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Ergocalciferol from Mushrooms or Supplements Consumed with a Standard Meal Increases 25-Hydroxyergocalciferol but Decreases 25-Hydroxycholecalciferol in the Serum of Healthy Adults[1–3]

Charles B. Stephensen,[4,5]* Melissa Zerofsky,[4,5] Dustin J. Burnett,[4,5] Yan-ping Lin[6] Bruce D. Hammock,[6] Laura M. Hall,[7] and Tara McHugh[8]

[4]USDA-Agricultural Research Service Western Human Nutrition Research Center, [5]Nutrition Department, and [6]Department of Entomology, University of California, Davis, CA; [7]Food Science and Nutrition Department, California Polytechnic State University, San Luis Obispo, CA; and [8]USDA-Agricultural Research Service Western Regional Research Center, Processed Foods Research Unit, Albany, CA

Abstract

Few foods contain ergocalciferol or cholecalciferol. Treatment of mushrooms with UV light increases ergocalciferol content and could provide a dietary source of vitamin D. We evaluated the impact of consuming UV-treated white button mushrooms (*Agaricus bisporus*) on the vitamin D status of healthy adults. Thirty-eight volunteers were randomized to 4 treatments consumed with a standard meal for 6 wk: the control (C) group received untreated mushrooms providing 0.85 μg/d ergocalciferol (n = 10); groups M1 and M2 received UV-treated mushrooms providing 8.8 (n = 10) and 17.1 μg/d (n = 9), respectively; and the supplement (S) group received purified ergocalciferol plus untreated mushrooms, providing a total of 28.2 μg/d (n = 9). Serum total 25-hydroxyvitamin D [25(OH)D] and 25-hydroxyergocalciferol [25(OH)D2] were 83 ± 38 and 2.4 ± 2.0 nmol/L, respectively, at baseline (mean ± SD). At wk 6, 25(OH)D2 had increased and was higher in all treatment groups than in the C group, whereas 25-hydroxycholecalciferol [25(OH)D3] had decreased and was lower in the M2 and S groups than in the C group. Increases in 25(OH)D2 for groups C, M1, M2, and S were 1.2 ± 5.2, 13.8 ± 7.3, 12.7 ± 3.7, and 32.8 ± 3.3 nmol/L and decreases in 25(OH)D3 were −3.9 ± 16.3, −10.4 ± 6.4, −20.6 ± 14.6, and −29.5 ± 15.9 nmol/L, respectively. Concentrations did not change in group C. In summary, ergocalciferol was absorbed and metabolized to 25(OH) D2 but did not affect vitamin D status, because 25(OH)D3 decreased proportionally. J. Nutr. 142: 1246–1252, 2012.

Introduction

Vitamin D is required for regulation of calcium balance and bone mineralization. Recent work also suggests that vitamin D may protect against chronic inflammatory and infectious diseases (1). There are 2 forms of vitamin D. Cholecalciferol is synthesized from 7-dehydrocholesterol in the skin of humans and other animals following exposure to UV light, whereas ergocalciferol is synthesized from ergosterol in plants and fungi following UV exposure (2). National survey data indicate that vitamin D deficiency, defined by measurement of 25-hydroxyvitamin D [25(OH)D][9] in serum, is common in the U.S. population (3). In many individuals, cutaneous synthesis maintains serum 25-hydroxycholecalciferol [25(OH)D3] at an adequate level, although substantial intake from food or supplements is required by others with lower sun exposure (4).

The RDA for vitamin D is 15 μg/d for children and adults <70 y of age (5), but most Americans consume substantially less than this amount. National survey data indicate that the mean vitamin D intake from food is <50% of the RDA for children and <40% of the RDA for adults (6). The principal natural food

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[3] Supplemental Table 1 and Figures 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

* To whom correspondence should be addressed. E-mail: charles.stephensen@ars.usda.gov.

[9] Abbreviations used: C, control group receiving untreated mushrooms; M1, group receiving the lower dose of ergocalciferol from UV-treated mushrooms; M2, group receiving the higher dose of ergocalciferol from UV-treated mushrooms; NIST, National Institute of Standards and Technology; 24,25(OH)$_2$D3, 24,25-hydroxycholecalciferol; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D2, 25-hydroxyergocalciferol; 25(OH)D3, 25-hydroxycholecalciferol; S, group receiving purified ergocalciferol and untreated mushrooms; UPLC-MS/MS, ultra-performance liquid chromatography-tandem MS; WHNRC, Western Human Nutrition Research Center.

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sources of vitamin D in the U.S. diet are fish and shellfish. Fortified foods, which account for the majority of vitamin D intake, include milk, other dairy products, breakfast foods, and a few other products. These natural and fortified foods largely contain cholecalciferol (7).

Mushrooms can be an important dietary source of ergocalciferol. For example, ergocalciferol from mushrooms accounts for ~5% of vitamin D intake in Japan and Taiwan (8,9). Whereas commercially produced mushrooms [e.g., white button mushrooms (*Agaricus bisporus*)] typically contain little ergocalciferol (10), wild mushrooms and sun-dried mushrooms may contain large amounts of the vitamin (11,12). The ergocalciferol content of commercially produced mushrooms can be increased by exposure to a UV source during processing (13), and methods have been developed for postharvest treatment that are applicable to commercial production (14). Thus, ergocalciferol-enriched mushrooms may be useful for increasing the vitamin D content of the U.S. diet.

In the study reported here, we sought to determine if consuming one serving of cooked, UV-treated mushrooms per day for 6 wk would increase serum 25-hydroxyergocalciferol [25(OH)D2] levels and improve vitamin D status [assessed by measuring total 25(OH)D] in healthy adults. Two levels of UV-treated mushrooms were compared to ergocalciferol from capsules and to untreated mushrooms.

Participants and Methods

Participant recruitment. Healthy adults of either sex 20–59 y of age were recruited on the University of California, Davis campus and in the Davis community (**Supplemental Fig. 1**). Potential participants were screened by telephone for age, pregnancy status, and willingness to comply with protocol requirements. Individuals were then asked to come to the Western Human Nutrition Research Center (WHNRC) for a screening visit to assess other exclusion criteria. Weight and height were measured and dietary intake of ergocalciferol and cholecalciferol was assessed using a FFQ adapted from a previous study (4). Information on sun exposure, use of supplements and medications, and medical conditions that would affect vitamin D absorption or metabolism was collected. Blood was taken for a complete blood count and metabolic panel. Exclusion criteria were as follows: *1*) BMI >27 or <18.5 kg/m^2; *2*) regular consumption of ergocalciferol-containing foods, including mushrooms and fortified foods (e.g., soy milk) or supplements; *3*) sun exposure or use of tanning beds likely to produce high serum 25(OH)D3 levels; *4*) regular use of supplements containing >10 μg cholecalciferol; *5*) self-report of health conditions or use of medications that would interfere with vitamin D absorption or metabolism; *6*) pregnancy or lactation; *7*) <110 pounds body weight; and *8*) anemia or abnormal liver function. This study was approved by the Institutional Review Board at the University of California, Davis.

Study design. The study was a double-blind, randomized feeding trial incorporating one serving (1/2 cup, 87.9 g) of cooked mushrooms into a standard lunch 7 d/wk for 6 wk. The study had 4 groups with a goal of recruiting 10 participants per group, for a total of 40 participants. The 4 groups were: C, negative control group receiving untreated mushrooms plus placebo capsule; M1, group receiving the lower dose of ergocalciferol from UV-treated mushrooms, containing a target value of 10 μg ergocalciferol/serving, plus placebo capsule; M2, group receiving the higher dose of ergocalciferol from UV-treated mushrooms, with a target value of 25 μg ergocalciferol/serving, plus placebo capsule; and S, the supplement group receiving untreated mushrooms plus 25 μg purified ergocalciferol in a supplement capsule (identical to the placebo).

Participants were recruited in 4 cohorts in 2010. Cohort 1 consisted of 8 participants (6 completed) who began on June 8 and ended on July 13. Cohort 2 consisted of 7 participants (6 completed) who began on July 20 and ended on August 24. Cohort 3 consisted of 10 participants (all completed) who began on August 31 and ended on October 5, and Cohort 4 consisted of 13 participants (all completed) who began on October 12 and ended on November 23.

Blood draws. The principal outcome variables were serum 25(OH)D2, 25(OH)D3, 24,25-hydroxycholecalciferol [24,25(OH)$_2$D3], and total 25(OH)D measured at 0, 3, and 6 wk. Blood was drawn (20 mL) without an anticoagulant. Serum was stored at −80°C until analysis.

Randomization and blinding. Participants were randomized to the 4 treatments in the order that they qualified for the study using a randomized block design with a block size of 4. Treatment groups were designated with 4 arbitrarily chosen, sequential letters used by the study personnel during meal preparation and service to participants. Mushrooms with the same letter designations were delivered weekly to the WHNRC. Study personnel were unaware of the vitamin D content of the mushrooms and capsules. The metabolic kitchen supervisor (D.J.B.) was aware of this information in order to facilitate communication with the mushroom supplier.

Sample size determination. The desired sample size (10/group; 40 total participants) was based on a previous study conducted in Helsinki, Finland (60°N latitude), which fed the following preparations daily for 3 wk with lunch: *1*) freeze-dried mushroom powder containing 14 μg ergocalciferol/serving; *2*) no mushroom powder; and *3*) a supplement capsule containing 14 μg purified ergocalciferol. The 3-wk study had 9 participants/group and reported significant increases over baseline in total serum 25(OH)D in the 2 groups receiving supplemental vitamin D as well as differences between these groups and the C group at the end of the study (13). Serum 25(OH)D2 was not measured. The Finnish study was conducted under conditions of low UV exposure (Helsinki in January and February) that would preclude cutaneous vitamin D synthesis (15). Participants with serum 25(OH)D >60 nmol/L were excluded. Participants with high dietary vitamin D intake or supplement use were excluded and participants were asked to avoid vitamin D-rich foods during the study. The mean intake of vitamin D from nonstudy sources during the study period was <1.2 μg/d. Thus, the vitamin D provided by the Finnish study was the principal source of vitamin D during the study period. Similarly, we recruited participants with low ergocalciferol intake to ensure low serum 25(OH)D2 at baseline (4) and asked that participants avoid ergocalciferol during the study period. Our principal dependent variable was serum 25(OH)D2 rather than total 25(OH)D. Thus, the ergocalciferol provided by our study would be the principal source of ergocalciferol during the study period. We concluded that these similarities between the studies were sufficient to adopt the same sample size for our study (10 participants/group with a possible 10% drop-out) in order to detect significant within-group increases over baseline in serum 25(OH)D2 in the treatment groups, and to detect significant differences between the treatment and C groups. An important difference between the 2 studies was that ours occurred at a lower latitude (Davis, CA; 38.6°N latitude) and was not planned for winter. Thus, cutaneous synthesis could be an important source of serum 25(OH)D3 for our participants, though we screened out participants reporting high sun exposure.

Study meals. Study lunches were designed by a registered dietitian (D.J.B.) using Nutrition Data System for Research software version 2009 developed by the Nutrition Coordinating Center, University of Minnesota, and they were prepared by the Metabolic Kitchen and Human Feeding Lab at the WHNRC. Menus were controlled for fat (32% of total energy by calculation) and were designed to not contain ergocalciferol (by selecting manufactured meals that did not list mushrooms or foods fortified with ergocalciferol as an ingredient on the product label). Additionally, menus were designed to provide ~25–30% of a 2000-kcal diet. Study participants ate the study lunch at the WHNRC every weekday for 6 wk. Weekend lunches were packed out for participant preparation at home.

Each study meal consisted of a Lean Cuisine entrée (Nestlé USA), dinner roll (Pepperidge Farm, Campbell's Soup), soybean oil (Ventura Foods), 87.9 ± 2.8-g portions of white button mushrooms (Monterey

Mushrooms), and in the soup on the menu for d 6 was reduced-sodium beef broth concentrate (Savory Creations International), fresh spring onions (Safeway), Accent seasoning (B&G Foods), table salt, and ground black pepper. Participants also consumed an ergocalciferol supplement or matching placebo at each meal.

The mushrooms were harvested, treated with UV light (groups M1 and M2 only), packaged, coded into 1 of 4 treatment groups with arbitrary letter designations in 87.9-g servings (1 1/4 cups) by Monterey Mushrooms, and delivered to the WHNRC by truck (a distance of ~270 km). Mushrooms were typically harvested on a Thursday, shipped on a Friday, and used to prepare meals beginning the next Tuesday. The shipments were made every week. This 5-d lag before serving is similar to the lag that would occur between production and consumption by consumers under normal commercial conditions. In addition, the ergocalciferol levels in mushrooms treated with UV according to the conditions of this study decrease initially but are stable by d 4 after treatment (14). Upon delivery to the WHNRC, mushrooms were stored in their original coded containers at 3°C. The mushroom samples were frozen at −20°C on Tuesdays (5 d after UV treatment) for later analysis of ergocalciferol content. Systematically selected samples (to proportionally represent each group) from each cohort were shipped frozen on dry ice to Heartland Assays, where they were analyzed for ergocalciferol content.

Mushrooms prepared for eating "in-house" at the WHNRC were cooked for 60 s at full power in a 1000-watt output programmable microwave oven (Panasonic Commercial Microwave Oven model NE-1064T) immediately prior to service. The mushrooms and portioned soybean oil were then mixed into the cooked entrée and served to the participants. Mushrooms for meals packed "to go" remained in their original packaging, similar to how they would be found by the consumer. Participants were instructed to heat their meals according to the manufacturer's instructions and to microwave the mushrooms on high for up to 2 min before mixing with the soybean oil and entrée.

On a few occasions, participants could not consume 5 meals/wk at the WHNRC and, with advance notice, additional to-go meals were provided. Participants were also provided with an extra to-go meal to keep at home in the event they could not come to the WHNRC for a regular meal without advance notice.

UV treatment of mushrooms. White button mushrooms were used for the trial. The mushrooms were exposed to UV-B light for exposure times <30 s to achieve targeted vitamin D levels of ~10 and 25 μg/serving. The lamps used were fluorescent bulbs that operate only in the UV-B range. The intensity output was adjusted by adjusting the height of the unit.

Serum vitamin D metabolite analysis. Serum 25(OH)D2, 25(OH)D3, and 24,25$(OH)_2$D3 were determined using Diels-Alder derivatization and ultra-performance liquid chromatography-tandem MS (UPLC-MS/MS), as previously described (16). The mean limits of detection for these 3 compounds during this study were 0.31, 0.15, and 0.16 nmol/L, respectively. Samples were analyzed in 2 batches: cohorts 1 and 2 together and cohorts 3 and 4 together. Standard vitamin D material from the National Institute of Standards and Technology (NIST; Standard Reference Material 972) was analyzed to characterize the accuracy of the method. Three of the NIST standards had certified concentration values for 25(OH)D2 (4.1, 64.1, and 5.8 nmol/L) and all 4 had certified values for 25(OH)D3 (59.6, 30.8, 46.2, and 82.3 nmol/L). Each of the 4 NIST standards was analyzed twice by UPLC-MS/MS in different batches. For 25(OH)D2, the mean measured value as a percentage of the certified NIST value was 144 ± 27% (n = 6; CV = 19%). For 25(OH)D3, the mean measured value as a percentage of the certified NIST value was 112 ± 22% (n = 8; CV = 19%). Total serum 25(OH)D was measured by the Clinical Chemistry Laboratory in the Department of Pathology at the University of Davis Medical Center using the Diasorin LIAISON assay system.

Ergocalciferol and placebo capsules. Gelatin capsules containing a target of 25 μg ergocalciferol in powder or identical placebos were manufactured by Tishcon with a shelf-life of at least 1 y. The vitamin D content was measured at Heartland Assays as described (17).

Statistical analysis. Vitamin D metabolite concentrations for the 35 participants completing all 3 visits were analyzed using repeated-measures 2-way ANOVA to compare means between visits (wk 0, 3, and 6) and among the 4 treatment groups. When the group × visit interaction was significant ($P < 0.05$), group means were compared between visits within treatment groups and between groups at each of the 3 visits. Adjustment for multiple comparisons was made using the Holm-Sidak procedure for all pairwise multiple comparisons. Variables that were not normally distributed were transformed to achieve normality and equal variance ($P > 0.05$). Total 25(OH)D, 25(OH)D3, and 24,25$(OH)_2$D3 concentrations were analyzed as square root values and 25(OH)D2 concentrations as rank values (P = 0.042 for normality).

In addition to comparing group means, individual differences between wk 3 and baseline (n = 38 participants) and between wk 6 and baseline (n = 35 participants) were calculated for all metabolites for each participant and comparisons among treatment groups were made independently at wk 3 and 6 using 1-way ANOVA. All difference data were normally distributed except for 25(OH)D2 at wk 6, where a square root transformation was used, and 25(OH)D3 at wk 6, where rank data were analyzed using the Kruskal-Wallis ANOVA. All pairwise multiple comparisons were made using the Holm-Sidak method.

Our principal preplanned comparisons were to determine which groups had significant changes in vitamin D metabolite concentrations (relative to baseline) and to determine if change in metabolite levels differed among groups at 3 and 6 wk as a result of the intervention. Student's t test was used for 2-group comparisons. Statistical analysis was performed with SigmaStat software (SigmaStat 11.0, Systat Software). Values reported in the text are mean ± SD unless otherwise indicated.

Results

Effect of cooking on vitamin D content of mushrooms. Three servings of mushrooms treated with UV at the lower level (group M1) were independently cooked on the same day and 3 identical servings were not cooked. The vitamin D contents of the cooked and uncooked mushrooms were 11.6 ± 1.1 and 10.9 ± 0.70 μg/serving, respectively (P = 0.41). Based on this lack of difference as a result of cooking, we froze uncooked mushrooms for later analysis of ergocalciferol content during the course of the study.

Participant characteristics. Of 250 potential participants screened by telephone, 71 were judged potentially eligible and came for a screening visit (Supplemental Fig. 1). Of these, 45 were eligible and were invited to participate; 38 accepted the invitation and began the study. Three participants dropped out following the 3-wk blood draw and 35 completed the study. The demographic characteristics of the participants enrolled in the study are shown in **Table 1**.

Vitamin D content of mushrooms and capsules. A systematic sample of mushrooms from each treatment group and cohort (**Supplemental Table 1**) was selected for ergocalciferol analysis. The overall mean ergocalciferol contents of mushrooms used for the C, S, M1, and M2 treatment groups were 0.85 ± 0.95, 0.42 ± 0.30, 8.8 ± 2.6, and 17.1 ± 4.3 μg/serving, respectively (**Supplemental Fig. 2**). The C and S means did not differ from one another, as expected, because both of these groups received untreated mushrooms from the same production pool. The M1 and M2 means differed from one another and each differed from both the C and S groups. The placebo capsule contained no detectable ergocalciferol and the supplement contained 27.8 ± 1.8 μg ergocalciferol/capsule on delivery (n = 4). Based on these data, the estimated mean ergocalciferol intakes from mushrooms plus capsules for groups C, M1, M2,

TABLE 1 Characteristics of study participants[1]

	Group C	Group S	Group M1	Group M2	*P* value[2]
Participants beginning study (*n* = 38)					
n	10	9	10	9	—
Sex, *F/M*	7/3	4/5	7/3	6/3	0.61
Race/ethnicity, *White/Asian/Latino/mixed*	6/2/2/0	2/3/3/1	5/2/2/1	4/4/1/0	0.74
Age, *y*	30.1 ± 11.7	22.7 ± 2.9	32.7 ± 11.9	37.3 ± 15.9	0.096[3]
Body weight, *kg*	65.3 ± 9.5	69.9 ± 10.1	65.7 ± 15.0	67.0 ± 9.4	0.81
BMI, *kg/m²*	22.8 ± 2.1	24.7 ± 1.7	22.5 ± 2.7	23.4 ± 2.7	0.19
Daily vitamin D intake, *μg*	7.6 ± 4.2	4.4 ± 3.4	10.1 ± 9.1	7.5 ± 5.0	0.25[4]
Participants completing study (*n* = 35)					
n	10	7	9	9	—
Sex, *F/M*	7/3	3/4	7/2	6/3	0.52
Race/ethnicity, *White/Asian/Latino/mixed*	6/2/2/0	2/2/2/1	5/2/1/1	4/4/1/0	0.78
Age, *y*	30.1 ± 11.7	22.9 ± 3.3	33.4 ± 12.3	37.3 ± 15.9	0.13
Body weight, *kg*	65.3 ± 9.5	68.3 ± 10.4	63.5 ± 14.1	67.0 ± 9.4	0.83
BMI, *kg/m²*	22.8 ± 2.1	24.5 ± 1.9	22.1 ± 2.5	23.4 ± 2.7	0.22
Daily vitamin D intake, *μg*	7.6 ± 4.2	4.9 ± 3.7	10.0 ± 9.7	7.5 ± 5.0	0.51[4]

[1] Continuous data reported as mean ± SD. C, control group receiving untreated mushrooms; M1, group receiving the lower dose of ergocalciferol from UV-treated mushrooms; M2, group receiving the higher dose of ergocalciferol from UV-treated mushrooms; S, group receiving purified ergocalciferol and untreated mushrooms.
[2] Categorical variables were compared by chi-squared test and continuous variables by ANOVA.
[3] ANOVA performed on rank data.
[4] ANOVA performed with square root transformation of data.

and S were 0.85, 8.8, 17.1, and 28.2 μg/d, respectively. The target intakes for groups M1, M2, and S were 10, 25, and 25 μg/d, respectively; thus, the actual intakes as a percentage of the target were 88, 68, and 113%, respectively.

Effect of treatment on serum 25(OH)D2 concentration. Serum 25(OH)D2 concentrations were low at baseline, ranging from 0.6 to 8.7 nmol/L (n = 38). The median (25th, 75th percentiles) was 1.8 (1.2, 2.5) nmol/L and the mean ± SD was 2.3 ± 2.0 nmol/L. There were no differences among treatment groups or cohorts. Consumption of the UV-treated mushrooms caused a significant increase in serum 25(OH)D2 in groups M1 and M2 at 3 and 6 wk (**Fig. 1**). These increases were similar in magnitude and did not differ from one another but were significantly greater than the change in the C group (**Table 2**). Serum 25(OH)D2 remained low in the C group throughout the study, though a small (<2 nmol/L), significant increase was seen at wk 3. Group S, with the highest intake of ergocalciferol, had a significantly higher mean serum 25(OH)D2 at both 3 and 6 wk than any of the other groups.

Change in serum 25(OH)D2 per 2.5 μg (100 IU) ergocalciferol intake. The change in serum 25(OH)D2 over the 6-wk study period was divided by the median intake of ergocalciferol (for each cohort) to compare the relative effectiveness of the different levels of ergocalciferol intake in increasing serum 25(OH)D2 concentration. The median change (25th, 75th percentile) in serum 25(OH)D2 for groups M1, M2, and S were 3.8 (2.1, 5.6), 1.8 (1.5, 2.3), and 2.9 (2.6, 3.3) nmol/L per 2.5 μg of intake, respectively. These median changes differed from one another (P = 0.002 by 1-way ANOVA on ranks), with post hoc comparisons showing that the M2 group, with the lowest relative increase, differed significantly from the M1 and S groups but that the M1 and S groups did not differ from one another.

Effect of treatment on serum total 25(OH)D concentration. Total serum 25(OH)D was measured using a standard, antibody-based assay to assess overall vitamin D status. Mean concentrations at baseline for the C, S, M1, and M2 groups were 102 ± 56, 71 ± 16, 74 ± 24, and 85 ± 40 nmol/L, respectively, and did not differ by treatment group or cohort. Mean serum 25(OH)D decreased significantly in the M1 and M2 groups (**Fig. 2**) but not in the C or S groups. However, the mean serum 25(OH)D concentrations in the M1 and M2 groups did not differ from either the C or S groups at wk 3 or 6. In addition, the mean change from baseline did not differ among the groups at wk 3 or 6 (Table 2). Thus, the M1, M2, and S treatments did not affect overall vitamin D status relative to the C group.

Effect of treatment on serum 25(OH)D3 concentration. Whereas consumption of ergocalciferol resulted in higher 25(OH)D2 concentrations in the treatment groups than in the C group at wk 3 and 6, the concentration of total 25(OH)D did not differ between the treatment and C groups at either time point. These findings suggest that ergocalciferol intake may cause a decrease in serum 25(OH)D3. Mean 25(OH)D3 concentrations at baseline for the C, S, M1, and M2 groups were 93 ± 38, 69 ± 14, 68 ± 19, and 84 ± 31 nmol/L, respectively, and did not differ by treatment group or cohort. At wk 3 and 6, the mean serum 25(OH)D3 concentrations did not differ from baseline for the C group. However, the mean serum 25(OH)D3 concentrations decreased from wk 0 to 3 in the M2 and S groups and decreased from wk 0 to 6 for all 3 treatment groups (Fig. 1). The mean 25(OH)D3 concentration in the S group was significantly lower than that of the C group at wk 3, and the means for both the M1 and S groups differed from the C at wk 6. The mean change from baseline did not differ among the treatment groups at wk 3 but did differ significantly at wk 6 when the M2 and S groups had greater decreases in 25(OH)D3 concentrations than those of either the C group or group M1 (Table 2).

The magnitude of the mean increase in serum 25(OH)D2 for treatment groups was proportional to the magnitude of the decrease in serum 25(OH)D3 (Table 2). To determine if this trend is also seen at the level of individual participants, the

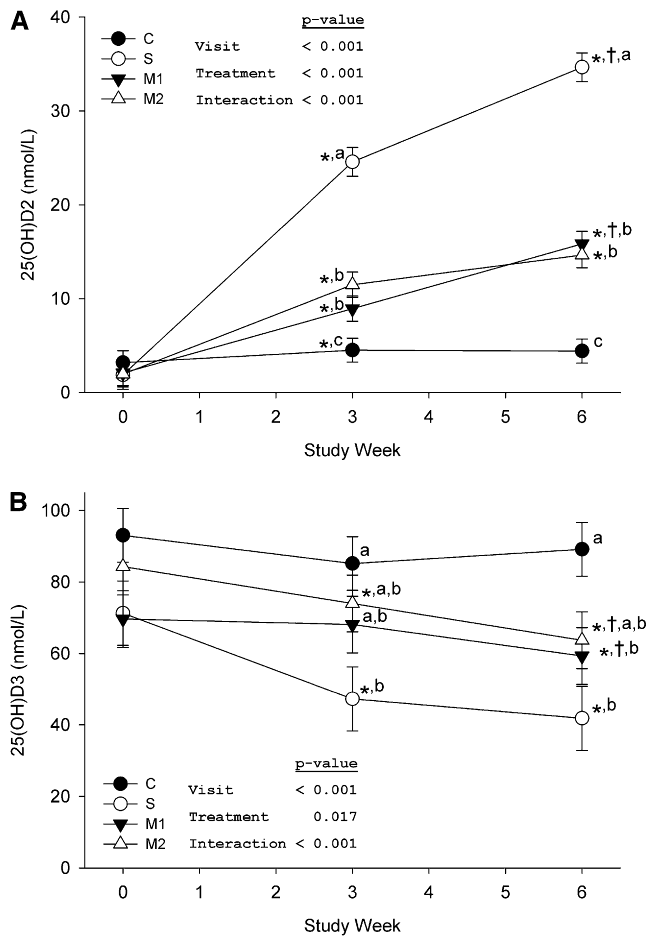

FIGURE 1 Serum 25(OH)D2 (*A*) and 25(OH)D3 (*B*) concentrations measured by UPLC-MS/MS in healthy adults who consumed ergocalciferol from mushrooms or supplements with a meal for 6 wk. Values are mean ± SE, n = 8–10/group. Labeled means at a time without a common letter differ, $P < 0.05$. *Different from baseline, $P < 0.05$; †different from wk 3, $P < 0.05$. Statistical analysis was performed by repeated-measures 2-way ANOVA. 25(OH)D2, 25-hydroxyergocalciferol; 25(OH)D3, 25-hydroxycholecalciferol; UPLC-MS/MS, ultra-performance liquid chromatography-tandem MS.

change in 25(OH)D2 from baseline to wk 6 was used to predict the change in serum 25(OH)D3 over the same period. Linear regression analysis demonstrated a significant negative association, with a 1.0-nmol/L increase in serum 25(OH)D2 predicting a decrease in serum 25(OH)D3 of ~0.8 nmol/L (**Fig. 3**).

Effect of treatment on serum 24,25(OH)$_2$D3 concentration. 25(OH)D undergoes catabolism via hydroxylation at the 24 position (18). Because ergocalciferol caused a decrease in serum 25(OH)D3, it is possible that catabolism of 25(OH)D3 may have increased, which could result in increased serum 24, 25(OH)$_2$D3 concentrations. For this reason, we compared changes in serum 24,25(OH)$_2$D3 concentrations among the treatment groups. Our analysis did not demonstrate differences or an interaction between treatment group and visit, but the overall mean concentration decreased over time (**Supplemental Fig. 3**). The mean for all participants at baseline, wk 3, and wk 6 were 6.9 ± 4.1, 6.0 ± 3.4, and 5.8 ± 3.5 nmol/L, respectively. The change from baseline did not differ among treatment groups at either wk 3 or 6 (Table 2).

Discussion

The purpose of this study was to investigate the bioavailability of ergocalciferol from mushrooms under normal market conditions among healthy adults. Fresh mushrooms were delivered to the WHNRC from the producer under similar conditions that consumers would encounter, in that postharvest UV treatment, packaging, shipping, storage, and cooking were similar to commercial production and home preparation. According to our analysis, microwave cooking did not affect ergocalciferol content (P = 0.41). Because all meals were not prepared from a single batch of mushrooms, the ergocalciferol content of mushrooms was assessed throughout the study. Two other studies have also reported on the availability of ergocalciferol from mushrooms. Outila et al. (19) demonstrated that ergocalciferol is available from lyophilized wild mushrooms naturally exposed to sunlight. In a second study, which was published after the conclusion of our study, the bioavailability of ergocalciferol from mushrooms irradiated with artificial UV light was demonstrated by Urbain et al. (20). Both studies measured serum total 25(OH)D rather than 25(OH)D2, but because both studies were conducted under conditions of minimal UV exposure (wintertime in Northern Europe), the increase in total 25(OH)D can be attributed to the dietary intervention and would not be affected by changes in serum 25(OH)D3 due to sun exposure.

The present study, the Outila et al. study (19), and the Urbain et al. study (20) also show that the availability of ergocalciferol

TABLE 2 Change in serum vitamin D metabolite concentrations from baseline in healthy adults who consumed ergocalciferol from mushrooms or supplements with a meal for 6 wk[1]

Treatment group	n	25(OH)D2 wk 3	25(OH)D2 wk 6	25(OH)D3 wk 3	25(OH)D3 wk 6	Total 25(OH)D wk 3	Total 25(OH)D wk 6	24,25(OH)$_2$D3 wk 3	24,25(OH)$_2$D3 wk 6
					nmol/L				
C	10	1.32 ± 1.03^{c,*}	1.22 ± 1.65^{c}	−7.89 ± 6.98	−3.93 ± 5.16^{a}	−1.29 ± 4.20	2.60 ± 3.30	−1.87 ± 0.89	−1.59 ± 0.79
M1	9	6.72 ± 0.92^{b,*}	13.8 ± 2.42^{b,*}	−1.49 ± 3.06	−10.4 ± 2.14^{a,b,*}	2.85 ± 2.78	−6.50 ± 3.71	0.73 ± 0.65	−0.51 ± 0.56
M2	10	9.53 ± 0.87^{b,*}	12.7 ± 1.22^{b,*}	−10.3 ± 1.75*	−20.6 ± 4.86^{b,*}	−6.08 ± 3.70	−10.5 ± 6.02*	−1.28 ± 0.63	−1.18 ± 0.73
S	9	22.1 ± 1.64^{a,*}	32.8 ± 1.26^{a,*}	−17.1 ± 5.45*	−29.5 ± 6.01^{b,*}	3.36 ± 3.17	−7.29 ± 2.86	−0.63 ± 0.55	−1.19 ± 0.86
P value[2]		<0.001	<0.001	0.17	0.004	0.23	0.15	0.066	0.76

[1] Data are mean ± SE. Labeled means in a column without a common letter differ, $P < 0.05$. *Different from baseline, $P < 0.05$. C, control group receiving untreated mushrooms; M1, group receiving the lower dose of ergocalciferol from UV-treated mushrooms; M2, group receiving the higher dose of ergocalciferol from UV-treated mushrooms; 24,25(OH)$_2$D3, 24,25-hydroxycholecalciferol; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D2, 25-hydroxyergocalciferol; 25(OH)D3, 25-hydroxycholecalciferol; S, group receiving purified ergocalciferol and untreated mushrooms.

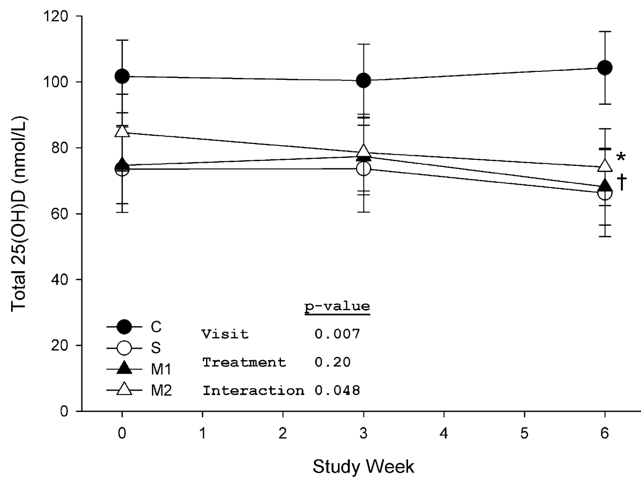

FIGURE 2 Total serum 25(OH)D concentrations measured by Diasorin Liaison system in healthy adults who consumed ergocalciferol from mushrooms or supplements with a meal for 6 wk. Values are mean ± SE, n = 8–10 per group. *Different from baseline, $P < 0.05$; †different from wk 3 (at wk 6), $P < 0.05$. Statistical analysis was performed by repeated-measures 2-way ANOVA. 25(OH)D, 25-hydroxyvitamin D.

from mushrooms is similar to that of ergocalciferol from capsule supplements, though the present study is complicated by the fact that vitamin D intake from supplements was higher than intake from the M1 and M2 groups providing UV-treated mushrooms. In the present study, we found that the low- and high-UV mushroom treatments produced similar changes in serum 25(OH)D2 despite containing different levels of ergocalciferol (8.8 and 17.1 μg/serving, respectively). Thus, the increase in serum 25(OH)D2 per unit of intake differed between the 2 groups (with median values of 3.8 and 1.8 nmol/L per 2.5 μg of intake for the low- and high-UV treatments, respectively), whereas the capsule group (receiving 28.2 μg/serving) was intermediate (2.9 nmol/L per 2.5 μg). The explanation for the differences in serum 25(OH)D2 response between the 2 mushroom groups is not apparent. The observed increases in serum 25(OH)D2 are all higher than the increase in total 25(OH)D (0.5 nmol/L per 2.5 μg of intake) reported by the Urbain et al. study (20), but the values are not directly comparable. The values reported here for a 6-wk intervention with ergocalciferol are not dissimilar from the mean increase in total serum 25(OH)D reported for cholecalciferol taken as supplements, 1.8 nmol/L per 2.5 μg (21), at doses ranging from 25 to 250 μg/d for a longer period (20 wk). From these 3 studies, we concluded that ergocalciferol is as available from cooked mushrooms, that the availability is similar to that of ergocalciferol and cholecalciferol from supplements, and that ergocalciferol from mushrooms will improve vitamin D status in winter months (i.e., under low-UV conditions) when baseline 25(OH)D levels are relatively low. Ergocalciferol intake did not improve vitamin D status in the present study. The key differences between this and previous studies are that baseline 25(OH)D levels were relatively high in the present study, with 94% of participants beginning the study at >50 nmol/L, and cholecalciferol input from cutaneous synthesis was presumably higher in the present study than in the previous studies given the differences in latitude and season.

A novel aspect of the present study is that we measured the effect of 3 different levels of ergocalciferol intake from both food and supplement on total 25(OH)D as well as on 25(OH)D3 and 25(OH)D2 separately. Unexpectedly, we found that consuming ergocalciferol not only caused an increase in serum 25(OH)D2 but also caused a proportionate decrease in serum 25(OH)D3. When we analyzed all participants in a regression model, we found an increase of 1 nmol/L in serum 25(OH)D2 predicted a 0.8-nmol/L decrease in serum 25(OH)D3. The net result was a small but significant decrease in total serum 25(OH)D in 2 of the groups consuming ergocalciferol (Fig. 2), though the change in total serum 25(OH)D did not significantly differ from the C group. In contrast, Holick et al. (22) saw no change in serum 25(OH)D3 in participants receiving a daily 25-μg ergocalciferol supplement for 11 wk. We postulate that this difference may be due to the fact that the study by Holick et al. (22) was performed at the end of winter in Boston, with minimal cholecalciferol from cutaneous synthesis entering the system, whereas our trial was conducted from June to November at a lower latitude where we previously demonstrated a significant association of sun exposure with serum 25(OH)D3 levels (4). Although we intentionally recruited participants with limited sun exposure, the serum 25(OH)D3 levels at baseline are consistent with some sun exposure for most participants.

A number of studies have investigated the effects of ergocalciferol and D3 intake from supplements on serum total 25(OH)D. In particular, a study by Tjellesen et al. (23) working in Copenhagen found that consuming 100 μg/d of ergocalciferol for 8 wk increased serum 25(OH)D2 but apparently caused a corresponding decrease in serum 25(OH)D3 such that there was no net change in serum total 25(OH)D. However, a parallel control group not receiving vitamin D supplements was not included to control for the expected seasonal decrease in serum 25(OH)D3 during the September through November study period, though the actual decrease was stated to be greater than would be expected based on seasonality. The ability of ergocalciferol to depress serum 25(OH)D3 under some circumstances may explain the observations of other studies showing that high-dose ergocalciferol supplements are less effective at increasing serum total

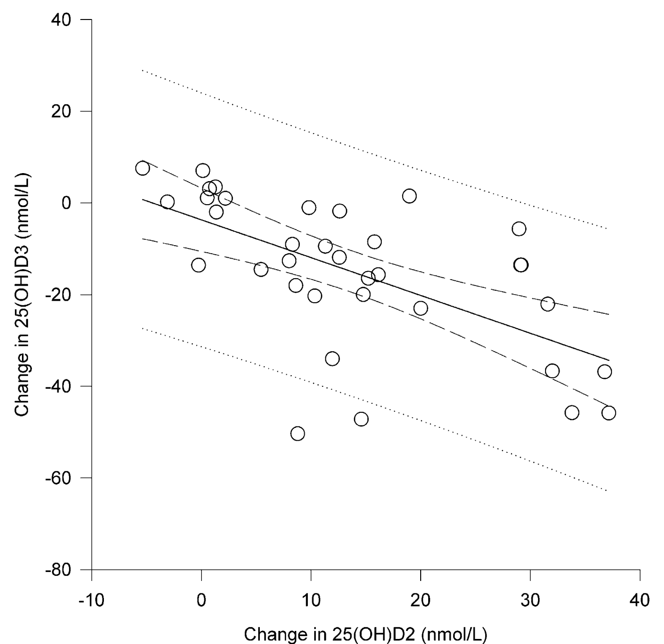

FIGURE 3 Predicted change in serum 25(OH)D3 from change in serum 25(OH)D2 for healthy adults who consumed ergocalciferol from mushrooms or supplements with a meal for 6 wk (n = 35). Dashed lines, 95%CI for the regression line; dotted lines, prediction of individual values. The regression equation was as follows: Δ 25(OH)D3 = $-3.667 - [0.825 \cdot \Delta$ 25(OH)D2] ($R^2 = 0.37$; $P < 0.001$).

25(OH)D than are cholecalciferol supplements of the same dose (24–26).

One hypothesis to explain the negative effect of ergocalciferol intake on serum 25(OH)D3 is that increased intake of ergocalciferol increases the catabolism of 25(OH)D3. To indirectly test this hypothesis, we measured the 24-hydroxylation product 24,25 $(OH)_2D3$, which is produced by the first step in 25(OH)D3 catabolism (2). We found no treatment effect on the concentration of $24,25(OH)_2D3$, suggesting that ergocalciferol does not increase the catabolism of 25(OH)D3 via 24-hydroxylation. Alternatively, the negative interaction between ergocalciferol and cholecalciferol could occur at the 25-hydroxylation step. Consistent with this thought, cholecalciferol has been shown to inhibit activity of the rat liver vitamin D 25-hydroxylase enzyme (27). Future studies are needed to evaluate this potential mechanism. Finally, it is possible that competition between vitamins D2 and D3 occurred at the level of absorption, but this seems unlikely for these participants because the principal source of cholecalciferol was likely to be cutaneous synthesis.

In summary, these results indicate that ergocalciferol is absorbed from UV-treated mushrooms and converted to 25(OH)D2 with an efficiency similar to that seen for ergocalciferol or D3 from supplements. However, serum 25(OH)D3 decreased in proportion to the increase in 25(OH)D2, with the net effect that there was no improvement in overall vitamin D status in participants receiving the UV-treated mushrooms or supplements. The decrease in serum 25(OH)D3 does not appear to result from increased catabolism but could result from competition of ergocalciferol with cholecalciferol for 25-hydroxylation. Previous studies with ergocalciferol-containing mushrooms conducted under conditions of low cutaneous cholecalciferol synthesis showed improved vitamin D status. Thus, ergocalciferol intake from mushrooms is beneficial for participants at risk of deficiency but may not improve status cholecalciferol participants with considerable sun exposure and resulting cutaneous synthesis of cholecalciferol.

Acknowledgments

C.B.S. and T.M. designed the research (project conception, development of overall research plan, and study oversight); M.Z., D.J.B., and Y.L. conducted the research (hands-on conduct of the experiments and data collection); B.D.H. provided essential expertise, equipment, and materials for vitamin D analysis; C.B.S., M.Z., Y.L., and L.M.H. analyzed data and performed statistical analysis; C.B.S., M.Z., and D.J.B. wrote the paper (only authors who made a major contribution); and C.B.S. had primary responsibility for final content. All authors have read and approved the final manuscript.

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