

# Bioavailability of vitamin D from wild edible mushrooms (*Cantharellus tubaeformis*) as measured with a human bioassay<sup>1-3</sup>

Terhi A Outila, Pirjo H Mattila, Vieno I Piironen, and Christel JE Lamberg-Allardt

## ABSTRACT

**Background:** The bioavailability of vitamin D from mushrooms in humans is unknown.

**Objective:** We investigated the bioavailability of vitamin D from wild edible mushrooms (*Cantharellus tubaeformis*) using the increase in serum 25-hydroxyvitamin D concentrations as a measure of vitamin D bioavailability.

**Design:** Twenty-seven volunteers with serum 25-hydroxyvitamin D concentrations <60 nmol/L ( $\bar{x}$ : 38.5 nmol/L; range: 15–60 nmol/L) were randomly divided into 3 groups of 9 persons each. For 3 wk, excluding Saturdays and Sundays, group 1 received mushrooms (*C. tubaeformis*) providing 14  $\mu\text{g}$  ergocalciferol/d with their lunch, group 2 (control) received an ergocalciferol supplement providing 14  $\mu\text{g}/\text{d}$ , and group 3 (also a control) received no supplementation.

**Results:** At the beginning of the study, mean serum 25-hydroxyvitamin D concentrations did not differ significantly among the groups ( $P = 0.280$ ). When all 3 groups were considered, serum 25-hydroxyvitamin D concentrations showed different time-related changes among the groups during the study: group ( $P = 0.388$ ), time ( $P = 0.000$ ), and group  $\times$  time ( $P = 0.001$ ). When groups 1 and 2 were compared with group 3, serum 25-hydroxyvitamin D concentrations at 3 wk differed significantly between groups 1 and 3 ( $P = 0.032$ ) as well as between groups 2 and 3 ( $P = 0.004$ ). Serum 25-hydroxyvitamin D concentrations at 3 wk did not differ significantly between groups 1 and 2 ( $P = 0.317$ ).

**Conclusions:** We showed for the first time that ergocalciferol was well absorbed from lyophilized and homogenized mushrooms in humans and that vitamin D bioavailability can be studied in humans with such an experimental protocol. *Am J Clin Nutr* 1999;69:95–8.

**KEY WORDS** Vitamin D, ergocalciferol, bioavailability, edible mushrooms, bioassay, humans, *Cantharellus tubaeformis*, sunlight, Finland

## INTRODUCTION

In humans, vitamin D is obtained from the diet and through cutaneous synthesis in the presence of ultraviolet light supplied by sunlight or other ultraviolet B light sources. An adequate source of dietary vitamin D is especially important for healthy, active persons during the winter, especially in northern latitudes,

because of their reduced exposure to sunlight during this time. In addition, dietary vitamin D intake is important for persons spending most of their time indoors, such as those who are institutionalized. Vitamin D<sub>3</sub> (cholecalciferol) is present in animal sources and is the main form of vitamin D in food. Vitamin D<sub>2</sub> (ergocalciferol), which is usually of plant origin, is of minor importance because it is not abundant in foodstuffs (1). The biological activities of cholecalciferol and ergocalciferol are considered to be equal in humans. Both cholecalciferol and ergocalciferol are inactive themselves, but are metabolized in the liver to 25-hydroxyvitamin D and further in the kidney to the biologically active form, 1,25-dihydroxyvitamin D. The serum concentration of 25-hydroxyvitamin D is considered to be a good indicator of vitamin D status in humans.

In Finland, only margarine (0.07  $\mu\text{g}/\text{g}$ ) and fat-free and 1%-fat milk (0.0008  $\mu\text{g}/\text{g}$ ) are slightly fortified with vitamin D. Fish and vitamin-fortified margarines supply 70–80% of the daily dietary vitamin D intake in Finland (2, 3). In a previous study we showed that some wild mushrooms, especially *Cantharellus tubaeformis*, contain high amounts (0.298  $\mu\text{g}/\text{g}$  fresh weight) of ergocalciferol (4). The intake of mushrooms in Finland is only 0.6  $\text{kg} \cdot \text{person}^{-1} \cdot \text{y}^{-1}$  (5), but for some groups, such as vegetarians and persons allergic to fish, mushrooms can be an important natural dietary source of vitamin D. The bioavailability of vitamin D from this source in humans, however, is unknown.

It is generally known that, depending on the vitamin D source, the absorption of vitamin D in humans varies between 55% and 99%, and that absorption does not decrease significantly with age (6–8). However, vitamin D bioavailability studies have been conducted in animals. Because there might be differences in the bioavailability of vitamin D between humans and animals, results from animal studies are not necessarily applicable to humans. In humans, the bioavailability and absorption of vitamin D from supplements has been studied, but not from natural food sources.

<sup>1</sup>From the Division for Nutrition and the Division for Food Chemistry, University of Helsinki.

<sup>2</sup>Supported by the Academy of Finland and 'Juho Vainio' Foundation.

<sup>3</sup>Address reprint requests to CJE Lamberg-Allardt, Division of Nutrition, Department of Applied Chemistry and Microbiology, Calcium Research Unit, University of Helsinki, PO Box 27, FIN-00014 Helsinki, Finland. E-mail: christel.lamberg-allardt@helsinki.fi.

Received January 13, 1998.

Accepted for publication July 2, 1998.

The bioavailability of vitamin D from edible mushrooms in humans is unknown. The aim of our study was to investigate the bioavailability of ergocalciferol from wild edible mushrooms (*C. tubaeformis*) by using the increase in total serum 25-hydroxyvitamin D concentrations as a measure of vitamin D absorption. Because the absorption of vitamin D from supplements is known to be good (9–11), the efficacy of absorption from mushrooms was compared with that from an ergocalciferol supplement. In addition, we attempted to develop a bioassay for human vitamin D absorption studies to be used for different foodstuffs.

## SUBJECTS AND METHODS

### Subject selection

The study was performed during January and February when serum 25-hydroxyvitamin D concentrations are expected to be lowest because of low solar exposure in Finland. Thirty-one healthy female volunteers were recruited through advertising at the University of Helsinki. All subjects signed a written consent form in which the intentions and procedures of the study were explained, which were in accordance with the Helsinki Declaration of 1975. In the preexperimental interview, dietary vitamin D intake was estimated with a food-frequency questionnaire (FFQ), administered by a nutritionist. The FFQ was used previously in dietary studies (12). The subjects' use of vitamin D-containing supplements was determined. Those with a low dietary intake of vitamin D were asked to give a blood sample. The 25-hydroxyvitamin D concentrations in these serum samples were measured ( $\bar{x}$ : 42.3 nmol/L; range: 15–87 nmol/L) and 3 persons with a serum 25-hydroxyvitamin D concentration >60 nmol/L were excluded from the study; 27 subjects, who had a mean serum 25-hydroxyvitamin D concentration of 38.5 nmol/L (range: 15–60 nmol/L), were included in the study.

### Study design

The subjects were randomly divided into 3 groups of 9 persons each. During the 3-wk supplementation period, all subjects were served lunch—on weekdays—at the Division of Nutrition at the University of Helsinki. With their lunch, group 1 received mushrooms providing 14  $\mu$ g ergocalciferol/d, group 2 (control)

received a supplement providing 14  $\mu$ g ergocalciferol/d (VITOL drops, 2  $\mu$ g ergocalciferol/drop; Oriola, Espoo, Finland), and group 3 (also a control) received no supplementation (Table 1). The participants were asked to maintain their habitual diets, but not to eat vitamin D-containing foods (mainly fish and vitamin-fortified margarines) or supplements during the study period.

The dose of ergocalciferol given to subjects in groups 1 and 2 daily, 14  $\mu$ g, meant that each of these subjects received a total dose of 210  $\mu$ g ergocalciferol over the 3-wk study period. This dosage was estimated, on the basis of previous results (11), to be enough to increase serum 25-hydroxyvitamin D concentrations. The mushrooms were obtained during the autumn of 1995 from local retail stores. The ergocalciferol content of the batch of mushrooms was analyzed at the Division of Food Chemistry at the University of Helsinki according to a method described previously (4). Before the mushrooms were analyzed, they were pooled, lyophilized, and homogenized. The analyzed content of ergocalciferol in the mushroom homogenate was 2.34  $\mu$ g/g lyophilized weight. The lyophilized, homogenized, and uncooked mushrooms were mixed with broth (20 g/L at 37–40°C) just before being served to group 1. Overnight, fasting serum samples and 24-h urinary samples were collected from all subjects at the beginning (day 1 or 2), in the middle (day 10 or 11), and at the end (day 20 or 21) of the 3-wk study.

### Laboratory measurements

Serum 25-hydroxyvitamin D concentrations were analyzed with a competitive protein-binding assay described previously (13). The assay does not discriminate between 25-hydroxyergocalciferol (ercalcidiol) and 25-hydroxycholecalciferol (calcdiol). The reference range for serum 25-hydroxyvitamin D was 25–125 nmol/L. Inter- and intraassay CVs for 25-hydroxyvitamin D were 14% and 15%, respectively. Urinary calcium was measured by using a standard automated procedure. Serum intact parathyroid hormone (iPTH) concentrations were measured with an immunoradiometric method (Nichols Institute, San Juan Capistrano, CA). The reference range for iPTH was 10–60 ng/L. Inter- and intraassay CVs for iPTH were 3.7% and 1%, respectively. Intestinal calcium absorption was studied with a stable strontium isotope method (14). Serum iPTH concentrations and strontium absorption were measured only at the beginning and

TABLE 1

Background and dietary data of the 3 study groups<sup>1</sup>

	Group 1 (ergocalciferol-containing mushrooms)	Group 2 (ergocalciferol supplement)	Group 3 (no supplement)	P (significant main effect)
Age (y)	25 ± 2	29 ± 4	29 ± 3	NS
Height (cm)	166 ± 3	164 ± 2	169 ± 2	NS
Weight (kg)	57 ± 3 <sup>a</sup>	60 ± 2 <sup>b</sup>	65 ± 2 <sup>c</sup>	0.033
BMI (kg/m <sup>2</sup> )	20.4 ± 0.5	22.5 ± 0.9	23.1 ± 1.2	NS
Dietary intake				
3-d Dietary records (during the study)				
Energy (kJ)	7355 ± 568 <sup>a</sup>	6735 ± 640 <sup>b</sup>	8775 ± 478 <sup>c</sup>	0.047
Calcium (mg)	902 ± 139	951 ± 106	1326 ± 136	NS
Vitamin D ( $\mu$ g)	1.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	NS
Food-frequency questionnaire (at the end of the study)				
Vitamin D ( $\mu$ g)	0.8 ± 0.2	0.8 ± 0.1	1.2 ± 0.3	NS

<sup>1</sup> $\bar{x}$  ± SEM; n = 9. Means with different superscript letters are significantly different, P < 0.05. Statistical analyses were based on ANOVA and post hoc analyses with Student-Neuman-Keuls test.

at the end of the study; all other variables were also measured in the middle of the study.

### Dietary intake

Mean energy and nutrient intakes were calculated from 3 dietary records. These records were collected on the day before blood was sampled: once at the beginning, once in the middle, and once at the end of the study. Two of the days were weekdays and one was a weekend day. Dietary vitamin D intake was estimated by an FFQ at the beginning and at the end of the study.

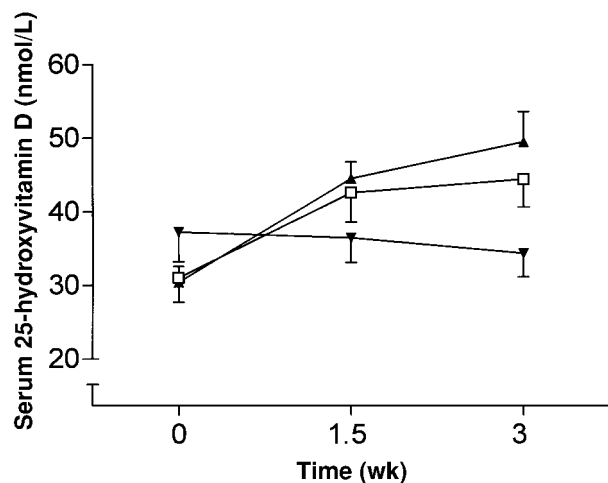
### Statistical analyses

The data are expressed as means  $\pm$  SEMs. The distribution of the variables was checked for normality, and logarithmic transformation was used for skewed data. The effects of supplementation on serum and urinary variables among groups were analyzed by repeated-measures analysis of variance (ANOVA) and analysis of covariance (ANCOVA). Differences in background and dietary data among the groups were analyzed with a Student-Neuman-Keuls post hoc test with 95% CIs. A paired *t* test was used to analyze the differences within groups. Analyses were made with BMDP statistical software (15). All tests were considered significant at  $P < 0.05$ .

## RESULTS

### Background data

As calculated from the dietary records, mean energy intakes differed among the groups ( $P = 0.047$ , ANOVA) and significant differences were found between groups 1 and 2, between groups 1 and 3, and between groups 2 and 3 (all  $P < 0.05$ ). Calcium intake did not differ significantly among the studied groups ( $P = 0.551$ ). Because consumption of vitamin D-containing foods was avoided during the study, dietary vitamin D intakes were low in all 3 groups during the study period (Table 1).



**FIGURE 1.** Changes in serum 25-hydroxyvitamin D concentrations in subjects who consumed ergocalciferol-containing mushrooms (group 1, □), ergocalciferol-containing supplements (group 2, ▲), or no supplement (group 3, ▼) over the 3-wk study period. Concentrations at 1.5 and 3 wk were significantly different (by ANCOVA) between groups 1 and 3 (1.5 wk:  $P = 0.007$ ; 3 wk:  $P = 0.032$ ) and between groups 2 and 3 (1.5 wk:  $P = 0.002$ ; 3 wk:  $P = 0.004$ ) when the initial values were used as covariates.

### Changes in serum 25-hydroxyvitamin D concentrations

At the beginning of the study, mean serum 25-hydroxyvitamin D concentrations were within the reference range and did not differ significantly among the groups ( $P = 0.280$ , Figure 1). When all groups were considered, serum 25-hydroxyvitamin D concentrations showed different time-related changes among the groups during the study (group:  $P = 0.388$ ; time:  $P = 0.000$ ; group  $\times$  time:  $P = 0.001$ ; repeated-measures ANOVA). When initial values were used as covariates, final serum 25-hydroxyvitamin D concentrations differed significantly among the groups ( $P = 0.011$ , ANCOVA). Furthermore, when group 1 or group 2 was compared with group 3, the ANCOVA showed that final serum 25-hydroxyvitamin D concentrations differed significantly between groups 1 and 3 as well as between groups 2 and 3 when the initial values were used as covariates. However, final serum 25-hydroxyvitamin D concentrations did not differ significantly between groups 1 and 2. Moreover, after 1.5 wk of supplementation, serum 25-hydroxyvitamin D concentrations differed significantly among the groups ( $P = 0.004$ ) and changes in serum 25-hydroxyvitamin D concentrations differed significantly between groups 1 and 3 and between groups 2 and 3 when initial values were used as covariates.

### Other serum and urinary variables

Serum iPTH concentrations did not change significantly over the study among the groups (group:  $P = 0.235$ ; time:  $P = 0.971$ ; group  $\times$  time:  $P = 0.961$ ; repeated-measures ANOVA, Table 2). Regardless of the comparatively high mean serum iPTH concentrations, no individual value was outside the reference range for any of the 3 groups. Strontium absorption, which reflects calcium absorption, did not differ significantly among the groups during the study (group:  $P = 0.959$ ; time:  $P = 0.081$ ; group  $\times$  time:  $P = 0.404$ ). Time-related changes in urinary calcium excretion did not differ significantly among the groups over the study, but a significant difference was seen in urinary calcium excretion among the groups (group:  $P = 0.021$ ; time:  $P = 0.356$ ; group  $\times$  time:  $P = 0.076$ ). However, the ANCOVA showed that urinary calcium excretion did not differ significantly among the groups at the end of the study when initial values were used as covariates ( $P = 0.875$ , Table 2).

**TABLE 2**

Serum intact parathyroid hormone (iPTH), strontium absorption, and urinary calcium in the 3 groups at the beginning and end of the study<sup>1</sup>

	Group 1	Group 2	Group 3
Serum iPTH (ng/L)			
Beginning	51 $\pm$ 6	47 $\pm$ 7	60 $\pm$ 9
End	51 $\pm$ 7	46 $\pm$ 5	61 $\pm$ 5
Strontium absorption (%)			
Beginning	9.1 $\pm$ 0.8	8.7 $\pm$ 1.2	9.5 $\pm$ 1.0
End	8.7 $\pm$ 0.4	8.5 $\pm$ 0.9	8.1 $\pm$ 0.6 <sup>2</sup>
Urinary calcium (mmol/d) <sup>3</sup>			
Beginning	1.95 $\pm$ 0.34	1.75 $\pm$ 0.29	3.48 $\pm$ 0.47
End	2.14 $\pm$ 0.40	1.87 $\pm$ 0.25	2.57 $\pm$ 0.33

<sup>1</sup> $\bar{x} \pm$  SEM;  $n = 9$ . Group 1, ergocalciferol-containing mushrooms; group 2, ergocalciferol supplement; group 3, no supplement.

<sup>2</sup>Significantly different from at the beginning,  $P < 0.05$  (paired *t* test).


<sup>3</sup>There was a significant group effect,  $P = 0.021$  (repeated-measures ANOVA).

## DISCUSSION

The bioavailability of vitamin D has been studied previously, mainly in vitamin D-deficient animals, with changes in bone used as an indicator of the vitamin D activity of the food. In humans, the bioavailability of vitamin D has been investigated only from supplements, not from natural food sources. In most of these studies, the dose of vitamin D was high and thus the serum response of native vitamin D, which has a short half-life, could be measured after only a few hours. In foodstuffs, vitamin D activity is comparably low and thus the serum response of native vitamin D cannot be measured. In addition, other metabolites of vitamin D could contribute to the total vitamin D activity of a foodstuff. In this study, for the first time, we investigated the bioavailability of vitamin D from wild edible mushrooms (*C. tubaeformis*) in healthy, young subjects by using the increase in serum 25-hydroxyvitamin D concentrations as the measure of bioavailability. The study was conducted in a semicontrolled setting, assuming that only minor amounts of other metabolites are present in the mushrooms, as indicated by the studies of Mattila et al (4). Serum 25-hydroxyvitamin D is the major vitamin D metabolite in the circulation and other metabolites cannot be considered important markers of vitamin D status. The response of 25-hydroxyvitamin D concentrations in serum is not immediate, and thus the experiment had to be carried out for a few weeks. To study the effect of moderate vitamin D supplementation on serum 25-hydroxyvitamin D concentrations, the basal concentration of serum 25-hydroxyvitamin D has to be comparatively low. In addition, foods containing vitamin D as well as solar exposure need to be avoided.

In this study, we showed that ergocalciferol in mushrooms increased serum 25-hydroxyvitamin D concentrations as effectively as did the ergocalciferol supplement. A significant difference was seen in serum 25-hydroxyvitamin D concentrations between group 3 and groups 1 and 2 after only 1.5 wk of supplementation, which seemed to plateau during the next 1.5 wk of supplementation. The findings of our study agree well with those of earlier studies, suggesting that 10 µg ergocalciferol/d is sufficient to elevate serum 25-hydroxyvitamin D concentrations significantly higher than concentrations seen in control subjects (11, 16, 17).

We also studied whether the supplement affected other markers of calcium metabolism. The high mean serum iPTH concentrations found in all 3 study groups could be partly explained by the groups' initially low serum 25-hydroxyvitamin D concentrations (18). On the basis of our results, a 3-wk treatment period with vitamin D was too short to decrease serum iPTH concentrations. Van der Klis et al (19) reported that after 4 wk of ergocalciferol supplementation (20 µg/d), serum iPTH concentrations unexpectedly increased in premenopausal Dutch women. We did not find any significant differences in the other markers of calcium metabolism between the groups during the period of supplementation.

Our results showed for the first time that ergocalciferol was well absorbed from lyophilized and homogenized wild mushrooms in humans. In addition, the results indicated that the bioavailability of vitamin D from dietary sources can be conveniently studied in humans with such an experimental protocol. In conclusion, mushrooms can be reliably recommended as a natural vitamin D source. However, the bioavailability of vitamin D from fresh, nonlyophilized mushrooms was not conclusive and should be evaluated further. 

## REFERENCES

1. Boland RL. Plant as a source of vitamin D<sub>3</sub> metabolites. *Nutr Rev* 1986;44:1-8.
2. Lamberg-Allardt C. Vitamin D intake, sunlight exposure and 25 hydroxyvitamin D levels in the elderly during one year. *Ann Nutr Metab* 1984;28:144-50.
3. Mattila P. Analysis of cholecalciferol, ergocalciferol and their 25-hydroxylated metabolites in foods by HPLC. Helsinki: Helsinki University Printing House, 1995. (EKT series 995.)
4. Mattila PH, Piironen VI, Uusi-Rauva EJ, Koivistoinen PE. Vitamin D contents in edible mushrooms. *J Agric Food Chem* 1994;42:2449-53.
5. Statistics Finland. Household survey 1990: income and consumption. Helsinki: Statistical Office, 1993.
6. Thompson GR, Lewis B, Booth CC. Absorption of D<sub>3</sub>-<sup>3</sup>H in control subjects and patients with intestinal malabsorption. *J Clin Invest* 1966;45:94-102.
7. Horst RL, Goff JP, Reinhardt TA. Advancing age results in reduction of intestinal and bone 1,25-dihydroxyvitamin D receptor. *Endocrinology* 1990;126:1053-7.
8. Van den Berg H. Bioavailability of vitamin D. *Eur J Clin Nutr* 1997;51:S76-9.
9. Stamp TCB, Haddad JG, Twigg CA. Comparison of oral 25-hydroxycholecalciferol, vitamin D, and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. *Lancet* 1977;1:1341-3.
10. Whyte MP, Haddad JG, Walters DD, Stamp TC. Vitamin D bioavailability: serum 25-OHD levels in man after oral administration. *J Clin Endocrinol Metab* 1979;48:906-11.
11. Lamberg-Allardt C. The vitamin D<sub>2</sub> metabolism of healthy subjects. In: Cohn DV, Martin TJ, Meunier PJ, eds. Calcium regulation and bone metabolism: basic and clinical aspects. Vol 9. Amsterdam: Excerpta Medica, 1987.
12. Välimäki M, Tiihonen M, Laitinen K, et al. Bone mineral density measured by dual-energy X-ray absorptiometry and novel markers of bone formation and resorption in patients on antiepileptic drugs. *J Bone Miner Res* 1994;9:631-7.
13. Törnquist K, Lamberg-Allardt C. Systemic effects of 1,25-dihydroxy-vitamin D<sub>3</sub> on the pituitary-hypothalamic axis in rats. *Acta Endocrinol (Copenh)* 1987;115:225-8.
14. Milsom S, Ibbertson K, Hannan S, Shaw D, Pybus J. Simple test of intestinal calcium absorption measured by stable strontium. *Br Med J* 1987;295:231-4.
15. Dixon WJ, Brown MB, Engelman L, Hill MA, Jennrich RI, eds. BMDP statistical software manual. Berkeley, CA: University of California Press, 1988.
16. Lips P, Wiersinga A, van Ginkel FC, et al. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. *J Clin Endocrinol Metab* 1988;67:644-50.
17. Dubbelman R, Jonxis JH, Muskiet FA, Saleh AE. Age-dependent vitamin D status and vertebral condition of white women living in Curaçao (The Netherlands Antilles) as compared with their counterparts in The Netherlands. *Am J Clin Nutr* 1993;58:106-9.
18. Lips P, Hackeng WHL, Jongen MJM, van Ginkel FC, Netelenbos C. Seasonal variation in serum concentrations of parathyroid hormone in elderly people. *J Clin Endocrinol Metab* 1983;57:204-6.
19. Van der Klis FRM, Jonxis JHP, van Doormaal JJ, Sikkens P, Saleh AEC, Muskiet FAJ. Changes in vitamin-D metabolites and parathyroid hormone in plasma following cholecalciferol administration to pre- and postmenopausal women in the Netherlands in early spring and to postmenopausal women in Curacao. *Br J Nutr* 1996;75:637-46.

