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A 25-Hydroxycholecalciferol–Fortified Dairy Drink Is More Effective at Raising a Marker of Postprandial Vitamin D Status than Cholecalciferol in Men with Suboptimal Vitamin D Status

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Abstract

Background: One strategy for improving population vitamin D status is consumption of fortified foods. However, the effects of dairy products fortified with different vitamin D isoforms on postprandial vitamin D status and metabolic outcomes have not been addressed.

Objective: We investigated whether consumption of dairy drinks fortified with either 25-hydroxycholecalciferol [25(OH)D_3] or cholecalciferol (vitamin D_3) had differential effects on 24-h circulating plasma 25(OH)D_3 concentration (a marker of vitamin D status) and cardiometabolic risk markers.

Methods: A randomized, controlled, 3-way crossover, double-blind, postprandial study was conducted in 17 men with suboptimal vitamin D status [mean $\pm$ SEM age: 49 $\pm$ 3 y; body mass index (in kg/m²): 26.4 $\pm$ 0.6; and plasma 25(OH)D_3 concentration: 31.7 $\pm$ 3.4 nmol/L]. They were randomly assigned to consume 3 different test meals (4.54 MJ, 51 g fat, 125 g carbohydrate, and 23 g protein), which contained either a nonfortified dairy drink (control), 20 μg 25(OH)D_3-fortified (+HyD$_3$) dairy drink, or 20 μg vitamin D_3–fortified (+D_3) dairy drink with toasted bread and jam on different occasions, separated by a 2-wk washout. Plasma 25(OH)D_3 concentrations and cardiometabolic risk markers, including vascular stiffness, serum lipids, and inflammatory markers, were measured frequently within 8 h postprandially and 24 h after the dairy drink was consumed.

Results: Plasma 25(OH)D_3 concentrations (the primary outcome) were significantly higher after the +HyD$_3$ dairy drink was consumed compared with +D_3 and control ($P = 0.019$), which was reflected in the 1.5-fold and 1.8-fold greater incremental area under the curve for the 0–8 h response, respectively. The change in plasma 25(OH)D_3 concentrations from baseline to 24 h for the +HyD$_3$ dairy drink was also 0.9-fold higher than the +D_3 dairy drink and 4.4-fold higher than the control ($P < 0.0001$), which were not significantly different from each other.

Conclusion: The dairy drink fortified with 25(OH)D_3 was more effective at raising plasma 25(OH)D_3 concentrations postprandially than was the dairy drink fortified with vitamin D_3 in men with suboptimal vitamin D status. This trial was registered at clinicaltrials.gov as NCT02535910. *J Nutr* 2017;147:2076–82.

Keywords: vitamin D_3, 25(OH)D_3, dairy drink, milk, butter, vascular function, augmentation index, vitamin D status

Introduction

Vitamin D deficiency has been reported to be associated with an increased risk of many common and chronic diseases, including cardiovascular diseases (CVDs), some cancers, and diabetes (1). Circulating plasma 25-hydroxyvitamin D concentration is commonly used as the measure of vitamin D status (2). The Institute of

Medicine has reported circulating concentrations of $\geq$50 nmol/L as adequate for sustaining musculoskeletal health outcomes (3). Hypovitaminosis D is now increasingly common in the general European population (4), with 23% of UK adults presenting with a vitamin D status <25 nmol/L (5). Due to diet and lifestyle changes and the frequent use of sunscreen, many individuals do not endogenously synthesize sufficient vitamin D from sunlight exposure (6). Therefore, vitamin D from dietary sources has become more important for maintenance of adequate vitamin D status. However, there are only a few foods that are naturally rich in vitamin D, such as egg yolk and oily fish (7). Thus, one strategy used in some countries, including the United States and Canada, to improve population vitamin D status is the fortification of milk with vitamin D, which has resulted in milk being the major contributor to vitamin D intake in these countries (8).

The relative efficacy of 25-hydroxycholecalciferol [$25(OH)D_3$] and cholecalciferol (vitamin D_3) for improving vitamin D status is inconsistent between studies (9–15), yet it has generally been found that $25(OH)D_3$ supplementation can increase vitamin D status more efficiently than vitamin D_3 after a single dose (15) or after longer-term supplementation from 1 mo to 1 y (9–14). In addition, Jetter et al. (15) have studied both the pharmacokinetics of a single dose and chronic supplementation for 15 wk with vitamin D_3 and $25(OH)D_3$. Their data showed that longer-term $25(OH)D_3$ supplementation was superior to vitamin D_3 for increasing vitamin D status. Moreover, Bischoff-Ferrari et al. (10) showed that 20 μg $25(OH)D_3$/d over 4 mo had significant benefits for lowering systolic blood pressure (SBP) compared with vitamin D_3 in 20 healthy postmenopausal women.

To our knowledge, there are no human studies that have compared the efficacy of foods fortified with these 2 forms of vitamin D_3 to increase postprandial circulating $25(OH)D_3$ concentrations or their differential effects on chronic disease risk markers in the short term. Therefore, our study aimed to address this knowledge gap by comparing the acute effect of consuming test meals containing dairy drinks that were fortified with either 20 μg vitamin D_3 (+D_3) or 20 μg $25(OH)D_3$ (+HyD_3) with a nonfortified dairy drink (control) on changes in postprandial plasma vitamin D_3 and $25(OH)D_3$ concentrations and cardiometabolic risk markers, including vascular reactivity, blood pressure (BP), lipid profile, indexes of insulin resistance, and inflammatory and vascular biomarkers. In addition, ex vivo whole blood culture cytokine production was examined as a real-time measure of inflammatory status. Our hypothesis was that a +HyD_3 dairy drink would be more effective at raising vitamin D status than would a +D_3 dairy drink and would have beneficial effects on cardiometabolic risk markers.

Methods

Subjects. The study was conducted according to the Declaration of Helsinki and approved by the University of Reading Research Ethics Committee (approval no. 15/15) and was registered at www.clinical-trials.gov (NCT02535910). Nonsmoking men (*n* = 18) aged 30–65 y with a BMI (in kg/m^2) between 20 and 35 with suboptimal vitamin D status (plasma 25-hydroxyvitamin D concentration $\leq$50 nmol/L) were recruited from the population in Reading, United Kingdom, and the surrounding areas from May to October 2015 by e-mail, internet, poster, or newspaper advertisements. Subjects who expressed an interest in the study were asked to complete a medical, lifestyle, and ethnicity questionnaire. The key exclusion criteria included: female sex (to avoid the potential impact of the menstrual cycle on the study outcomes); cardiovascular, renal, gastrointestinal, respiratory, and endocrine diseases, diabetes, or cancer; hypertension; use of nutritional supplements;

use of long-term medication; milk allergy or intolerance or lactose intolerance; outdoor workers and those who used tanning beds; overseas vacation 2 mo before or during the study period; vigorous exercise (>3 instances of 30 min aerobic exercise/wk); and excessive alcohol intake (>14 U/wk). Those who complied with the inclusion criteria were invited to attend a screening visit after a 12-h overnight fast during which nothing but water was consumed. All subjects provided written informed consent. Blood samples were taken by venipuncture for determination of the full blood count at the Royal Berkshire Hospital (Reading, United Kingdom), and men who had anemia (hemoglobin <125 g/L) were excluded. Blood samples were also collected for measuring vitamin D status [plasma $25(OH)D_3$ concentration, performed at the Royal Berkshire Hospital] and fasting serum glucose concentration, total cholesterol, TGs, and markers of liver and kidney function through the use of an automated clinical chemistry analyzer (ILAB 600; Werfen UK Ltd.). Furthermore, clinical BP was measured during the screening visit to exclude subjects with abnormal BP. Normal BP was considered to be a SBP of 90–120 mm Hg and a diastolic blood pressure (DBP) of 60–80 mm Hg.

Study design. This study was a short-term, randomized, controlled, 3-way crossover, double-blinded study conducted between October 2015 and February 2016. Men were randomly assigned to 1 of 3 treatments (control, +HyD_3 dairy drink, or +D_3 dairy drink) at each visit by using a Web-based random letter sequence generator (https://www.randomizer.org/) by JG. A dose of 20 μg HyD_3 or D_3 (in a dairy drink) was used because it represented achievable vitamin D intake from a single meal containing vitamin D–fortified foods at the approximate fortification amount used in the United States and Canada. Furthermore, a single supplement of 20 μg $25(OH)D_3$ or vitamin D_3 was previously shown to have a significant differential impact on circulating plasma $25(OH)D_3$ concentrations (15).

After participants were accepted into the study, they were invited to the clinical unit of the Hugh Sinclair Unit of Human Nutrition at the University of Reading for a familiarization visit to become acquainted with the clinical facilities and vascular function study measurements. Before the first study visit, the participants were asked to complete a 4-d diet diary (including 3 weekdays and 1 weekend day within the same week), and Dietplan version 6.6 software was used to assess habitual dietary intake, including dietary vitamin D. The first study day was 2 wk after the familiarization visit, and there was a 2-wk washout period between the 3 study visits (**Supplemental Figure 1**). A double-blinded protocol was maintained throughout the study until all of the statistical analysis was completed. Throughout the study, participants were asked to maintain their normal diet and lifestyle, to avoid taking any dietary supplements, and to minimize sun exposure. The participant flow chart is shown in **Figure 1**.

Participants were asked to avoid alcohol, caffeine, or any vigorous physical activity for 24 h before each visit and to consume a standard low-fat evening meal (<10 g fat) provided by the researchers. In addition, no foods that were fortified with or high in vitamin D (such as egg yolk or oily fish) were permitted for the 24-h study period, and low-nitrate water (Buxton Mineral Water Company Ltd.) was provided to the subjects to consume the day before the study visit and throughout the postprandial day until the 24-h time point.

For each study visit, participants arrived at the clinical unit of the Hugh Sinclair Unit of Human Nutrition at ~0800 after a 12-h overnight fast. Height, weight, and waist and hip circumferences were measured before a cannula was inserted into the antecubital vein of the dominant arm. BP and vascular reactivity measurements were performed after a 30-min rest in a temperature-controlled (23°C ± 1°C) clinical room before a fasting blood sample was taken. After the baseline measurements were completed, the test meal was provided and consumed within 15 min. Ten postprandial blood samples were collected and 4 BP and 4 vascular reactivity measurements were performed $\leq$8 h after the test meal (Supplemental Figure 1). Subjects remained in the clinical unit for the duration of the 8-h study visit, and no additional food was consumed during the postprandial study period. A standard controlled evening meal (Marks and Spencer Ltd.) was consumed at the end of the study visit (no vitamin D–enriched or –fortified foods), after which the
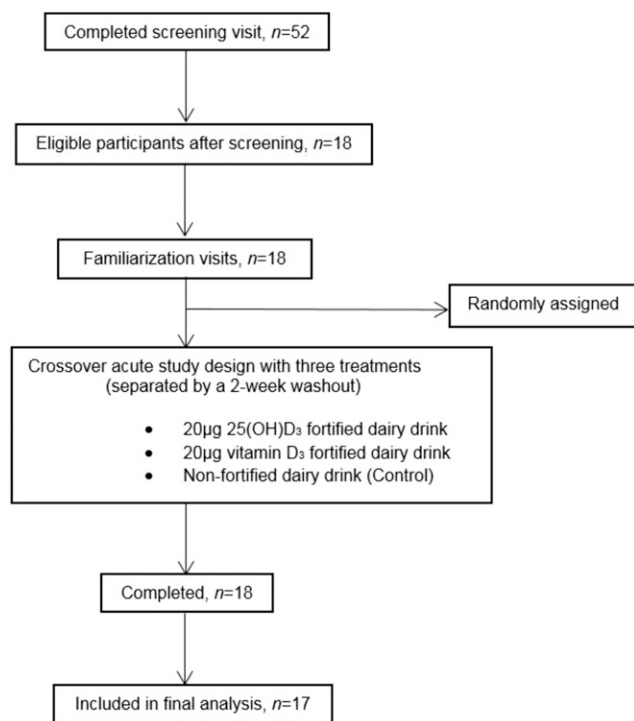
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FIGURE 1 Participant flow chart. 25(OH)D$_3$, 25-hydroxycholecalciferol.

participants fasted overnight. The following morning, they returned to the clinical unit for their 24-h assessment, during which a fasting blood sample was collected, and BP and vascular reactivity were measured.

Acute test meals. The 3 treatment drinks were control, +HyD$_3$, and +D$_3$ dairy drinks. The manufactured crystalline vitamin D$_3$ and 25(OH)D$_3$ were supplied by Dishman Netherlands BV, both of which were packed in glass vials under a nitrogen atmosphere. Vitamin D$_3$ and 25(OH)D$_3$ were dissolved in refined olive oil (Sainsbury's Supermarkets Ltd.) to achieve a concentration of 1 μg/100 μL vitamin D$_3$ or 25(OH)D$_3$ stock fortified oil. Aliquots of vitamin D$_3$ test oil (containing 20 μg vitamin D$_3$), 25(OH)D$_3$ test oil [containing 20 μg 25(OH)D$_3$], and control (olive oil only) were assigned a random code and stored at −20°C.

On the morning of each study visit, the dairy drink was prepared with the use of 300 mL full-fat, nonfortified milk (Co-operative Ltd.), 32 g unsalted butter (Co-operative Ltd.), and 25 g Askeys Treat Strawberry Sauce (The Silver Spoon Ltd.). Milk and strawberry sauce were warmed and mixed with melted butter through the use of a hand blender (Sainsbury's Supermarkets Ltd.) before 2 mL of the defrosted test or control oil was added into the warm dairy drink and well homogenized.

Subjects were given a test breakfast that included the dairy drink, 3 slices (120 g) of toasted white bread (Hovis Ltd.) with 40 g strawberry jam (Sainsbury's Supermarkets Ltd.), and 15 g unsalted butter (Co-operative Ltd.). Each of the test meals contained 51 g fat, 125 g carbohydrate, 23 g protein, and 4.54 MJ. The nutrient composition of the foods was obtained from the product labels.

Assessment of vascular function, BP, and anthropometric measures. Height and weight were measured with the use of a wall-mounted stadiometer and a Tanita BC-418 digital scale (Tanita Europe BV), respectively. Clinical BP was measured on the upper left arm with the use of a BP monitor (TM-2430; A&D Ltd.) in triplicate after a minimum of 10 min of rest in a supine position at baseline (0 h), at 1.5, 3, 6, and 8 h after breakfast, and at the 24-h visit. An Endo-PAT 2000 device (Itamar Medical Ltd.) was used to assess the peripheral artery tonometry at baseline (before breakfast) and at the 24-h visit, as described elsewhere (16, 17). In addition, digital volume pulse photoplethysmography (Pulse Trace; Micro Medical) was measured at baseline (0 h), at 1.5, 3, 6, and 8 h after breakfast, and at the 24-h visit

to determine the arterial stiffness index, reflection index, peak-to-peak time, and heart rate; the method of the assessment is described elsewhere (18).

Plasma and serum collection and analysis. Blood samples collected from the cannula were placed into serum-separating tubes [for the analysis of serum lipids, apoB, C-reactive protein (CRP), glucose, and insulin], lithium heparin tubes (for the analysis of plasma total nitrates and nitrites), and tripotassium-EDTA–coated tubes [for the analysis of plasma TNF-α, IL-6, vitamin D$_3$, and 25(OH)D$_3$]. After blood collection, the serum-separating tubes were stored at room temperature for 15 min, whereas those containing anticoagulant were stored on ice. All blood samples were centrifuged within 30 min at $1700 \times g$ for 15 min at room temperature (serum) or 4°C (plasma). After centrifugation, the serum or plasma were aliquoted and stored at −20°C [−80°C for the 25(OH)D$_3$ and vitamin D$_3$] until analysis.

Plasma vitamin D$_3$ and 25(OH)D$_3$ [as the sum of 25(OH)D$_3$ and 3-epi-25(OH)D$_3$] were analyzed by DSM Nutritional Products Ltd. with the use of a method validated according to FDA (19) and European Medicines Agency (20) bioanalytical guidelines. In brief, after the addition of a deuterated internal standard solution [d$_6$-25(OH)D$_3$ and d$_3$-vitamin D$_3$], a protein precipitation was performed with a mixture of tetrahydrofuran (50%), acetonitrile (40%), and methanol (10%). After centrifugation, the supernatant was evaporated, and the residue was reconstituted with an acetonitrile–methanol solution. An aliquot was then injected into an LC-MS/MS system (Agilent 1290, C^{18} column) with an APPI source (ABSciex 4000), and the detection of the specific fragment ions was performed with the use of the multiple reactions monitoring mode. To assess the daily and long-term laboratory performances of the method, dedicated standard and quality control samples were analyzed daily with the unknown samples to ensure the accuracy and precision of the method. Data acquisition of the extracted ion chromatograms, integration, and quantification were performed with the use of Analyst software from ABSciex.

Serum total cholesterol, HDL-cholesterol, nonesterified FAs (NEFAs), TGs, apoB, and CRP were determined with the use of the ILAB 600 autoanalyzer with standard kits and appropriate quality controls (reagents and analyzer: Werfen UK Ltd.; NEFA reagent: Alpha Laboratories; and apoB reagent: Randox Laboratories). The fasting LDL-cholesterol concentration was calculated from total cholesterol, HDL-cholesterol, and TGs by using the Friedewald formula (21). ELISA kits were used to measure plasma TNF-α (R&D Systems Europe Ltd.), IL-6 (R&D Systems Europe Ltd.), and serum insulin (Dako Ltd.). Insulin resistance markers, quantitative insulin sensitivity check index, revised quantitative insulin sensitivity check index, and homeostasis for insulin resistance, were calculated by using standard equations (22). Plasma samples were analyzed for nitrites and nitrates with the use of the Eicom NOx Analyzer (ENO-30), as described elsewhere (23).

Blood was collected into dipotassium-EDTA tubes (Greiner BioOne Ltd.) at baseline and at 8 and 24 h after the consumption of the test meal to measure ex vivo LPS–stimulated whole blood culture cytokine production, as previously described (24). The cytokines TNF-α and IL-6 were measured in whole-blood culture supernatants with the use of ELISA kits (R&D Systems Europe Ltd.). The data were normalized for monocyte numbers, and only samples stimulated for 24 h with LPS (0.5 μg/mL) were used in the final analysis.

Study power. According to earlier research by Jetter et al. (15), the expected difference between the treatments [i.e., a single dose of 20 μg vitamin D$_3$ or 20 μg 25(OH)D$_3$] for plasma 25(OH)D$_3$ is 3.7 ng/mL (peak concentration within the 24 h) with an SD of 13.2 ng/mL. Thus, it was estimated that 15 subjects were required to detect a significant change in this primary outcome measurement with a power of 80% and a 5% significance level. A total of 18 subjects were recruited to allow for a drop-out rate of 15%.

Statistical analysis. All data analyses were conducted with the use of STATA (version 13.0; STATA Corporation, 2014). The results are expressed as means ± SEs (SEMs). Data were checked for normality, and natural logarithm transformation was performed if needed. The primary

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analysis of the time courses from baseline to 8 h for outcome variables were analyzed by 2-factor repeated measures ANOVA to assess the effect of treatment, time, and treatment by time interactions with Bonferroni correction to control for multiple pairwise comparisons.

For the secondary data analysis, postprandial summary measures were calculated, which included AUC, incremental AUC (iAUC), maximum concentration (maxC), increment from baseline to maximal concentration [imaxC (imaxC = maxC – fasting value)], and the time to reach maxC. These measures were analyzed by one-way ANOVA, and subsequently, Bonferroni correction was applied if post hoc multiple pairwise comparisons were performed. The Kruskal-Wallis equality-of-populations rank test was applied to data that could not be normalized.

For NEFAs, the postprandial summary measures AUC, iAUC, maxC, imaxC, and time to reach the maxC were calculated from the mean minimum concentration ($\sim$2 h) to 8 h (25).

Results

Of the 18 men who completed the study, the data for 1 subject, whose baseline plasma $25(OH)D_3$ concentration (indicator of vitamin D status) on the study visit was >50 nmol/L, was excluded from the statistical analysis. Therefore, 17 men were included in the current study data set (**Table 1**) with a mean $\pm$ SEM plasma $25(OH)D_3$ concentration of 31.7 $\pm$3.4 nmol/L and a low dietary vitamin D intake of 4.4 $\pm$1.5 μg/d assessed from the 4-d diet diary.

There were no differences in fasting (0 h) $25(OH)D_3$ concentration, lipids, indexes of insulin resistance and glycemia, vascular biomarkers, SBP, stimulated whole-blood culture cytokine production, or vascular function measurements between study visits (**Supplemental Tables 1–4, Table 2**). However, the fasting DBP and pulse pressure (PP) were significantly different between study visits (Supplemental Table 1). Thus, only the iAUC was calculated to determine the effects of the fortified and control dairy drinks on DBP and PP.

Postprandial response of plasma vitamin D_3 and $25(OH)D_3$. Following the test dairy drinks, there was a significant time-by-treatment interaction for the postprandial plasma $25(OH)D_3$ concentrations ($P < 0.0001$) (**Figure 2**). After the consumption of the +HyD_3 dairy drink, imaxC (0–8 h) was 1.2-fold higher than after the control and 1.7-fold higher than after the +D_3 dairy drink ($P = 0.0001$) (Table 2). Furthermore, the iAUC (0–8 h) for

TABLE 1 Baseline characteristics of men with suboptimal vitamin D status[1]

	Value
Age, y	49 $\pm$ 3
BMI, kg/m^2	26.4 $\pm$ 0.6
Systolic blood pressure, mm Hg	122 $\pm$ 2
Diastolic blood pressure, mm Hg	64 $\pm$ 2
Serum total cholesterol, mmol/L	5.04 $\pm$ 0.21
Serum LDL cholesterol, mmol/L	3.61 $\pm$ 0.09
Serum HDL cholesterol, mmol/L	1.21 $\pm$ 0.03
Serum TG, mmol/L	1.48 $\pm$ 0.21
Serum glucose, mmol/L	5.42 $\pm$ 0.14
Serum insulin, pmol/L	47.7 $\pm$ 3.2
Vitamin D dietary intake,[2] μg/d	4.40 $\pm$ 1.51
Plasma $25(OH)D_3$[3] concentration, nmol/L	31.7 $\pm$ 3.4

[1] Values are means $\pm$ SEMs, n = 17 (means of 3 baseline periods).
[2] Derived from 4-d diet diaries.
[3] $25(OH)D_3$, 25-hydroxycholecalciferol.

TABLE 2 Baseline and postprandial changes in plasma $25(OH)D_3$ concentrations from baseline in men after consumption of a +HyD_3 dairy drink, a +D_3 dairy drink, and an unfortified dairy drink (control)[1]

Measures of $25(OH)D_3$	Dairy drink			P[2]
	Control	+D_3	+HyD_3	
Baseline, nmol/L	28.5 $\pm$ 2.8	31.0 $\pm$ 3.4	30.4 $\pm$ 3.3	0.85
maxC (0–8 h), nmol/L	32.9 $\pm$ 3.6	34.6 $\pm$ 3.8	40.2 $\pm$ 3.9	0.37
imaxC (0–8 h), nmol/L	4.4 $\pm$ 1.1^b	3.6 $\pm$ 0.7^b	9.8 $\pm$ 1.2^a	0.0001
AUC (0–8 h), nmol/L $\times$ 8 h	238.2 $\pm$ 24.9	259.6 $\pm$ 29.1	272.3 $\pm$ 28.4	0.68
iAUC (0–8 h), nmol/L $\times$ 8 h	10.3 $\pm$ 5.8^b	11.7 $\pm$ 4.2^b	29.2 $\pm$ 5.2^a	0.019
Change from baseline to 24 h	1.6 $\pm$ 1.1^b	4.5 $\pm$ 0.8^b	8.7 $\pm$ 0.9^a	<0.0001
Change from 8 to 24 h	−0.2 $\pm$ 1.2	3.1 $\pm$ 0.9	1.6 $\pm$ 1.0	0.10

[1] Values are means $\pm$ SEMs, n = 17. Labeled means in a row without a common superscript letter differ, $P < 0.05$. iAUC, incremental AUC; imaxC, maximal change of the variables; maxC, maximum concentration; $25(OH)D_3$, 25-hydroxycholecalciferol; +D_3, 20 μg vitamin D_3–fortifiied dairy drink; +HyD_3, 20 μg 25-hydroxycholecalciferol–fortified dairy drink.
[2] One-factor ANOVA was applied to the outcome variable. Bonferroni multiple pairwise comparison post hoc tests were used to identify the significant differences between the treatments.

the +HyD_3 dairy drink was 1.5-fold higher than the +D_3 dairy drink and 1.8-fold higher than the control ($P = 0.019$), whereas the iAUC (0–8 h) for the +D_3 dairy drink was not different than the control. The change in plasma $25(OH)D_3$ concentration calculated from baseline to 24 h after the +HyD_3 dairy drink was also 0.9-fold higher than after the +D_3 dairy drink and 4.4-fold higher than after the control ($P < 0.0001$) (Table 2).

Statistical analysis of the plasma vitamin D_3 responses was not conducted because only 42 of 648 plasma samples had vitamin D_3 concentrations above the limit of detection of the LC-MS/MS technique (2.5 nmol/L).

Vascular function and postprandial BP. The treatment effects on vascular function and postprandial BP are presented in Supplemental Table 1. There were no differences in the change from baseline to 24 h for the vascular function measurements by EndoPAT and digital volume pulse devices. There were no significant effects of treatments on postprandial BP (SBP and DBP) or PP.

Blood lipid profile and indexes of insulin resistance and glycemia. There were no treatment effects on postprandial blood lipids or indexes of insulin resistance and glycemia determined over the 8 h (Supplemental Table 2). In addition, there was no difference in the change from baseline to 24 h for any of these measures.

Postprandial responses of vascular and inflammatory biomarkers. No significant effects of treatments on serum CRP, plasma total nitrates and nitrites, and IL-6 were observed (Supplemental Table 3). Statistical analysis of plasma TNF-α was not conducted because 37% of the samples had concentrations below the lower level of detection of the ELISA kit (0.11 pg/mL).

Ex vivo cytokine production. There was no effect of the fortified or control dairy drinks on ex vivo production of IL-6 or TNF-α after stimulation of whole-blood cultures with LPS, measured with the use of blood samples collected at baseline, 8 h, or 24 h, or calculated as the change from baseline to 8 or 24 h (Supplemental Table 4).

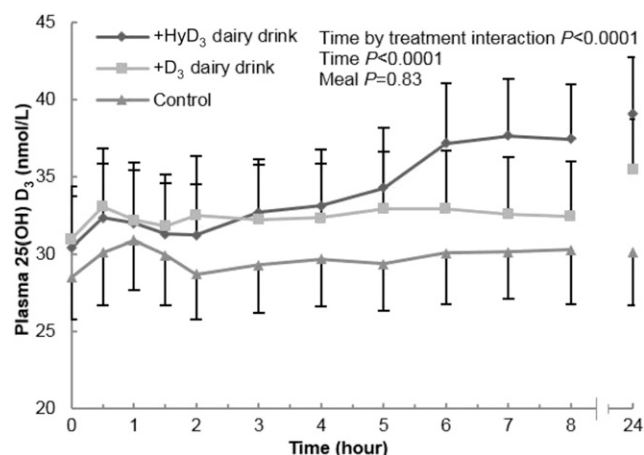Dairy drink fortification with vitamin D isoforms   2079

FIGURE 2 Postprandial plasma $25(OH)D_3$ concentrations in men after consumption of a $+HyD_3$ dairy drink, a $+D_3$ dairy drink, and an unfortified dairy drink (control). Values are means $\pm$ SEMs, $n = 17$ for each treatment. Two-factor repeated measure ANOVA was used to access treatment, time, and time-by-treatment interaction effects. $25(OH)D_3$, 25-hydroxycholecalciferol; $+D_3$, 20 μg vitamin D_3–fortified dairy drink; $+HyD_3$, 20 μg 25-hydroxycholecalciferol–fortified dairy drink.

Discussion

This study is the first to our knowledge to compare the postprandial responses to vitamin D_3– and $25(OH)D_3$-fortified dairy drinks on plasma $25(OH)D_3$ concentrations in addition to markers of cardiometabolic risk. It was observed that consumption of a $+HyD_3$ dairy drink resulted in higher and more sustained plasma $25(OH)D_3$ concentrations over 8 h and at 24 h compared with the control and $+D_3$ dairy drink, showing that $25(OH)D_3$-fortified dairy foods were more effective at increasing plasma $25(OH)D_3$ than vitamin D_3–fortified foods. However, we did not detect changes in vascular function measurements or cardiometabolic risk markers after consumption of the test meals containing the 3 different types of dairy drink.

To date, to our knowledge, no randomized controlled trials have evaluated the effects of $25(OH)D_3$-fortified dairy products on the change in acute vitamin D status. The postprandial change in plasma $25(OH)D_3$ concentrations offers an important insight into the absorption kinetics of $25(OH)D_3$ and vitamin D_3, which may influence the form of vitamin D chosen for fortification. Jetter et al. (15) compared the effect of capsules containing 20 μg of $25(OH)D_3$ and vitamin D_3 on plasma $25(OH)D_3$ concentrations in healthy postmenopausal women who had similar baseline plasma $25(OH)D_3$ concentrations [30.7 $\pm$ 10.2 nmol/L (mean $\pm$ SD)] to the participants in the current study. A significant 1.3-fold higher plasma $25(OH)D_3$ AUC (0–24 h) was observed after the $25(OH)D_3$ supplementation compared with the vitamin D_3 supplement (15). This direction of effect was in line with the current study, where the $+HyD_3$ dairy drink resulted in a significant 1.5-fold higher plasma $25(OH)D_3$ iAUC compared with the $+D_3$ dairy drink, which was evident within 8 h of ingestion. The differences between the studies may be due in part to the characteristics of the study participants. The current study was conducted in men aged 30–54 y, whereas Jetter et al. (15) studied postmenopausal women aged 50–70 y, although there is no evidence from any previous studies of an age or sex effect on the absorption of vitamin D supplements. In addition, the form of the $25(OH)D_3$ may have influenced absorption, with a preferential absorption of $25(OH)D_3$ with a fat-containing meal rather than

taking it from capsules with water. This speculative explanation would require further confirmation.

We were unable to quantify plasma vitamin D_3 concentrations because plasma concentrations were below the detection limit of the LC MS/MS assay. One explanation may relate to the findings of Barger-Lux et al. (9). Their study investigated the dose response to supplemental vitamin D_3 (25, 250, and 1250 μg/d) and $25(OH)D_3$ (10, 20, and 50 μg/d) for 8 and 4 wk, respectively. It was observed that both serum vitamin D_3 and $25(OH)D_3$ increased with all of the vitamin D_3 supplementations, whereas only serum $25(OH)D_3$ increased after the $25(OH)D_3$ supplementations. In addition, the subjects in the study of Barger-Lux et al. (9) had higher mean fasting $25(OH)D_3$ concentrations (67 compared with 32 nmol/L in the current study) considered to be in the optimal range, which may have been associated with higher circulating concentrations of vitamin D_3, the precursor of $25(OH)D_3$. Furthermore, our study was performed in the postprandial phase, and so a longer-term intervention period [as in Barger-Lux et al. (9)] may be required for detectable plasma vitamin D_3 concentrations.

A study by Stamp (26) investigated the acute effect of a single dose of supplemental $25(OH)D_3$ at 10 μg/kg body weight in healthy subjects over 24 h. The peak concentration of circulating $25(OH)D_3$ was reached between 4 and 8 h. In contrast, Jetter et al. (15) reported the time to reach peak plasma $25(OH)D_3$ concentrations for a supplemental dose of 20 μg of $25(OH)D_3$ and vitamin D_3 to be 10.8 and 22.2 h, respectively. In the current study, the peak circulating concentration of $25(OH)D_3$ could not be identified precisely because blood samples were not collected between 8 and 24 h, although 24-h concentrations were still above baseline concentrations. Thus, we speculate that the peak concentration was reached earlier, after ingestion of the $+HyD_3$ dairy drink compared with the $+D_3$ dairy drink, although this would need to be confirmed in a study with frequent blood sampling over 8–36 h.

The mechanism for the more rapid absorption of $25(OH)D_3$ is unclear, but it might be because hepatic metabolism of vitamin D_3 to $25(OH)D_3$ is circumvented (6), and so the bioactive form of vitamin D, $1,25(OH)_2D_3$, can be more rapidly synthesized by the kidneys, whereas vitamin D_3 needs to be transported from the gut to the liver for further metabolism (3).

Effective dietary strategies to increase population vitamin D status are required to address the high incidence of suboptimal vitamin D status within the population (5). The UK Scientific Advisory Committee for Nutrition published new dietary guidance in 2016 (1), recommending a daily vitamin D intake of 10 μg/d for adults, which is challenging to achieve through diet unless fortified foods are consumed. The mean daily intake of vitamin D for UK adults is only 3.1 μg for men and 2.6 μg for women, respectively (5). Therefore, vitamin D–fortified foods are one strategy that would increase vitamin D dietary intake. Milk and dairy are ideal foods for fortification because they are consumed by the majority of the population within Europe and the United States (5, 27). The current study verified that dairy products are suitable vehicles for fortification with $25(OH)D_3$, resulting in a more rapid increase in markers of vitamin D status than vitamin D_3 in the 24 h after consumption. Although we could not determine whether long-term consumption of $25(OH)D_3$-fortified foods would lead to more favorable vitamin D status than vitamin D_3–fortified foods, a previous study reported that $25(OH)D_3$ given as a daily or weekly supplement, or as a single bolus, was 2- to 3-fold more potent at increasing plasma $25(OH)D_3$ concentrations than vitamin D_3 supplementation (15). However, further studies investigating the effects of $25(OH)D_3$-compared with vitamin D_3–fortified foods over a longer intervention

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period are required to determine the efficacy of $25(OH)D_3$ fortification on reversing suboptimal vitamin D status.

Evidence on the associations of low vitamin D status and CVD risk factors, such as hypertension and elevated lipids, is predominantly from epidemiological studies, which may be influenced by confounding factors or reverse causality (1). In the current study, no treatment effects on postprandial arterial stiffness, a key CVD risk factor (28), in men with suboptimal vitamin D status were observed, which is in line with a previous study (29) that also reported no changes in arterial stiffness after consumption of a single dose of 7500 or 1875 μg of vitamin D_3. In addition, a recent systematic review and meta-analysis (30) summarized 28 randomized controlled trials on vitamin D_3 supplementation and concluded that there was no effect of vitamin D supplementation (doses ranged from 25 μg/d to 3000 μg/mo) on arterial stiffness after administration periods ranging from 2 to 12 mo.

In contrast with our study of no effect on BP, Bischoff-Ferrari et al. (10) reported a 5.7–mm Hg decrease (P = 0.0002) in SBP after daily supplementation with 20 μg $25(OH)D_3$ compared with 20 μg vitamin D_3 over 4 mo in subjects who had normal BP. In our study, the effect of the test meal containing the dairy drink was only followed up for 24 h as opposed to the study of Bischoff-Ferrari et al. (10), which had a 4-mo intervention period. This suggests that a chronic intervention period may have been required for significant changes in BP to be observed.

The determination of postprandial outcomes is important because an elevated nonfasting serum TG concentration is now recognized as an independent CVD risk factor (31). Our findings on a lack of effect of the fortified dairy drinks on the postprandial lipid profiles (TGs and NEFAs) are in line with a previous intervention study (29), which also reported that there were no effects of a single higher dose of vitamin D_3 of 7500 or 1875 μg on postprandial lipid profiles (TGs and total HDL and LDL cholesterol) after ≤8 h in overweight vitamin D–deficient women [vitamin D concentration (mean ± SD) of 27.1 ± 13.8 nmol/L]. Furthermore, the current study is the first to investigate the effects of vitamin D–fortified dairy drinks on the ex vivo production of the inflammatory cytokines, IL-6 and TNF-α, in whole-blood culture following stimulation with LPS. No differences between the dairy drinks were observed, suggesting that higher doses or a longer supplementation period may be required to determine the effects of vitamin D forms on inflammation.

This study has some potential limitations. First, the study was powered to detect a significant difference in the primary outcome of postprandial plasma $25(OH)D_3$, however, it may not have been suitably powered to detect changes in the secondary outcomes. In addition, this study was conducted in generally healthy men, which may have contributed to the null findings with respect to the cardiometabolic risk markers following the 3 treatments. Second, blood samples were not collected between 8 and 24 h, which restricted the estimation of the peak $25(OH)D_3$ concentration. Furthermore, the participants were men with suboptimal vitamin D concentrations, and the results may not be representative of responses in women or those individuals with adequate vitamin D concentrations.

In conclusion, the current study confirmed that a $+HyD_3$ dairy drink was able to increase the postprandial marker of vitamin D status more efficiently than a $+D_3$ dairy drink. Although the magnitude of change in the vitamin D status was significantly higher after the $+HyD_3$ dairy drink than after the $+D_3$ dairy drink, additional longer-term intervention studies are needed to determine whether $25(OH)D_3$ preferentially increases

vitamin D status. This may have important public health implications for addressing hypovitaminosis D in the population. It is important that future studies investigate the impact of daily $+HyD_3$ dairy drink consumption on longer-term vitamin D status in both men and women.

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