



Published in final edited form as:

*Arch Biochem Biophys.* 2007 April 15; 460(2): 213–217. doi:10.1016/j.abb.2006.12.017.

## Factors that Influence the Cutaneous Synthesis and Dietary Sources of Vitamin D

Tai.C. Chen<sup>1</sup>, Farhad Chimeh<sup>1,3</sup>, Zhiren Lu<sup>1</sup>, Jeffrey Mathieu<sup>1</sup>, Kelly S Person<sup>1</sup>, Anqi Zhang<sup>1</sup>, Nathan Kohn<sup>1</sup>, Steven Martinello<sup>2</sup>, Roger Berkowitz<sup>2</sup>, and Michael F. Holick<sup>1</sup>

<sup>1</sup> Vitamin D, Skin and Bone Research Laboratory, Endocrine Section, Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA

<sup>2</sup> Legal Sea Foods, Boston, MA, USA

### Abstract

The major sources of vitamin D for most humans are casual exposure of the skin to solar ultraviolet B (UVB;290–315 nm) radiation and from dietary intake. The cutaneous synthesis of vitamin D is a function of skin pigmentation and of the solar zenith angle which depends on latitude, season, and time of day. In order to mimic the natural environment of skin to sunlight exposure, we therefore measured serum 25-hydroxyvitamin D levels in volunteers with different skin types following repeated UV irradiation. Because melanin pigment in human skin competes for and absorbs the UVB photons responsible for the photolysis of 7-dehydrocholesterol to previtamin D<sub>3</sub>, we also studied the effect of skin pigmentation on previtamin D<sub>3</sub> production in a human skin by exposing type II and type V skin samples to noon sunlight in June when the solar zenith angle is most acute. Vitamin D is rare in food. Among the vitamin D-rich food, oily fish are considered to be one of the best sources. Therefore, we analyzed the vitamin D content in several commonly consumed oily and non-oily fish. The data showed that farmed salmon had a mean content of vitamin D that was ~25% of the mean content found in wild caught salmon from Alaska, and that vitamin D<sub>2</sub> was found in farmed salmon, but not in wild caught salmon. The results provide useful global guidelines for obtaining sufficient vitamin D<sub>3</sub> by cutaneous synthesis and from dietary intake to prevent vitamin D deficiency and its health consequences.ensuing illness, especially, bone fractures in the elderly.

### Keywords

Vitamin D; Nutrition; Sunlight; Photobiology; Skin Pigmentation

### Introduction

Vitamin D plays an essential role for the growth and maintenance of a healthy skeleton by increasing calcium absorption (1). Vitamin D deficiency leads to rickets in children, and osteomalacia and osteoporosis in adults. Vitamin D-deficiency is a potential health problem for the aged (2), especially at the end of winter (3), and causes increasing risk of hip fracture (4–10). Vitamin D supplementation with calcium has been shown to prevent hip and non-vertebral fractures in elderly women and men (10–13). The principle causes of vitamin D

<sup>3</sup>Current Address: Nephrology Division, Department of Medicine, Harvard Medical School, Boston MA 02215, USA

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

deficiency in the elderly in the US and Canada is due to a decrease or complete abstinence of consumption of vitamin D containing foods, supplements and lack of exposure to sunlight (1,2,12).

The major source of vitamin D for most humans is casual exposure of the skin to sunlight. During exposure the UVB (290 to 315 nm) portion of sunlight photolyzes 7-dehydrocholesterol (7-DHC) in the epidermis to previtamin D<sub>3</sub> (14). Once formed, previtamin D<sub>3</sub> undergoes thermal isomerization to form vitamin D<sub>3</sub>. The amount of solar UVB radiation reaching the biosphere is a function of wavelength and the amount of ozone the solar radiation travels through the atmosphere, which is a function of the solar zenith angle and depends on latitude, season, and time of day (15,16,17). In addition, the effectiveness of cutaneous synthesis of vitamin D<sub>3</sub> is also determined by the skin pigmentation, because melanin efficiently absorbs UVB radiation (18).

Because of the concern about skin cancer many may have avoided direct sun exposure and they would depend on dietary sources for their vitamin D requirement. Most diets are low in vitamin D. Among the vitamin D-rich foods, oily fish, such as salmon, are considered to be one of the best sources. Few foods are fortified with vitamin D. In the US and Canada milk is fortified with vitamin D while in Europe margarine is fortified in some countries. However, a study from our laboratory demonstrating that 49% of 173 milk samples collected in the United States and British Columbia, Canada, contained less than 80% of the vitamin D content on the label and 14% did not contain any detectable vitamin D raises a serious question about the role of milk in providing the consumers with their vitamin D requirement (19).

In this report we determined serum 25-hydroxyvitamin D [25(OH)D] concentrations at baseline compared with the last visit 3 months later in adults with different skin types following repeated exposures to UV irradiation. We also evaluated the cutaneous synthesis of previtamin D<sub>3</sub> in human type II and type IV skin after exposure to sunlight in June in Boston, Massachusetts. In addition, we measured several species of commonly consumed oily and non-oily fish for their vitamin D content, and evaluated the effect of various cooking methods on the vitamin D content of fish.

## Methods and Materials

### Human Study

Healthy adults with different skin types (II, III, IV and V) were recruited in the beginning of winter to participate in our study. They were asked to lie in a tanning bed (source of UV irradiation) to expose whole body to a light similar to sunlight. Based on the manufacturer recommendation each volunteer received a total of 0.75 of MED (minimal erythema dose) in each session. To achieve that goal, the skin type II, III, IV and V received an average of 6, 8, 11 and 12 minutes of UV irradiation during the first session respectively. The sessions were held three times a week for twelve weeks for a total of 36 sessions. The blood was drawn at the baseline visit (before exposure to UVB radiation), during and at the conclusion of the study. The samples were analyzed for 25(OH)D as previously described (20). We compared the levels at the baseline versus the end of the study and calculated the percentage increase in 25(OH)D levels for different skin types.

### Human Skin Model

To study the conversion of endogenous 7-DHC to previtamin D<sub>3</sub> in human skin samples, Black skin (type V, 57 yr) and Caucasian skin (type II, 60 yr) samples (1 cm<sup>2</sup>) in triplicate were placed on gauze moistened with saline in quartz petri dishes and exposed to noon sunlight on a cloudless day on June 20<sup>th</sup>. After the exposure the epidermis and stratum basal were separated

from the dermis (14). The combined epidermis and stratum basal fraction was then extracted and prepared for HPLC analysis as described (14). The detection limit for determining previtamin D<sub>3</sub> formation on this HPLC system was 0.3 % of the epidermal 7-DHC concentration.

### Determination of Vitamin D Content in Fish

Pieces of flesh from different parts of fish were obtained from Legal Sea Foods. Vitamin D analysis was performed as described previously with minor modification (21). Briefly, one gram of fish flesh was homogenized, followed by saponification and a lipid extraction. The lipid extracts were sequentially purified by C-18 reverse phase cartridge chromatography, normal phase HPLC and finally by reversed phase HPLC which can separate vitamin D<sub>2</sub> from vitamin D<sub>3</sub> for quantification (21).

### Liquid Chromatography-Tandem Mass Spectroscopy (LC-MS/MS) Analysis of Vitamin D<sub>2</sub> and Vitamin D<sub>3</sub> in Fish tissue

A separate and rapid method was also developed using LC-MS/MS which can detect 5pg/50mg of fish sample. A Cohesive Technologies Aria™ TX series HTLC™ system was used for the analysis. Samples were separated using 50 mm 2.1 mm Agilent C18 column with a 5 μm particle size. A binary gradient consisting of purified water (A) and methanol (B) at flow rate of 5 ml/min was used. Injection volume of 50 μL was used for all analyses. The gradient was 70% –98% of B within 7 min. Mass spectrometry was performed with TSQ quantum ultra equipped with APCI source using MRM (Thermo USA). Tandem mass spectrometer main working parameters were set as following: Discharge current 3.0 μA, vaporize temperature 293° C, Sheath gas 23 Arb. capillary temperature 200° C, collision energy 1.2 m Torr. MRM was used for the multiple products ion of vitamin D<sub>3</sub> (385.3 >91.1, 105.0 and 159.2) and vitamin D<sub>2</sub> (397.0 >105.1, 130.9 and 147.1)

### Serum 25(OH)D Measurement

Serum 25(OH)D was measured by competitive protein binding without prior chromatography as described previously (20). Duplicate samples that had been frozen only one time were measured. The intra- and inter-assay CVs are 5.0–10% and 10%–15%, respectively. The reference range is 30–100 ng/ml.

### Statistical Analysis

The results are represented as means ± SEM. The data were analyzed by using Microsoft Excel (Office 2000; Microsoft Corp, Redmond, WA) and ANALYSE IT (version 1.71; Analyse-it Software Ltd, Leeds, United Kingdom) software. Differences in concentrations of serum 25 (OH)D were evaluated by using a two-tailed unpaired Student's *t* test.

## RESULTS

To investigate the effect of skin pigmentation on previtamin D synthesis in humans, we exposed type II and type V skin along with ampoules containing 7-DHC solution to noon sunlight in June on a cloudless day in Boston, Massachusetts. We found that in June 0.67 ± 0.11% of 7-DHC in epidermis was converted to previtamin D<sub>3</sub> in type II skin, but no detectable amount was found in type V skin samples after 5 minutes of sunlight exposure (Fig. 1). A small amount (0.18 ± 0.06%) of epidermal 7-DHC was converted to previtamin D<sub>3</sub> in Type V skin after 10 min of sun exposure. Whereas in the same time period, 0.95% of 7-DHC was converted to previtamin D<sub>3</sub> in type II skin. The synthesis of previtamin D<sub>3</sub> in epidermis continued to increase to 2.01 ± 0.18% and 2.78 ± 0.09% after 20 min and 30 min sunlight exposure, respectively, in type II skin. Unlike type II skin, the conversion of epidermal 7-DHC to previtamin D<sub>3</sub> in type

V skin samples only increased modestly to  $0.25 \pm 0.04$  and  $0.29 \pm 0.05\%$  after 20 and 30 minutes of exposure, respectively.

The effect of skin pigmentation on the cutaneous synthesis of previtamin D<sub>3</sub> was further investigated in volunteers with different skin types by measuring their serum 25(OH)D levels at the beginning and after 12 weeks of irradiation with UVB. Figure 2 demonstrates that UVB irradiation greatly increased the serum 25(OH)D in all skin types. The percentage increase in the 25(OH)D level at the end of the study for skin type II, III, IV and V were  $310\% \pm 107$ ,  $287\% \pm 157$ ,  $225\% \pm 96$  and  $140\%$  respectively.

Due to concern about skin cancer the public have been advised to avoid direct sun exposure in the US (22). Those people, therefore, are at high risk for vitamin D deficiency. Most diets are low in vitamin D and intake from the few vitamin D-rich or enriched foods typically occurs only intermittently. Among the vitamin D-rich food, oily fish are considered to be one of the best sources. Therefore, we analyzed several species of oily and non-oily fish for their vitamin D content. We also evaluated what the effect of various cooking methods have on the vitamin D content of fish. The analysis of 24 farmed salmon and 20 wild caught salmon samples reveals that the farmed salmon had about ~25% of the vitamin D content in their flesh compared to the wild caught salmon (Table 1). Farmed trout, blue fish, swordfish and Mahi had about 1/2 of the vitamin D content compared to the wild caught salmon. Cod, grey sole, haddock, squid and clams had less than 10% vitamin D content compared to the wild caught salmon (Table 1). Microwave or bake did not decrease the vitamin D content significantly (Table 2). However, when the salmon was fried in vegetable oil, only about half of vitamin D was recovered. Some farmed salmon samples were found to contain vitamin D<sub>2</sub> in addition to vitamin D<sub>3</sub> (Figure 3).

## DISCUSSION

Vitamin D<sub>3</sub> is unique among numerous vitamins and hormones in that it is derived from a precursor, previtamin D<sub>3</sub>, which is synthesized in the skin in response to solar irradiation (14). The photoproduction of previtamin D<sub>3</sub> is dependent on the concentration of 7-DHC in epidermis and melanin pigmentation (23,24). There is an inverse relation between the concentrations of provitamin D<sub>3</sub> in the epidermis with age (23). The percentage conversion of cutaneous 7-DHC is also influenced by the solar zenith angle, which is inversely related to the amount of UVB photons in the solar spectrum (15,25). An increase in the zenith angle either by the daily rotation of the Earth or by an increase in the distance north or south from the equator shifts the spectral distribution of sunlight toward longer wavelengths because of a greater subtraction of shorter wavelengths of UVB by atmospheric absorption and scattering (14,25).

We have previously reported that melanin pigmentation in skin, which acts as a sunscreen efficiently absorbs solar UVB radiation, can affect the cutaneous production of previtamin D<sub>3</sub> (24). When Blacks and Caucasians were exposed to the same amount of simulated sunlight, little vitamin D<sub>3</sub> was detected in the circulation from Blacks compared to the Caucasian positive controls (18). Using the Black and Caucasian skin samples irradiated under simulated sunlight, we reported the threshold at which the Black or the Caucasian skin began to synthesize detectable amounts of previtamin D<sub>3</sub> (15). In agreement with the *in vivo* finding, the threshold for the type V Black skin (1.8%) is higher than the type III Caucasian skin (0.8%). For people with less melanin pigmentation, such as type II skin, their threshold is expected to be less than 0.8%. Likewise, a person with skin type V would have threshold higher than 1.8%, and the one with the type IV skin would have a threshold between 0.8 and 1.8%.

Our current data demonstrate that the conversion of epidermal 7-DHC to previtamin D<sub>3</sub> in Type II skin is approximately 5–10 fold more efficient than the highly pigmented Type V skin (Figure 1), suggesting that sufficient previtamin D<sub>3</sub> can be synthesized in the Caucasian skin after a brief exposure to summer noon sunlight even in Boston, 42 °N. Since the sunlight spectrum (UVB radiation) responsible for the synthesis of previtamin D<sub>3</sub> also increases risk of skin cancers (1,22), dietary supplementation of vitamin D is advisable in order to prevent vitamin D-deficiency, especially elderly and highly pigmented skin individuals who will not be able to synthesize sufficient vitamin D<sub>3</sub>, even in the summer months.

It is believed that oily fish such as salmon, mackerel and bluefish are excellent sources of vitamin D<sub>3</sub>. However, our analysis of the vitamin D content in a variety of fish species indicates that farmed salmon, the most widely consumed fish in the US, contained about one quarter of the vitamin D<sub>3</sub> found in wild caught salmon from Alaska. Some farmed salmon even had vitamin D<sub>2</sub>, as verified by LC-MS/MS. There needs to be a reevaluation of the vitamin D content in all fish and other foods that have been traditionally recommended as good sources of naturally occurring vitamin D.

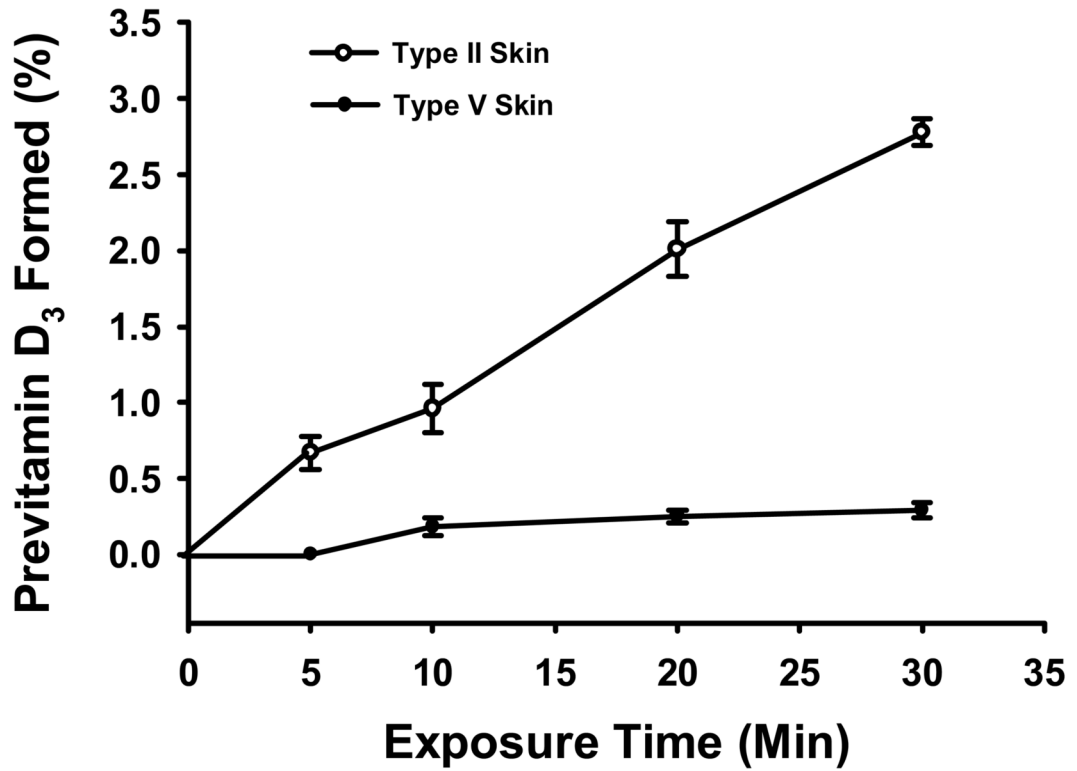
## Acknowledgments

This work was supported in part by grants (RO1AR-36963-05, R37AG6079-07 and MO1RR00053) from the National Institutes of Health, and a grant from the UV Foundation.

## References

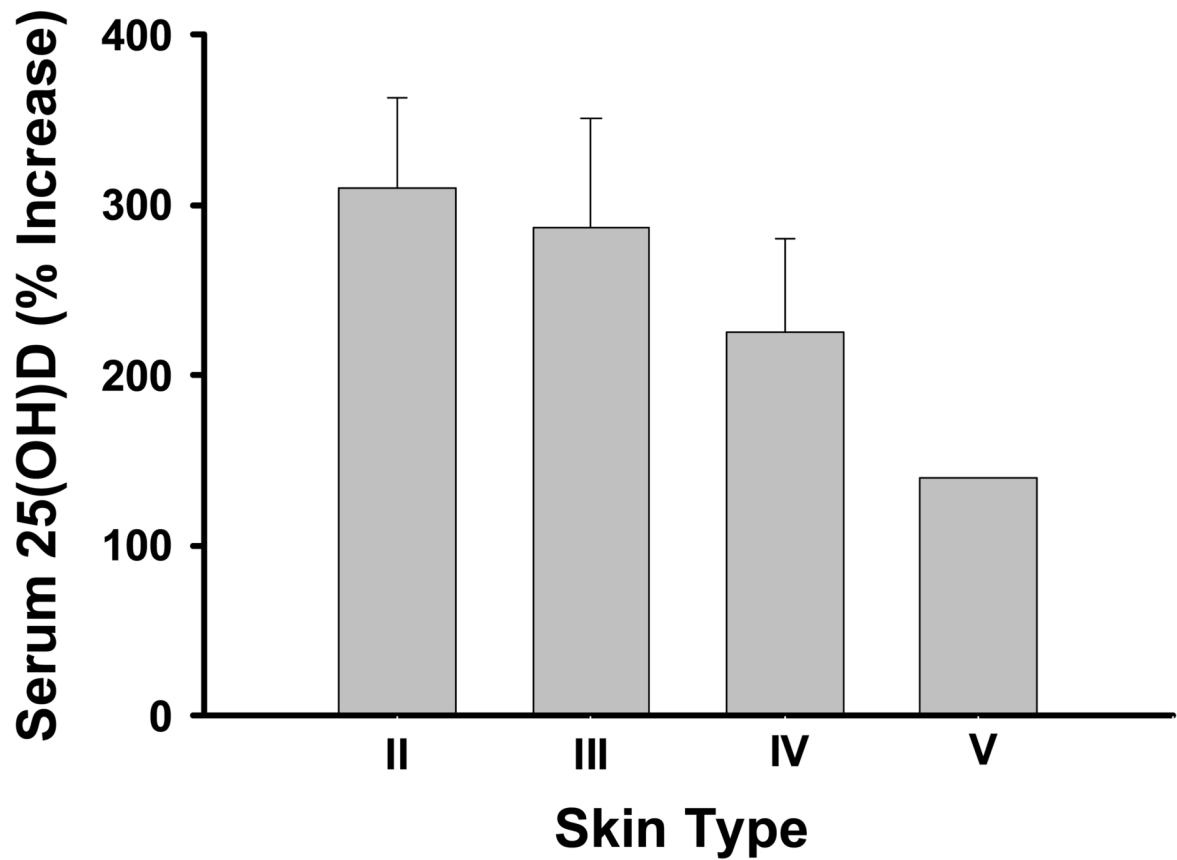
1. Holick MF. Resurrection of vitamin D deficiency and rickets. *J Clin Invest* 2006;116:2062–2072. [PubMed: 16886050]
2. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* 2006;81:353–373. [PubMed: 16529140]
3. Webb AR, Pilbeam C, Hanafin N, Holick MF. An evaluation of the relative contributions of exposure to sunlight and of diet to the circulating concentrations of 25-hydroxyvitamin D in an elderly nursing home population in Boston. *Am J Clin Nutr* 1990;51:1075–81. [PubMed: 2349922]
4. Chalmers J, Conacher DH, Gardner DL, Scott PJ. Osteomalacia—a common disease in elderly women. *J Bone Joint Surg (Br)* 1967;49B:403–23. [PubMed: 6037558]
5. Aaron JE, Gallagher JC, Anderson L, Stasiak EB. Frequency of osteomalacia and osteoporosis in fractures of the proximal femur. *Lancet* 1974;1:229–31. [PubMed: 4130245]
6. Hordon LD, Peacock M. Vitamin D metabolism in women with femoral neck fracture. *Bone and Min* 1987;2:413–26.
7. Sokoloff L. Occult osteomalacia in American patients with fracture of the Hip. *Am J Surg Pathol* 1978;2:21–30. [PubMed: 637186]
8. Doppelt SH, Neer RM, Daly M, Bourret L, Schiller A, Holick MF. Vitamin D deficiency and osteomalacia in patients with hip fractures. *Ortho Trans* 1983;7:512–3.
9. Whitelaw GP, Abramowitz AJ, Kavookjian H, Holick MF. Fractures and vitamin D deficiency in the elderly. *Complications Ortho* 1991;6:70–80.
10. Dawson-Hughes B, Dallal GE, Krall EA, Harris S, Sokoll LJ, Falconer G. Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. *Ann Intern Med* 1991;115:505–512. [PubMed: 1883119]
11. Chapuy MC, Arlot ME, Duboeuf F, et al. Vitamin D<sub>3</sub> and calcium to prevent hip fractures in elderly women. *New Engl J Med* 1992;327:1637–1642. [PubMed: 1331788]
12. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D<sub>3</sub> (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *BMJ* 2003;326:469–475. [PubMed: 12609940]
13. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. *Am J Clin Nutr* 2006;84:18–28. [PubMed: 16825677]

14. Holick MF, MacLaughlin JA, Clark MB, et al. Photosynthesis of previtamin D<sub>3</sub> in human skin and the physiological consequences. *Science* 1980;210:203–205. [PubMed: 6251551]
15. Chen, TC. Photobiology of Vitamin D in “Vitamin D”. Holick, MF., editor. Humana Press; 1998. p. 17-37.
16. Bener, P. Spectral intensity of natural UVR and its dependence on various parameters. In: Urbach, F., editor. *The Biologic Effects of Ultraviolet Radiation*. Oxford and New York: Pergamon Press; 1969. p. 351-8.
17. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D<sub>3</sub>: Exposure to winter sunlight in Boston and Edmonton will not promote vitamin D<sub>3</sub> synthesis in human skin. *J Clin Endocrinol Metab* 1988;67:373–8. [PubMed: 2839537]
18. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of the skin to synthesize vitamin D. *Lancet* 1982;1:74–76. [PubMed: 6119494]
19. Chen TC, Shao Q, Heath H, Holick MF. An update on the vitamin D content of fortified milk from the United States and Canada. *N Engl J Med* 1993;329:1507. [PubMed: 8413473]
20. Chen TC, Turner AK, Holick MF. Methods for the determination of the circulating concentration of 25-hydroxyvitamin D. *J Nutr Biochem* 1990;1:315–319. [PubMed: 15539221]
21. Chen TC, Turner AK, Holick MF. A method for the determination of the circulating concentration of vitamin D. *J Nutr Biochem* 1990;1:272–276. [PubMed: 15539215]
22. Wolpowitz D, Gilchrist BA. The vitamin D questions: How much do you need and how should you get it? *J Am Acad Dermatol*. 2006;10.1016/j.jaad.2005.11.1057
23. MacLaughlin JA, Holick MF. Aging decreases the capacity of skin to produce vitamin D<sub>3</sub>. *J Clin Invest* 1985;76:1536–8. [PubMed: 2997282]
24. Holick MF, MacLaughlin JA, Doppelt SH. Regulation of cutaneous previtamin D<sub>3</sub> photosynthesis in man: Skin pigment is not an essential regulator. *Science* 1981;211:590–93. [PubMed: 6256855]
25. Webb AR. Who, what, where and when-influences on cutaneous vitamin D synthesis. *Progress in Biophy Mol Biol* 2006:92.



**Figure 1.** The conversion of epidermal 7-dehydrocholesterol to previtamin D<sub>3</sub> in Type II and Type V skin after exposing to noon sunlight in June at Boston (42 °N), Massachusetts. The data represent the means  $\pm$  SEM of duplicate determinations.

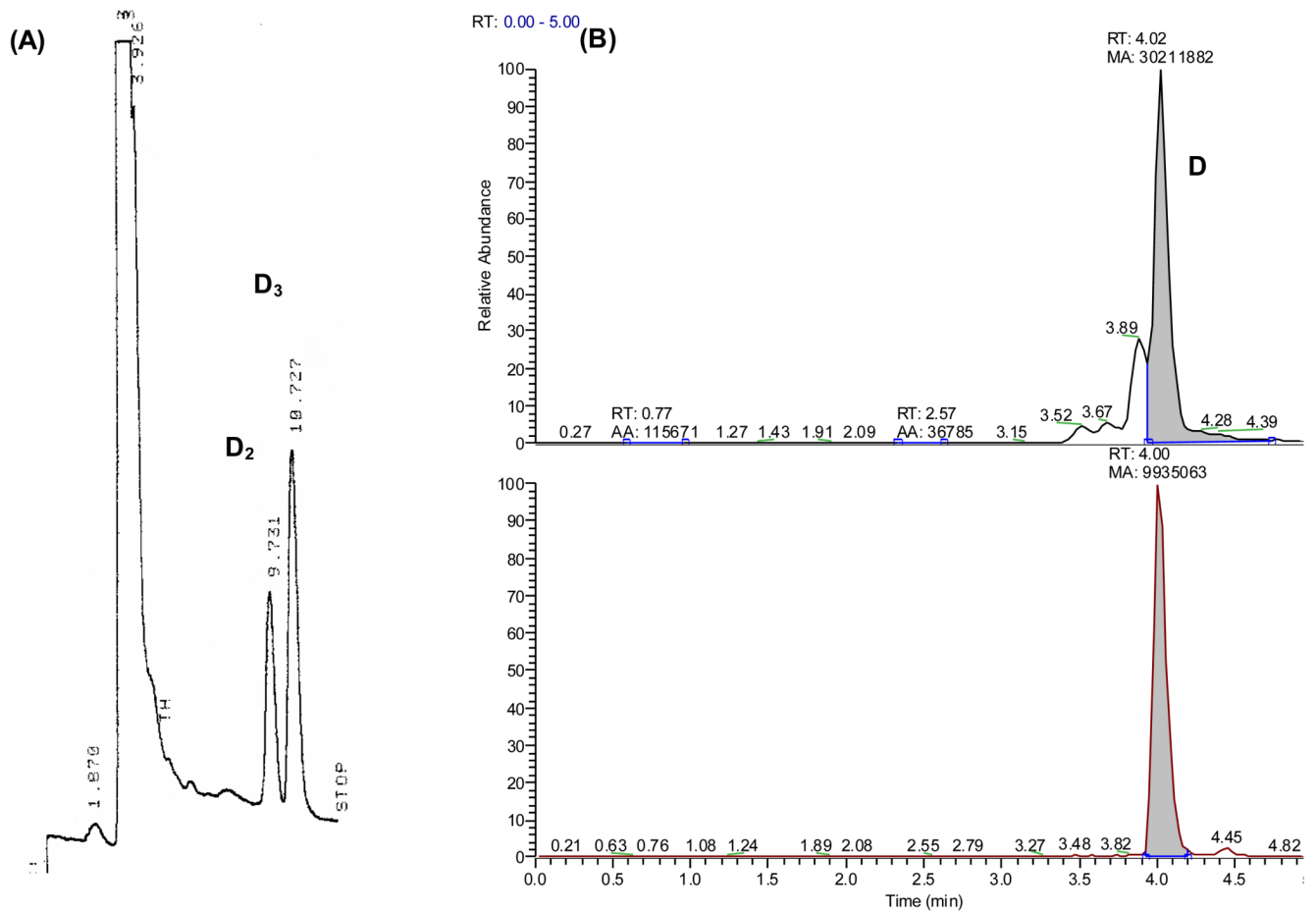




**Figure 2.**

The serum 25-hydroxyvitamin D levels in volunteers with different skin types after weekly exposure to simulated sunlight for 12 weeks. The data represent the means  $\pm$  SEM of duplicate determinations.





**Figure 3.** High Performance Liquid Chromatograph (A) and LC-MS/MS (B) chromatograms of farmed salmon lipid extracts. Tissue extraction and chromatographic conditions were described in the Methods Section.

**Table 1**

Vitamin D Content in Fish and other Sea Foods

	<b>IU/3.5 oz (N)</b>		<b>IU/3.5 oz (N)</b>
<b>Farmed Salmon</b>	249 ± 40 (24)	<b>Cod</b>	80 ± 14 (9)
<b>Wild Salmon</b>	981 ± 89 (20)	<b>Gray Sole</b>	45 ± 9 (13)
<b>Bluefish</b>	415 ± 112 (12)	<b>Haddock</b>	78 ± 22 (13)
<b>Mahi</b>	342 ± 96 (13)	<b>Clam</b>	59 ± 21 (4)
<b>Farmed Trout</b>	371 ± 63 (12)	<b>Mussels</b>	33 ± 4 (3)
<b>Swordfish</b>	447 ± 126 (12)	<b>Squid</b>	8 ± 0 (2)
<b>Tuna Ahi-YT</b>	164 ± 42 (9)	<b>Whiting</b>	48 (1)

**Table 2**

Effect of Cooking on Vitamin D Content in Fish

	<b>IU/3.5 oz (N)</b>
<b>Farmed Salmon, Raw</b>	274 ± 16 (6)
<b>Farmed Salmon, Microwaved</b>	272 ± 1 (2)
<b>Farmed Salmon, Baked</b>	248 ± 3 (2)
<b>Farmed Salmon, Fried</b>	142 ± 21 (2)
<b>Boston Mackerel, Raw</b>	10 ± 2 (2)
<b>Boston Mackerel, Microwaved</b>	8 ± 1 (2)