Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 95 (2006) 638–643
Food Chemistry
www.elsevier.com/locate/foodchem

Ultraviolet irradiation: The generator of Vitamin D_2 in edible mushrooms

Viraj J. Jasinghe, Conrad O. Perera *

Department of Chemistry, Food Science & Technology Program, National University of Singapore, 3 Science Drive, Singapore 117543

Received 29 November 2004; accepted 10 January 2005

Abstract

Fresh Shiitake mushrooms (*Lentinula edodes*), Oyster mushrooms (*Pleurotus ostreatus*), Button mushrooms (*Agaricus bisporus*), and Abalone mushrooms (*Pleurotus cystidus*) were irradiated with Ultraviolet-A (UV-A; wavelength 315–400 nm), Ultraviolet-B (UV-B; wavelength 290–315 nm), and Ultraviolet-C (UV-C; wavelength 190–290 nm). Irradiation of each side of the mushrooms for 1 h, was found to be the optimum period of irradiation in this conversion. The conversions of ergosterol to vitamin D_2 under UV-A, UV-B, and UV-C were shown to be significantly different ($p < 0.01$). The highest vitamin D_2 content (184 ± 5.71 μg/g DM) was observed in Oyster mushrooms irradiated with UV-B at 35 °C and around 80% moisture. On the other hand, under the same conditions of irradiation, the lowest vitamin D_2 content (22.9 ± 2.68 μg/g DM) was observed in Button mushrooms.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Vitamin D_2; Ergosterol; UV irradiation; Mushrooms; *Lentinula edodes*

1. Introduction

Vitamin D and calcium are well known to be vital for bone health. The deficiency of this vitamin, results in rickets in children and osteoporosis in adults. Vitamin D deficiency is an unrecognized epidemic among elderly (Holick, 2001). Most of the foods which are normally consumed by humans, are deficient in vitamin D and, especially, the vegetarians are at risk of vitamin D deficiency disorders.

Clinically, vitamin D deficiency has been proven to be associated with cancers (Grant, 2002; Hansen, Binderup, & Hamberg, 2001; Majewski, Kutner, & Jablonska, 2000; Mehta & Mehta, 2002; Mokady, Schwartz, & Shany, 2000; Platz, Hankinson, & Hollis, 2000; Polek & Weigel, 2002; Tuohimaa, Lyakhovich, & Aksenov, 2001), heart diseases (Mancini et al., 1996; Norman, Moss, Minder, Gosling, & Powell, 2002; Segall, 1989; Williams & Lloyd, 1989; Zittermann et al., 2003), obesity (Cantorna, 2000; Heldenberg, Gershon, & Weisman, 1992; Speer, Cseh, & Winkler, 2001), diabetes (Billaudel, Barakat, & Faure-Dussert, 1998; Bourlon, Billaudel, & Faure-Dussert, 1999; Hypponen, Laara, & Reunanen, 2001) and arthritis (Braun & Tucker, 1997; McAlindon & Felson, 1996). In addition, Vitamin D has been suggested for therapeutic applications in the treatment of several diseases, including hyperproliferative diseases, secondary hyperparathyroidism, post-transplant survival, and various malignancies (Peleg, 1997). However, hypercalcemic effects limits the therapeutic application of vitamin D_3 analogues, and, therefore, vitamin D_2 analogues may be used to replace D_3 analogues, since vitamin D_2 analogues do not have hypercalcemic effects (Mawer et al., 1995). Therefore studies on vitamin D_2 could be useful in future clinical applications.

* Corresponding author. Tel.: +65 68748821; fax: +65 67757895.
E-mail address: chmpco@nus.edu.sg (C.O. Perera).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.01.046

Mushrooms are highly prized in the Orient for their flavour and are reputed to have medicinal value. Cultivated mushrooms are deficient in vitamin D_2; however, they are found to be rich sources of ergosterol, the precursor of vitamin D_2 (Mattila, Lampi, Ronkainen, Toivo, & Piironen, 2002). Even though the conversion of ergosterol, in mushrooms, to vitamin D_2 is not a new idea, there are only a handful of experimental data reported in the literature. Mau, Chen, and Yang (1998) reported that UV-B was more effective than UV-C in conversion of ergosterol to vitamin D_2. Perera, Jasinghe, Ng, and Mujumdar (2003) reported higher values of vitamin D_2 under UV-B than those under UV-B and UV-C, previously reported by Mau et al. (1998). Moreover, we have optimized the conditions for this conversion (Jasinghe & Perera, 2004) in order to maximize the yield of vitamin D_2. A comparative study of conversion of ergosterol in mushrooms under UV-A, UV-B, and UV-C could be useful for further optimization studies. Hence, the focus of this study was to investigate how this conversion takes place under irradiation with different UV wavelength bands.

2. Materials and methods

2.1. Raw materials

Fresh Shiitake mushrooms (*Lentinula edodes*), Oyster mushrooms (*Pleurotus ostreatus*), Button mushrooms (*Agaricus bisporus*), and Abalone mushrooms (*Pleurotus cystidus*) were purchased fresh from a local grower and were used immediately in the experiments. In the experiments to study the conversion of ergosterol to vitamin D_2 under different bands of UV irradiations, the moisture contents of mushrooms were pre-adjusted to around 80% before the commencement of experiments as this was found to be the optimum moisture content for the conversion (Perera et al., 2003; Jasinghe & Perera, 2004). The moisture content of mushrooms was reduced to a value of around 80% by vacuum-drying at the ambient temperature of 27 °C.

2.2. Effect of duration of irradiation on the conversion of ergosterol to vitamin D_2 and optimization of radiation dose

Fresh shiitake mushrooms were used in this experiment. Each side of the mushrooms was irradiated with the UV-A source [Mineralight UVGL – 25, San Gabriel, U.S.A. with UV-A lamp (measured intensity at 15 cm, 3.5 W/m^2)], for different time periods from 10 to 120 min. These samples were freeze-dried and kept in a vacuum-desiccator for further analysis.

2.3. Effect of different orientations of mushrooms to the UV source and duration of irradiation on the conversion of ergosterol to vitamin D_2

Fresh Shiitake mushrooms were subjected to three different irradiations with UV-A. The first lot of mushrooms was irradiated for 2 h with their gills facing the UV source, the second lot of mushrooms was irradiated with their gills facing the UV source for 1 h and then they were further irradiated for another hour with their caps facing the UV source, and the third lot was irradiated with their gills facing the UV source for 2 h and then they were further irradiated for 2 h with their caps facing the UV source. These samples were separately freeze-dried to prepare for the analysis.

In these experiments, the source of irradiation was placed at a distance of 15 cm away from the samples in an irradiation chamber. The calculated irradiation dose was 0.21 kJ/m^2/min. All the above irradiation experiments were carried out at an ambient temperature of 27 °C, and average moisture content of mushrooms was found to be around 82% on a wet basis (w.b).

2.4. Conversion of ergosterol in mushrooms under different bands of UV irradiations

In this experiment, four different types of edible mushrooms were used. Each side of the mushrooms was irradiated for 1 h with UV-A [Mineralight UVGL – 25, San Gabriel, U.S.A. with a UV-A lamp (measured intensity at 15 cm, 3.5 W/m^2)], UV-B [MODEL UVM-57, UVP, Upland, CA, U.S.A. (measured intensity 4.9 W/m^2)], and UV-C [Mineralight UVGL – 25, San Gabriel, U.S.A. with UV-C lamp (measured intensity at 15 cm, 3.2 W/m^2)]. The rates of irradiation doses received by the mushrooms under UV-A, UV-B, and UV-C were 0.21, 0.29, and 0.19 kJ/m^2/min and the calculated radiation doses after 1 h of irradiation of each side were 25.2, 35.3, and 23.0 kJ/m^2, respectively. All the irradiations in this experiment were performed under the optimized conditions of temperature (35 °C) and moisture (80%). The moisture content of fresh mushrooms was measured gravimetrically by drying samples in an air convection drier at 105 °C for at least 20 h.

2.5. Analysis of vitamin D_2

The analysis and quantification of vitamin D_2 were performed by the method described previously (Perera et al., 2003).

Freeze-dried mushroom sample powders (0.5 g) were accurately weighed into 250 ml round bottom flasks and mixed with 4 ml of sodium ascorbate solution (17.5 g of sodium ascorbate in 100 ml of 1 M NaOH), 50 ml of

ethanol (95% pure, Riverbank Chemicals, Singapore), and 10 ml of 50% potassium hydroxide (85% pure, Merck Chemicals, Darmstadt, Germany). The mixture was saponified under reflux at 80 °C for 1 h, then, it was immediately cooled to room temperature and transferred into a separating funnel. The mixture was first extracted with 15 ml de-ionized water, followed by 15 ml ethanol and then with a three-stage *n*-pentane extraction of volumes 50, 50 and 20 ml, respectively. The pooled organic layers were washed three times with 50 ml of 3% KOH in 5% ethanol and then finally with de-ionized water until neutralized. The organic layer was transferred into a round-bottom flask, rotary-evaporated to dryness at 40 °C, and immediately re-dissolved in 5 ml ethanol.

The samples were passed through a 0.45 μm Non-Pyrogenic filter (Schleicher & Schuell, Dassel, Germany). A volume of 20 μl of filtered sample was injected into a Waters 600E HPLC system equipped with Waters 486 tunable absorbance UV detector (Waters, Milford, MA, USA) and eluted through a reverse phase C18 column (Maxsil 5 C18, 250×4.6 mm, Phenomenex, Torrance, CA, USA) using acetonitrile/methanol (HPLC grade Merck Chemicals, Darmstadt, Germany) (75:25) as the mobile phase at a flow rate of 2.3 ml/min. The UV detection of the eluate was performed at 282 nm. Vitamin D_2 qualitatively analyzed by comparing the retention times of standards obtained, and quantification was done using a calibration curve.

2.6. Statistical analysis

The results were statistically analyzed by analysis of variance (ANOVA, Vassar stats statistical computations). The data were expressed as means ± SD (standard deviation). The test results were considered significant at $p < 0.01$. The statistical analyses were based on ANOVA and Tukey's HSD test.

3. Results and discussion

3.1. Effect of duration of irradiation on the conversion of ergosterol to vitamin D_2

Fig. 1 shows the effect of period of irradiation of each side of the mushrooms on the conversion of ergosterol to vitamin D_2. The conversion was almost completed within 1 h when each side of the mushrooms was subjected to irradiation. The graph shows that prolonged irradiation does not increase vitamin D_2. Previtamin D intermediates also absorb UV radiation, producing tachysterol and lumisterol by photoisomerization (Havinga, 1973; Havinga, Kock, & Rappoldt, 1960), and prolonged irradiation produces irreversible "over-irradiation products" by dimerization and ring cleavage (Braun, Fub, & Kompa, 1991). These may be the reasons for the slight reduction in vitamin D_2 content close to a 2-h period of irradiation. In addition, irradiation also contributes to an oxidative atmosphere (Vayalil,

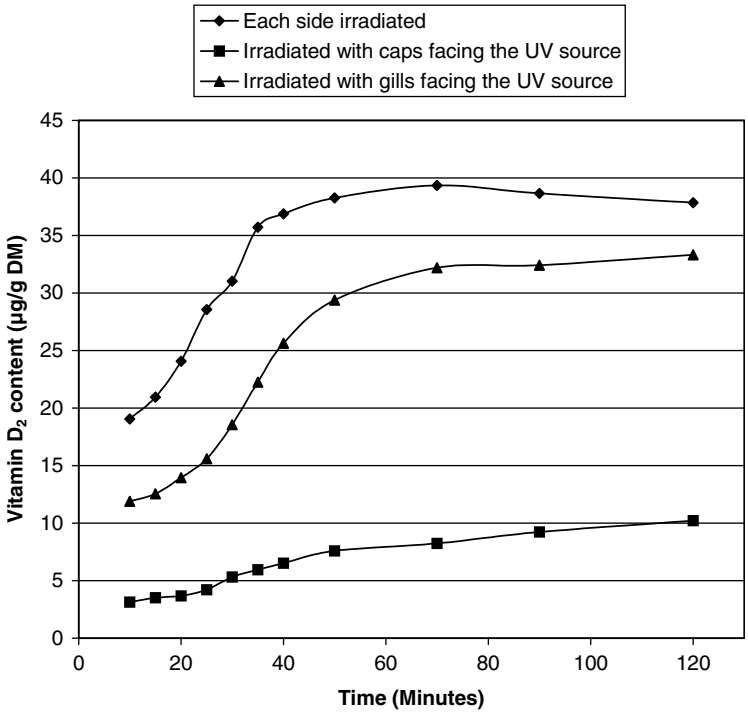

Fig. 1. The effect of time of UV-A irradiation of each side of the mushrooms on the conversion of ergosterol in Shiitake mushrooms to vitamin D_2.

Elmets, & Katiyar, 2003), and prolonged exposure of vitamin D to UV radiation may result in photo-degradation of vitamin D_2 (Webb, DeCosta, & Holick, 1989).

3.2. Investigation of duration of irradiation and the orientation of mushrooms on the conversion of ergosterol to vitamin D_2

Fig. 2 shows the effect of orientation of mushrooms and the duration of irradiation on this conversion. The yields of vitamin D_2, after the irradiation of each side of the mushrooms (cap and gills) for 1 and 2 h were shown to be significantly higher ($p < 0.01$) than the value observed from the mushrooms irradiated with their gills facing the UV source for 2 h. The vitamin D_2 yield obtained by irradiation of each side of the mushrooms for 2-h was 38.5 ± 3.18 μg/g DM, whereas it was 36.1 ± 2.32 μg/g DM when each side of the mushroom was irradiated for 1 h. However, the difference between the two values was not significant ($p = 0.154$). The conversion of ergosterol in mushrooms to vitamin D_2 is almost completed within an hour (Fig. 1), and this could be the reason why prolonged irradiation of each side, after 1 h, does not contribute much to this conversion.

3.3. Conversion of ergosterol to vitamin D_2 by different bands of UV (UV-A, UV-B, and UV-C)

In this experiment, the moisture content of mushrooms was adjusted to around 80% by removing the moisture in a vacuum dryer at ambient temperature. The irradiations were performed at 35 °C, since these are the optimum reaction conditions for this conversion (Jasinghe & Perera, 2004).

The yields of vitamin D_2 under UV-A, UV-B, and UV-C are significantly different. The calculated radiation doses of UV-A, UV-B, and UV-C after a 2-h period of irradiation (1 h each side) were 25.2, 35.3, and 23.0 kJ/m^2, respectively. Fig. 3 illustrates the effect of different bands of UV on the conversion of ergosterol to vitamin D_2. The results clearly indicate that the conversion of ergosterol to vitamin D_2 under UV-C was significantly higher ($p < 0.01$) than that under UV-A and the conversion under UV-B was significantly higher ($p < 0.01$) than those under UV-A or UV-C. The highest yields of vitamin D_2 were obtained under UV-B irradiation. However, under UV-B, mushrooms received 50% more irradiation dose than under UV-C. Therefore, the vitamin D_2 yields under UV-B and UV-C cannot be reconciled. Mau et al. (1998) have reported a value of 6.58 μg/g DM of vitamin D_2 from Shiitake mushrooms after a 2-h period of UV-B irradiation. However, the orientation of mushrooms to the UV source and the moisture content were not reported. The temperature of irradiation, reported in their study (12 °C) was much lower than that which we have maintained in the current study (35 °C). Temperature of irradiation plays an important role in this conversion and this may be one of the reasons why they obtained low conversion

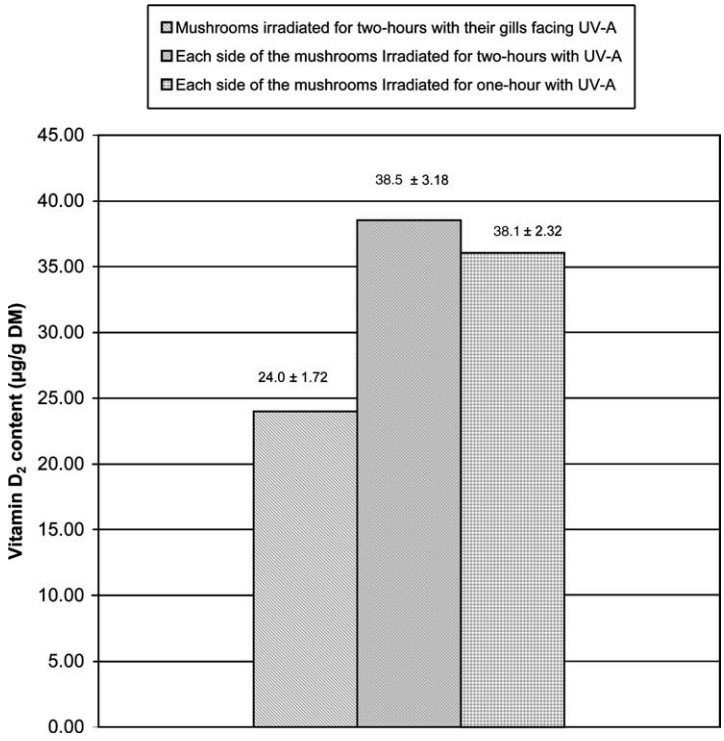

Fig. 2. Effect of orientation of mushrooms and the duration of irradiation on the conversion of ergosterol to vitamin D_2.

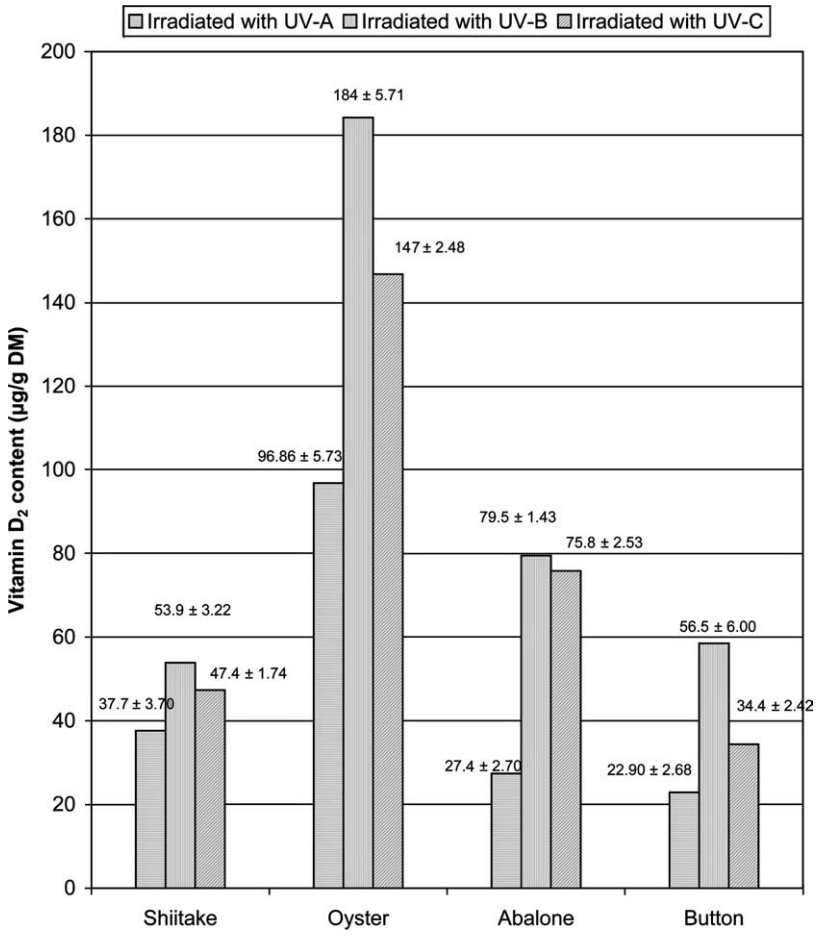

Fig. 3. The conversion of ergosterol to vitamin D_2 under UV-A, UV-B and UV-C.

rates. In addition, the irradiation dose used in their study (9.86 kJ/m^2) was much lower than the irradiation dose which we have used (35.3 kJ/m^2) and of course the orientation of the mushrooms to UV source is most important, as we have shown earlier (Jasinghe & Perera, 2004).

4. Conclusion

It can be concluded from the results that UV irradiation of each side of the mushrooms for 1 h is more than enough for this conversion. Furthermore, remarkably high amounts of vitamin D_2 could be obtained by UV irradiation of each side of the mushrooms under optimized conditions. In addition, intensity of the UV radiation and the dose of irradiation applied, also contributed to the conversion of ergosterol in mushrooms to vitamin D_2. Even under normal conditions, 5 g of fresh shiitake mushrooms irradiated for 15 min with UV-A, or UV-B is more than enough to obtain the recommended allowances of vitamin D for adults (10 µg/day).

Acknowledgement

This research was supported by an academic research grant from the National University of Singapore (R-143-000-138-112).

References

Billaudel, B., Barakat, L., & Faure-Dussert, A. (1998). Vitamin D_3 deficiency and alterations of glucose metabolism in rat endocrine pancreas. *Diabetes Metabolism, 24*, 344–350.

Bourlon, P. M., Billaudel, B., & Faure-Dussert, A. (1999). Influence of vitamin D_3 deficiency and 1,25 dihydroxyvitamin D_3 on de novo insulin biosynthesis in the islets of the rat endocrine pancreas. *Journal of Endocrinology, 160*, 87–95.

Braun, M. M., & Tucker, M. A. (1997). A role for photoproducts of vitamin D in the etiology of cutaneous melanoma? *Medical Hypotheses, 48*, 351–354.

Braun, M., Fub, W., & Kompa, K. L. (1991). Improved photosynthesis of previtamin D by wavelengths of 280–300 nm. *Journal of Photochemistry and Photobiology A: Chemistry, 61*, 15–26.

Cantorna, M. T. (2000). Vitamin D and autoimmunity: is vitamin D status an environmental factor affecting autoimmune disease prevalence? *Proceedings of The Society for Experimental Biology and Medicine, 223*, 230–233.

Grant, W. B. (2002). An ecologic study of dietary and solar ultraviolet-B links to breast carcinoma mortality rates. *Cancer, 94*, 272–281.

Hansen, C. M., Binderup, L., & Hamberg, K. J. (2001). Vitamin D and cancer: effects of 1,25(OH)2D3 and its analogues on growth control and tumorigenesis. *Frontiers in Bioscience, 6*, D820–D848.

Havinga, E. (1973). Vitamin D, example and challenge. *Experientia, 29*, 1181–1193.

Havinga, E., Kock, R. J. D., & Rappoldt, M. P. (1960). The photochemical interconversions of provitamin D, lumisterol, previtamin D and tachysterol. *Tetrahedron, 11*, 276–284.

Heldenberg, D., Gershon, T., & Weisman, Y. (1992). Effect of iron on serum 25-hydroxyvitamin D and 24, 25-dihydroxyvitamin D concentrations. *American Journal of Clinical Nutrition, 56*, 533–536.

Holick, M. F. (2001). Meeting the vitamin D needs of the elderly. *Nutrition and the M.D, 27*, 1–4.

Hypponen, E., Laara, E., & Reunanen, A. (2001). Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet, 358*(9292), 1500–1503.

Jasinghe, V. J., & Perera, C. O. (2005). Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D_2 by UV irradiation. *Food Chemistry, 92*(3), 541–546.

Majewski, S., Kutner, A., & Jablonska, S. (2000). Vitamin D analogues in cutaneous malignancies. *Current Pharmaceutical Design, 6*, 829–838.

Mancini, D., Aaronson, K., Foray, A., Addresso, V., Katz, S., & Elizabeth, S. (1996). Vitamin D deficiency is common in heart failure. *Journal of the American College of Cardiology, 27*, 338.

Mattila, P. H., Lampi, A. M., Ronkainen, R., Toivo, J., & Piironen, V. (2002). Sterol and vitamin D_2 contents in some wild and cultivated mushrooms. *Food Chemistry, 76*, 293–298.

Mau, J. L., Chen, P. R., & Yang, J. H. (1998). Ultraviolet irradiation increased vitamin D_2 content in edible mushrooms. *Journal of Agricultural and Food Chemistry, 46*, 5269–5272.

Mawer, E. B., Davies, M., Still, P. E., Jones, G., Knutson, J. C., & Bishop, C. W. (1995). 1, 24-Dihydroxyvitamin D2, a biologically active analog of vitamin D, is a naturally occurring metabolite in humans. *Bone, 17*, 321.

McAlindon, T. E., & Felson, D. T. (1996). Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. *Annals of Internal Medicine, 125*, 353–359.

Mehta, R. G., & Mehta, R. R. (2002). Vitamin D and cancer. *Journal of Nutritional Biochemistry, 13*, 252–264.

Mokady, E., Schwartz, B., & Shany, S. (2000). A protective role of dietary vitamin D_3 in rat colon carcinogenesis. *Nutrition and Cancer, 38*, 65–73.

Norman, P., Moss, I., Minder, S., Gosling, M., & Powell, J. (2002). Maternal and postnatal vitamin D ingestion influences rat aortic structure, function and elastin content. *Cardiovascular Research, 55*, 369–374.

Peleg, S. (1997). Molecular basis for differential action of vitamin D analogues. In D. Feldman, F. H. Glorieux, & J. W. Pike (Eds.), *Vitamin D* (pp. 1011–1025). New York, NY: Academic Press.

Perera, C. O., Jasinghe, V. J., Ng, F. L., & Mujumdar, A. S. (2003). The effect of moisture content on the conversion of ergosterol to vitamin D in shiitake mushrooms. *Drying Technology, 21*, 1093–1101.

Platz, E. A., Hankinson, S. E., & Hollis, B. W. (2000). Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and adenomatous polyps of the distal colorectum. *Cancer Epidemiology Biomarkers and Prevention, 9*, 1059–1065.

Polek, T. C., & Weigel, N. L. (2002). Vitamin D and prostate cancer. *Journal of Andrology, 23*, 9–17.

Segall, J. J. (1989). Latitude and ischaemic heart disease [letter]. *Lancet, 1*, 1146.

Speer, G., Cseh, K., & Winkler, G. (2001). Vitamin D and estrogen receptor gene polymorphisms in type 2 diabetes mellitus and in android type obesity. *European Journal of Endocrinology, 144*, 385–389.

Tuohimaa, P., Lyakhovich, A., & Aksenov, N. (2001). Vitamin D and prostate cancer. *Journal of Steroid Biochemistry and Molecular Biology, 76*, 125–134.

Vayalil, P. K., Elmets, C. A., & Katiyar, S. K. (2003). Treatment of green tea polyphenols in hydrophilic cream prevents UVB-induced oxidation of lipids and proteins, depletion of antioxidant enzymes and phosphorylation of MAPK proteins in SKH-1 hairless mouse skin.. *Carcinogenesis, 24*, 927–936.

Webb, A. R., DeCosta, B. R., & Holick, M. F. (1989). Sunlight regulates the cutaneous production of vitamin D_3 by causing its photodegradation. *Journal of Clinical Endocrinology and Metabolism, 68*, 882–887.

Williams, F. L., & Lloyd, O. L. (1989). Latitude and heart disease [letter]. *Lancet, 1*, 1072–1073.

Zittermann, A., Schleithoff, S. S., Tenderich, G., Berthold, H. K., Korfer, R., & Stehle, P. (2003). Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure?. *Journal of the American College of Cardiology, 41*, 105–112.