31.0 ± 15.1 | 30.5 ± 14.3 | 30.6 ± 14.5 | 0.982 |
| Baseline PTH (pg/ml) | 37.2 ± 18.3 | 38.1 ± 12.5 | 35.1 ± 17.9 | 0.623 |
| Physical activity score | 102.1 ± 53.6 | 111.5 ± 53.1 | 105.3 ± 44.9 | 0.642 |

unsaturation of fatty acids in the gut slowed the rate of vitamin $D_3$ absorption (3). In an uncontrolled, prospective study in 17 patients, some of whom had malabsorption, serum 25OHD levels increased when the patients were instructed to take their supplements with the largest meal of the day, as opposed to taking them at the time of their choosing (4). The diets of these patients were not characterized with respect to their content of fat or of other components.

This study was done to explore possible associations of dietary fat content and composition with the increment in plasma 25OHD in response to supplemental vitamin $D_3$.

## Subjects and Methods

The subjects in this analysis comprise the active treatment arm of a 3-yr, randomized, double-blind, placebo-controlled trial designed to determine the effect of 700 IU/d (17.5 $\mu$g/d) supplemental vitamin $D_3$ together with 500 mg/d (12.5 mmol/d) supplemental calcium on rates of bone loss and fractures in healthy older adults (5). Subjects were instructed to take their study pills at bedtime. As previously described, subjects were 65 yr of age or older, and exclusion criteria included selected medical conditions and medications related to bone metabolism, BMD more than 2 SD below the age- and sex-matched reference means, personal calcium or vitamin D supplement use, cod liver oil use, and dietary calcium intake above 1500 mg/d (37.5 mmol/d) (5). For the present analysis, we also excluded subjects missing a plasma 25OHD measurement at the 2-yr study visit (n = 32) or a food frequency questionnaire (n = 35) resulting in a sample size of 152. The protocol was approved by the Institutional Review Board at Tufts University, and all volunteers gave written informed consent. Adherence to treatment was assessed on the basis of pill counts.

All measurements were made at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University in Boston, MA. Blood was drawn between 0700 and 0930 h after a minimum 8-h fast. Plasma 25OHD measurements at baseline and after 2 yr were used in this analysis. Serum PTH was measured by immunometric assay (Nichols Institute, San Juan Capistrano, CA) and plasma total 25OHD ($25OHD_2$ and $25OHD_3$) was measured by the method of Preece *et al.* (6) with coefficients of variation of 5.6–7.7%. Fatty acid composition and vitamin D intake were assessed with a 126-item food frequency questionnaire conducted at the 18-month visit (7). The questionnaire was completed by study participants during the study visit and checked for completeness by study staff. Fatty acid intakes estimated from this questionnaire have been shown to correlate moderately well, after adjustment for total energy intake, with those from multiple 24-h recalls (8). Leisure, household, and occupational activity was estimated with use of the Physical Activity Scale for the questionnaire (9).

### Statistical analysis

Graphic inspection of the data revealed no important departures from normality in the key dependent or independent variables. Preliminary analyses suggested that both grams per day of fatty acid types [monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), and saturated fatty acid (SFA)] and the ratio of MUFA to PUFA (MUFA/PUFA) were related to the change in plasma 25OHD with supplementation, and we therefore present results in two ways. Baseline characteristics and dietary intakes are reported by tertile of MUFA/PUFA and compared by ANOVA (continuous variables) or the $\chi^2$ test (categorical variables). Separate regression models were constructed to describe the associations of MUFA/PUFA and grams per day of the fatty acids on the change in 25OHD after adjustment for baseline 25OHD, BMI, and total caloric intake. Sex was investigated as a potential confounder but had little influence on coefficients and was discarded from final models. The potential interaction of MUFA/PUFA and the fatty acids with baseline 25OHD was examined by including an interaction term in the regression models. Statistical tests were conducted at the two-tailed 0.05 level. SPSS version 17.0 (SPSS Inc., Chicago, IL) was used for the analyses.

## Results

The subjects ranged in age from 65–86 yr. Baseline characteristics including age, sex, measures of body size and adiposity, physical activity score, and circulating 25OHD and PTH levels were similar across MUFA/PUFA tertiles (Table 1). Total fat intake, percentage of total energy from fat, MUFA intake, and SFA intake all increased across MUFA/PUFA tertiles, and PUFA intake decreased (Table 2). Vitamin D intake, protein intake, carbohydrate intake, and total energy intake did not differ significantly across the tertiles. Mean compliance with vitamin D supplements

**TABLE 2.** Intake of macronutrients, fatty acids, and total energy by tertiles of the dietary MUFA/PUFA ratio

| | MUFA/PUFA | | | |
|---|---|---|---|---|
| | **<1.77** | **1.77–2.10** | **>2.10** | ***P*** |
| N | 50 | 51 | 51 | |
| PUFA/MUFA ratio | 1.5 ± 0.2 | 2.0 ± 0.1 | 2.5 ± 0.4 | <0.001 |
| Fat intake (g/d) | 48.2 ± 17.7 | 57.6 ± 27.5 | 60.5 ± 29.2 | 0.043 |
| MUFA (g/d) | 18.8 ± 7.2 | 23.9 ± 11.8 | 24.4 ± 10.7 | 0.010 |
| PUFA (g/d) | 12.7 ± 4.8 | 12.3 ± 6.1 | 9.8 ± 4.0 | 0.008 |
| SFA (g/d) | 16.7 ± 6.4 | 21.4 ± 10.0 | 26.2 ± 15.6 | <0.001 |
| Vitamin D intake (IU/d) | 315.5 ± 208.8 | 265 ± 142.2 | 301.1 ± 162.7 | 0.336 |
| Protein intake (g/d) | 74.3 ± 20.7 | 84.1 ± 47.9 | 83.8 ± 26.3 | 0.255 |
| Carbohydrate intake (g/d) | 265.0 ± 97.5 | 262.7 ± 128.9 | 246.0 ± 84.6 | 0.614 |
| Energy intake (kcal/d) | 1834.2 ± 526.4 | 1996.0 ± 936.9 | 1943.5 ± 667.8 | 0.528 |
| Fat, % of energy intake | 23.7 ± 6.3 | 25.9 ± 5.3 | 27.4 ± 5.6 | 0.006 |

over the 2-yr study period was 96.7 ± 16.6%. Mean change in BMI over the 2 yr was less than 0.2 kg/m$^2$ in each tertile and did not differ significantly across tertiles ($P = 0.660$). The mean change in plasma 25OHD was 15.3 ± 13.5 ng/ml and was inversely correlated with starting 25OHD level ($r = -0.38$; $P < 0.001$).

Total fat intake was not significantly associated with the change in 25OHD during supplementation (Table 3, model A). However, when examined simultaneously, MUFA was positively associated with the change in 25OHD, and PUFA and SFA were inversely associated with the change in 25OHD (Table 3, model B). Consistent with this, the MUFA/PUFA ratio was positively associated with the change in plasma 25OHD before (Table 3, model C) and after (Table 3, model D) adjustment for SFA. There was no interaction of MUFA/PUFA with baseline 25OHD when interaction terms were added to models C and D ($P > 0.944$).

## Discussion

In healthy older men and women who were instructed to take 700 IU supplemental vitamin $D_3$ daily at bedtime, we identified no association of total daily fat intake with 25OHD increment. In contrast, the increment in 25OHD was significantly positively associated with total daily MUFA intake and inversely associated with total PUFA intake.

**TABLE 3.** Associations of dietary fat intake with changes in 25OHD

| Model | $\beta$ | 95% CI | *P* |
|---|---|---|---|
| A, Total fat intake (g/d) | 0.05 | −0.10–0.20 | 0.530 |
| B, Type of dietary fat | | | |
| MUFA (g/d) | 0.94 | 0.18–1.71 | 0.016 |
| PUFA (g/d) | −0.93 | −1.81 to −0.05 | 0.038 |
| SFA (g/d) | −0.41 | −0.86–0.05 | 0.077 |
| C, MUFA/PUFA ratio | 4.71 | 0.58–8.84 | 0.026 |
| D, MUFA/PUFA ratio | 6.46 | 1.31–11.61 | 0.014 |

$\beta$ represents regression coefficients. All models are adjusted for baseline BMI (kilograms per square meter), baseline 25OHD (nanograms per milliliter), and total energy intake (kilocalories per day). In model B, fat variables are also adjusted for each other. In model D, SFA intake is also adjusted for.

The mechanisms by which fatty acid intake may influence vitamin $D_3$ absorption have not been completely delineated. Most of the available evidence comes from early work by Hollander and colleagues (10). Their gut perfusion studies in the rat revealed that vitamin $D_3$ is absorbed by passive diffusion in the proximal jejunum and the distal ileum (10). Absorption of physiological doses of vitamin $D_3$ in rats was reduced by 30% in the presence of a 4-fold increase in luminal fat (3, 10), and consistent with our findings, the PUFA, linoleic and linolenic acids, were particularly effective in decreasing vitamin $D_3$ absorption (3). Hollander offered several potential explanations for why these fatty acids impaired vitamin $D_3$ absorption. They may have increased the solubility of vitamin $D_3$ in the micelles and changed the partition coefficient such that the vitamin $D_3$ stayed in the micelle. Alternatively, they may have increased the size of the micelle and thereby reduced its diffusion rate and increased its difficulty in crossing the unstirred water layer lining the intestinal mucosa. We are aware of no previous evidence concerning the impact of MUFA on vitamin $D_3$ absorption.

Although we saw no effect of total fat intake on plasma 25OHD increment in this study, this does not constitute convincing evidence that some fat isn't needed to promote vitamin $D_3$ absorption. Absorption of other fat-soluble vitamins, E and K, is enhanced by the presence of small amounts of dietary fat, presumably because fat ingestion stimulates bile acid secretion. Grossmann and Tangpricha (11) recently reviewed available evidence for the importance of vehicle in the use of supplemental vitamin $D_3$. They concluded from limited available evidence that vitamin D solubilized in small amounts of fish oil, either in a capsule or liquid, produced a greater increment in

25OHD than vitamin $D_3$ as a powder or dissolved in ethanol. We agree with Grossmann that this evidence is inconclusive because the starting 25OHD levels, study durations, and dosing schedules in the available studies weren't matched and because increment in 25OHD rather than absorption of parent vitamin $D_3$ was measured. Of the two studies that compared the same dose of vitamin D as a powder and in fish oil, one found no difference in serum 25OHD increment (12) and the other found the serum 25OHD increment to be greater with the oil vehicle (13). The type of fish oil used in the available studies wasn't specified (12–14), and this may be relevant because fish oils vary widely in their MUFA/PUFA ratios (*e.g.* the ratio is 0.71 for salmon oil and 3.67 for herring oil). One study compared the serum parent vitamin $D_3$ response of healthy subjects to a single dose of 25,000 IU vitamin $D_3$ in 0.1 ml corn oil, in 240 ml whole milk, or in 240 ml fat-free milk, and found that increments in vitamin $D_3$ did not differ significantly across the different vehicles (15). It is difficult to relate this result to our findings, however, because, although the MUFA/PUFA ratio of corn oil and milk are quite different (0.41 and 7.0, respectively), the amount of oil relative to the amount of milk ingested differed greatly. Currently, the amount of fatty acid needed to significantly influence vitamin $D_3$ absorption in humans is unknown. The amounts in the studies cited above, on the order of 0.1 ml, were very small compared with the amounts of fat consumed daily, and they were intended to solubilize the vitamin $D_3$. Larger amounts of fat are likely needed to affect micelle content or migration rates.

The main limitation of our study is that our fat intake assessment was based on a food frequency questionnaire, and we were not able to define the fat content or composition of any specific meal. However, it seems plausible that there would be a positive association between both the amount and nature of fat consumed at dinnertime and that consumed throughout the day. We also don't know the time interval between dinner and bedtime when subjects were instructed to take their pills; a shorter interval might allow for more influence of the meal on vitamin $D_3$ absorption.

Both MUFA and PUFA are found in a variety of foods that are commonly consumed by Americans. Among U.S. adults, beef is the single biggest contributor of MUFA (11% of MUFA intake), followed by oils (8%) and baked goods (7%). Salad dressings provide 21% of PUFA, oils provide 13%, and margarine provides 8% (16). Although numerous health benefits have been attributed to the consumption of high-MUFA oils, particularly olive oil, it is unknown whether these benefits derive from MUFA or from other properties of the oil or whether MUFA derived from vegetable and animal sources are similarly beneficial (17). However, it is widely agreed that substitution of saturated fats with MUFA or PUFA is desirable for reducing the risk of heart disease (18). Given current knowledge, a reasonable strategy to increase the ratio of MUFA to PUFA in the diet would be to replace saturated fats with oils that have a high MUFA to PUFA ratio. This ratio is highest in olive oil (9.0), intermediate in canola oil (2.0) and peanut oil (1.4), and low in corn oil (0.4), soybean oil (0.4), and cottonseed oil (0.3). Our study suggests that an increase in the consumption of MUFA-rich oils may improve the bioavailability of vitamin $D_3$, but this remains to be demonstrated experimentally.

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