Relationship between skin color, sun exposure, UV protection, fish intake and serum levels of vitamin D in Japanese older adults

Maria Marina Serrao Cabral and Angelo Jose Goncalves Bos
Institute of Geriatrics and Gerontology,
Pontificia Universidade Catolica do Rio Grande do Sul, Porto Alegre, Brazil, and
Hidemori Amano, Satoshi Seino and Shoji Shinkai
Department of Social Participation and Health,
Tokyo-to Kenko Choju Iryo Center, Tokyo, Japan

Received 5 September 2016
Revised 28 November 2016
30 December 2016
Accepted 2 January 2017

Abstract

Purpose – This paper aims to observe the possible relationship between skin color, sun exposure level, UV protection and food intake and serum levels of 25(OH) D in Japanese older adults.

Design/methodology/approach – Elderly (n = 131; 65-93 years old), followed by the Tokyo Metropolitan Institute of Gerontology in the Kusatsu (36°N) received a self-applicable questionnaire about the quantity and quality of the daily sun exposure and behavior to avoid the sun. A color analyzer measured each red (R), green (G) and blue (B) component of skin color, and dietary vitamin D was estimated by food frequency questionnaire. Serum 25(OH) D levels were collected and categorized as sufficiency (>30 ng/mL), insufficiency (between 20 and 30 ng/mL) and deficiency (<20 ng/mL).

Findings – High proportion of participants had insufficiency (53 per cent) and deficiency (25 per cent) levels of 25(OH) D. Insufficiency levels were more prevalent in women (57 per cent, p = 0.048) and in participants that use gloves (49 per cent, p = 0.054) and sunscreen on face (76 per cent, p = 0.003) as a sun protection way. Participants with sufficiency levels of 25(OH) D presented lower values of R (p = 0.067), G (p = 0. 007) and B (p = 0.001) of skin color (what is meaning darker skin) and a higher fish intake (12 times per week).

Research limitations/implications – The study is a cross-sectional design and brings a potential for measurement error in the recorded subjective variables. There is a memory bias in self-reported sun exposure and food consumption; however, in the multivariate analysis, it was demonstrated a significant association. Second, although the authors have sought to evaluate a number of variables that could affect the skin's ability to synthesize vitamin D, there are many other factors that may affect this ability that could not be accounted for. Another limitation was the assessment of self-reported ultraviolet exposure data rather than direct measurement of exposure.

Practical implications – It was also concluded that darker skin color (a surrogate of longer-term sun exposure) participants had a lower prevalence of vitamin D insufficiency in this ethnic homogeneous population. When accessing patients' skin color, the clinician must account for his or her ethnicity.

Social implications – Governments should regulate supplementation or food fortification with vitamin D, with special focus in countries with geographical location of insufficient solar radiation for skin synthesis of this vitamin. With this, it becomes a priority that a safe sun exposure ensures the sufficient serum levels of 25 (OH) D without the use of supplements.

This study was sponsored by the TMIG.
Conflict of interest: This group has not cleared any potential conflicts.

Nutrition & Food Science
Vol. 47 No. 3, 2017
pp. 409-422

0034-6659
DOI 10.1108/NFS-09-2016-0136

Originality/value – This report was the first to analyze skin color components associated to vitamin D levels, finding that blue and green colors were significant. The clinical implication of this find is yet to understand. It was also concluded that darker skin color (a surrogate of longer-term sun exposure) participants had a lower prevalence of vitamin D insufficiency in this ethnic homogeneous population. When accessing patients' skin color, the clinician must account for his or her ethnicity.

Keywords Consumption, Solar energy, Vitamins, Fish (food), Age groups

Paper type Research paper

1. Introduction

Vitamin D is extremely important in maintaining normal bone metabolism in preventing falls and fractures (Holick and Chen, 2008) and exerts influence on muscle mass and strength (Girgis *et al.*, 2013). Its insufficiency and deficiency has been linked to cancer, cardiovascular disease (Bikle, 2014; Bouillon *et al.*, 2013), diabetes mellitus, metabolic syndrome (Bikle, 2014; Holick and Chen, 2008), obesity (Bikle, 2014), immune and cognitive function and dementia (Brouwer-Brolsma *et al.*, 2013). In addition, a low level of vitamin D is a risk factor for osteoporosis (Nakamura, 2006).

The worldwide prevalence of low vitamin D levels is about 1 billion people in all age groups (Holick and Chen, 2008). In Japanese population, the prevalence of vitamin D deficiency and insufficiency were 53.6 and 37.4 per cent, respectively (Nakamura *et al.*, 2015). Among the risk group for vitamin D deficiency, older adults (aged 65+ years) are included (Lips *et al.*, 2014). Recent Japanese epidemiologic researches on 25(OH) D have been conducted (Nakamura *et al.*, 2015; Yoshimura *et al.*, 2013), but only one had targeted older adults (Suzuki *et al.*, 2008). Thus, epidemiologic studies that select this specific population are scarce; however, the higher prevalence of osteoporosis and its related bone fracture are associated to this age group (Orimo *et al.*, 2012).

According to MacLaughlin and Holick, in older age, vitamin D insufficiency may be caused by physiological factors such as a decline in efficiency of vitamin D synthesis due to decreasing concentration its precursor, 7-dehydrocholesterol, transformed photochemically into cholecalciferol in the skin (MacLaughlin and Holick, 1985).

Japan is a country surrounded by sea and has a predominance of forests and mountains, with regions of different latitudes receiving different amounts of sunlight. The town of Kusatsu (36°N, 138°E) is located about 200 kilometers northwest of the capital Tokyo. In geographic regions closer to the equator (below 37°C), higher vitamin D3 synthesis occurs on the human skin throughout the year (Holick, 2004). Holick mentioned that skin synthesize of vitamin D is sufficiently produced in 5-15 min exposure to sunlight for between 10 a.m. and 3 p.m. during the spring, summer and autumn for most individuals (Holick, 2004).

Currently, there is a discussion in the literature on vitamin D, once multiple variables can interfere in the concentrations of this vitamin, such as sun exposure (Farrar *et al.*, 2013), diet, age, gender (Nakamura *et al.*, 2015; Yoshimura *et al.*, 2013), housing characteristics, sunscreen use (Nakamura *et al.*, 2015), among others. In addition, the traditional Japanese diet contains a large quantity of fish, (Nakamura *et al.*, 2002) including tuna cuttlefish, mackerel, saury and salmons (Sasaki *et al.*, 2003). On the other hand, Japanese, particularly women, are very concerned about the damage produced by the sun exposure, frequently using hats and long gloves (Kotodama, 2015). Those two factors make Japanese older adults an interesting population for a vitamin D study, once vitamin D is synthesized by the skin through sun exposure (Farrar *et al.*, 2013; Holick and Chen, 2008) and, one of the major vitamin D source foods are the fatty fishes as salmon (Nimitphong and Holick, 2013).

In this research, we observed the possible relationship between skin color, sun exposure level, UV protection and food intake and serum levels of 25(OH) D in Japanese older adults living in a small mountain area, the town of Kusatsu. Findings from the current investigation may be useful to health care, contributing to a proper orientation on sun exposure and dietary sources to obtain an adequate amount of vitamin D without supplementation.

2. Methods

2.1 Participants

This is a cross-sectional, quantitative, descriptive and analytical study. The sample consisted by 131 older adults (aged 65 years and older), living in the town of Kusatsu (located at 36°N, 138°E, with an average temperature around 30°C during summer). The current research invited older adults to participate during their regular assessment in the Kusatsu health check-up in July 2015 (summer). During the invitation, participants received a food intake and the sun-exposure questionnaires.

Older adults who have had some type of surgery in the gastrointestinal tract (duodenal or gastrostomy) were excluded because this may interfere on vitamin D intestinal absorption. Further, we excluded participants with skin allergy and skin burns scars evident in both hands and forearms. These factors may interfere in the cutaneous vitamin D synthesis and analysis of skin color and skin aging.

The present research was approved by Tokyo Metropolitan Institute of Gerontology (TMIG) ethics committee under the protocol number 27/1308, and written informed consent was obtained from all participants before enrolment.

2.2 Measurements

At enrollment, participants were asked to complete a lifestyle questionnaire detailing age, gender and supplemental vitamin D.

2.2.1 Body composition. A multifrequency impedance plethysmograph body composition analyzer (InBody 720® Biospace, Korea) was used to evaluate weight (Kg), height (cm), body mass index (BMI) in kg/m^2, fat free mass (Kg), soft lean mass (Kg), body fat mass (Kg) and body fat (per cent). InBody 720® takes readings from the body using an eight-point tactile electrode method, measuring resistance at five frequencies (1, 50, 250, 500 and 1 MHz) and reactance at three frequencies (5, 50 and 250 kHz). This body composition analyzer has previously been demonstrated to have high reliability and accuracy (Gibson *et al.*, 2008). During measures, participants were asked to wear normal clothes and advised to stand shoeless in erect position with their feet on the feet electrodes on the machine platform and their arms abducted with hands gripping on to the hands electrodes.

2.2.2 Vitamin D. Blood samples were collected in July 2015, during the regular Kusatsu health check-up. These samples were measured by SRL Inc. ® (Tokyo, Japan), by radioimmunoassay (RIA) using DiaSorin's kit which is certified for Vitamin D standardization program (Holmes *et al.*, 2013). This assay consists on an antibody with specificity to 25(OH) D. Sample, antibody and tracer are incubated for 90 min at 20-25°C. The separation phase is accomplished after 20 min of incubation at 20-25°C with a second antibody precipitating complex (DiaSorin, 2007). A buffer is added after this incubation prior to centrifugation to aid in reducing non-specific bindings (DiaSorin, 2007). The coefficient of variation (per cent CV), used as a quality measure of clinical tests, ensures a total imprecision of 8.2 up to 11.0 per cent and a sensitivity at or below 1.5 ng/mL (Kennedy *et al.*, 1999).

Serum 25(OH) D levels were classified as sufficiency (>30 ng/mL), insufficiency (between 20 and 30 ng/mL) and deficiency (<20 ng/mL). Those values were based on the Endocrine Society Clinical Practice Guideline (Holick *et al.*, 2011) and Institute of Medicine (Ross *et al.*, 2011).

2.2.3 Sun exposure. Participants were asked to fill a self-applicable questionnaire about the amount and quality of the sun exposure. To be highly accurate, the questionnaire was retained by the participants and completed day by day, filled with the amount of minutes exposed to the sun daily, during 30 days. The questionnaire also captured the use and the type of sun protection either by cosmetic products or by mechanical protection means (hat and gloves).

Our questionnaire was based on a recall that assessed daily time on sun and skin exposure for 1 week that predicted summer serum 25(OH) D concentrations, accounting for 38 per cent of the variability in 25(OH) D levels among healthy adults (Hanwell *et al.*, 2010).

2.2.4 Vitamin D intake. A food frequency questionnaire gathered the main food sources of this vitamin. The questions enquired about food and beverage items with standard portions/units (small, medium and large) and eating frequency (never, once-3 times/months, once-twice/week, 3-4 times/week, 5-6 times/week, once/day, once-twice/day, 4-6 times/day, 7+ times/day). Food frequency questionnaire has presented a correlation coefficient between 62 and 77 per cent for dietary vitamin D intake in a Japanese study (Ishihara *et al.*, 2006).

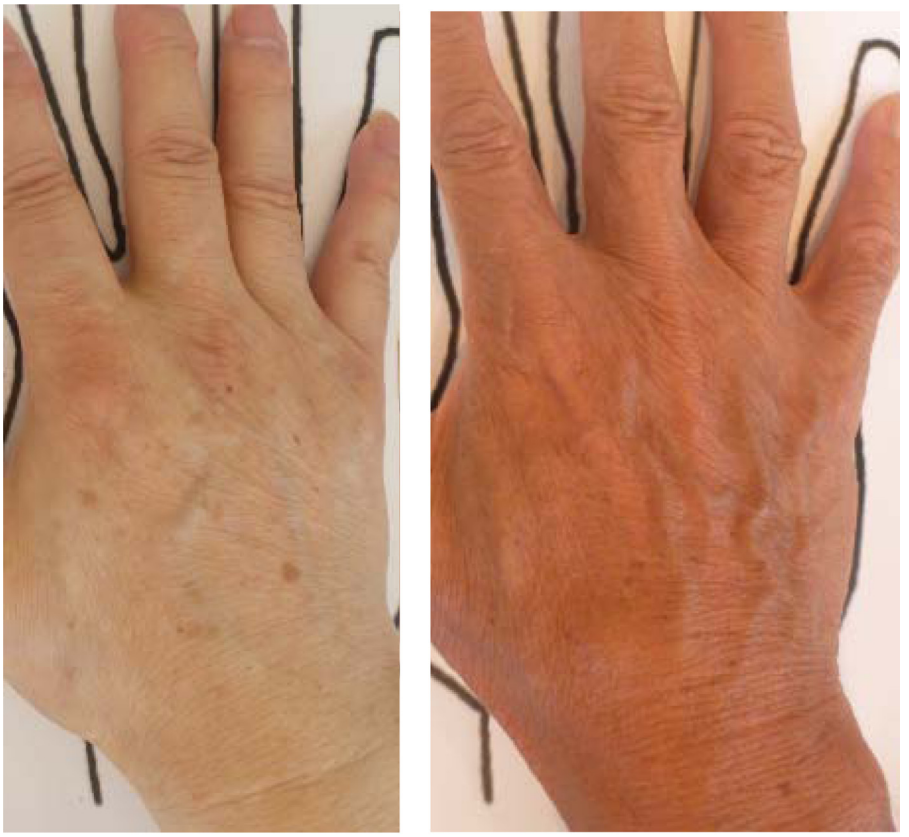
2.2.5 Skin color. Different methods have been used to differentiate individuals' skin colors. Suzuki and colleagues observed a homogeneous distribution on Asian individuals, where most participants (88.9 per cent) ranged between type III and IV on Fitzpatrick's classification (Suzuki *et al.*, 2011). Thus, we decided to use a least subjective measurement of skin color, the digital color analyzer (ACR-1023, Instrutherm®, São Paulo, Brazil). The equipment measures the pigments in an inner and hairless portion (below elbow) of the right arm. It is battery-portable color analyzer equipment that uses a spectral analysis method to determine the skin color. The instrument measures each value of red (R), green (G) and blue (B) ranging from zero to 1,023, where the minimum value (zero) represents the complete absence of color, and the maximum value (1,023) represents their presence complete. In this system, the total white has a value of R = 1,023, G = 1,023 and B = 1,023, and the total black has a value of R = 0, G = 0 and B = 0. The RGB skin values of each person were measured in triplicate and the average was recorded. The equipment was calibrated before each use by measuring a white plate.

2.3 Data analysis

Participants were classified according to their serum 25 (OH) D levels as sufficiency (>30 ng/mL), insufficiency (between 20 and 30 ng/mL) and deficiency (<20 ng/mL). Frequency distribution of the participants on 25(OH) D levels for different age group, sex, gloves or hat use, and use of sunscreen on face and hand were tested by chi-square. Differences on mean values of body composition (weight, height, BMI, fat free mass, soft lean mass, body fat mass and body fat), skin color (amount of green, red and blue), minutes per day of sun exposure (lower and higher UV), weekly intake of foods rich in vitamin D and daily estimated intake of vitamin D from food source were compared by analysis of variance. Finally, to test the possible correlation of serum levels of 25(OH) D and predictor parameters, we performed a linear regression analysis. An initial full model was tested with all possible confounders. The least significant variables were removed from the model enabling a final model if all significant variables to predict serum levels of 25(OH) D. The analysis was performed using Epi Info version 7.0. Significance levels than 5 per cent ($p < 0.05$) are considered significant.

3. Results

A total of 131 older-adult have participated in both steps of the study. The age of participants ranged from 65 to 93 years (mean 74.1 ± 6.44 years) and, most of them (61.1 per cent) were women. The mean level of serum 25(OH) D in the total participants was 24.6 (±6.93) ng/mL. All the blood samples were collected in July 2015 (summer). The overall prevalence of vitamin D insufficiency and deficiency was 52.7 and 25.2 per cent, respectively. The cut-offs for 25(OH) D levels were based on the Endocrine Society Clinical Practice Guideline (Holick *et al.*, 2011) and Institute of Medicine's (IOM) (Ross *et al.*, 2011), being the participants classified according to the 25(OH) D level as sufficiency (>30 ng/mL), insufficiency (between 20 and 30 ng/mL) and deficiency (<20 ng/mL). The mean minutes per day of sun exposure was 28.7 ± 19.15 on lower UV radiation time and 34.2 (±18.62) on higher UV radiation time. Skin color ranged from R 490, B 384 and G 437 (the most colored person) to R 206, B 116 and G 145 (the least colored person) (Plate 1).



(a) **(b)**

Notes: The smaller mean value of R, G and B meaning a darker skin and the bigger indicating a lighter skin. (a) R 490, B 384 and G 437 (the most color person); (b) R 206, B 116 and G 145 (the least color person)

Plate 1.
The smaller and bigger mean value of red (R), green (G) and blue (B) components of skin color from color analyzer (ACR-1023®)

Table I shows the distribution of 25(OH) D sufficiency, insufficiency and deficiency levels according to age groups, gender, sun protection habits and body composition. The age group was not significantly associated to serum 25(OH) D levels. Prevalence of insufficiency and deficiency was higher in women ($p = 0.048$). Among the women, 57 per cent presented insufficiency and 27.5 per cent deficiency levels. The use of gloves reached a trend of significance on its association to 25(OH) D levels. Participants who often wore sunscreen on their faces were also significantly associated to levels of 25(OH) D ($p = 0.003$). About body composition, despite no significant association, sufficiency levels of 25(OH) D were observed in participants with higher weight, height, BMI, fat free mass, soft lean mass and body fat mass.

Table II presents the mean and standard deviation value of skin color, sun exposure and vitamin D high content food intake related to 25 (OH) D levels. Sufficiency of 25(OH) D levels have presented significantly smaller mean value of R, G and B from spectrums, meaning a darker skin. Participants with deficiency serum 25(OH) D levels presented shorter exposure to higher UV radiation. Egg, salmon and mushrooms were the most frequent food items that have been intaken. Although not significant, participants classified with sufficiency levels of 25(OH) D have had a more frequent intake of vitamin D rich foods.

The daily amount of vitamin D intake according to 25(OH) D levels is demonstrated in Table III. Vitamin D food total intake ranged from 50.7 to 2133 UI (mean 401.4 ± 273.25 UI).

Variables	Sufficiency (%)	Insufficiency (%)	Deficiency (%)	Total (%)	*p*-value
	29 (22.1)	69 (52.7)	33 (25.2)	131	
Age group					
<70 years	10 (27.8)	17 (47.2)	9 (25.0)	36 (27.5)	0.731
70-79 years	13 (18.3)	42 (59.2)	16 (22.5)	71 (54.2)	
80+ years	6 (25.0)	10 (41.7)	8 (33.3)	24 (18.3)	
Sex					
Female	12 (15.0)	46 (57)	22 (27.5)	80 (61.1)	0.048
Male	17 (33.3)	23 (45.1)	11 (21.6)	51 (38.9)	
Sun protection habits					
Gloves use	6 (13.9)	21 (48.8)	16 (37.2)	43 (32.8)	0.055
Hat use	15 (19.2)	41 (52.6)	22 (28.2)	78 (59.5)	0.488
Sunscreen on arm	2 (12.5)	8 (50.0)	6 (37.5)	16 (12.2)	0.389
Sunscreen on face	3 (8.1)	28 (75.7)	6 (16.2)	37 (28.2)	0.003*
Body composition					
Weight (Kg)	58.8 ± 12.11	55.0 ± 10.71	54.6 ± 12.05	55.8 ± 11.4	0.270
Height (cm)	157.2 ± 9.73	153.8 ± 8.09	153.3 ± 8.13	154.4 ± 8.55	0.144
BMI (Kg/m^2)	23.6 ± 3.23	23.1 ± 2.98	23.1 ± 3.42	23.2 ± 3.13	0.732
Fat free mass (Kg)	41.2 ± 8.58	38.4 ± 7.43	38.1 ± 7.87	38.9 ± 7.84	0.214
Soft lean mass (Kg)	38.9 ± 8.16	36.2 ± 7.07	35.9 ± 7.47	36.7 ± 7.46	0.216
Body fat mass (Kg)	17.6 ± 6.02	16.8 ± 5.59	16.2 ± 6.60	16.8 ± 5.92	0.661
Body fat (%)	29.5 ± 7.68	30.0 ± 6.57	29.1 ± 9.29	29.7 ± 7.53	0.831

Notes: % distribution were tested by Chi-square and mean values by ANOVA; *tested by Fischer Exact Test; sufficiency 25(OH) D levels: >30 ng/mL; insufficiency 25(OH) D levels: between 20 and 30 ng/mL; deficiency 25(OH) D levels: <20 ng/mL; *p*-value, significance levels than 5% ($p < 0.05$) are considered significant; BMI = body index mass; BMI = Weight/height^2; 1 ng/ml = 0.40 nmol/l; Kg = Kilograms = 2.2 pounds (lb)

Table I.
Distribution of participants related to age group, sex, sun protection habits and body composition in relation to 25 (OH) D levels

Variables	Sufficiency	Insufficiency	Deficiency	*p*-value
Skin color				
Green	277.5 ± 77.6	300.5 ± 55.85	312.3 ± 46.40	0.067
Red	341.6 ± 58.8	370.3 ± 46.31	380.1 ± 47.17	0.007
Blue	224.3 ± 49.34	251.2 ± 42.34	266.5 ± 45.29	0.001
Sun exposure level (min/day)				
Lower UV radiation	27.98 ± 16,98	29.02 ± 20,59	28.82 ± 18,29	0.970
Higher UV radiation	36.50 ± 18.26	35.95 ± 19.40	32.37 ± 17.44	0.604
Food intake (tpw)				
Egg	5.07 ± 2.15	4.92 ± 2.79	5.29 ± 2.30	0.873
Egg yolk	4.79 ± 2.56	4.58 ± 3.03	5.25 ± 2.24	0.522
Mushroom	2.27 ± 4.53	1.66 ± 2.19	2.15 ± 2.69	0.568
Salmon	2.22 ± 2.03	2.17 ± 2.01	1.75 ± 1.51	0.523
Sardine	1.09 ± 3.89	0.43 ± 0.57	0.55 ± 0.72	0.294
Saury	0.93 ± 1.26	0.92 ± 1.26	0.59 ± 0.60	0.357
Tuna	0.77 ± 1.77	0.70 ± 1.13	0.78 ± 0.94	0.941
Swordfish	0.31 ± 0.54	0.34 ± 0.55	0.30 ± 0.53	0.924
Flatfish cutlass	0.21 ± 0.30	0.20 ± 0.28	0.11 ± 0.18	0.257
Oyster	0.12 ± 0.21	0.27 ± 0.86	0.15 ± 0.29	0.511
Eel	0.09 ± 0.13	0.08 ± 0.17	0.04 ± 0.11	0.403
Grunt	0.01 ± 0.04	0.04 ± 0.12	0.01 ± 0.08	0.392
All fishes	12.3 ± 11.32	11.0 ± 7.08	9.6 ± 5.67	0.428

Notes: Sufficiency 25 (OH) D levels: >30 ng/mL; insufficiency 25 (OH) D levels: between 20 and 30 ng/mL; deficiency 25 (OH) D levels: <20 ng/mL; *p*-value, significance levels than 5% ($p < 0.05$) are considered significant; min/day = minutes per day; Lower UV radiation = sun exposure before 10 a.m. and after 4 p.m.; Higher UV radiation = sun exposure between 10 a.m. and after 4 p.m.; tpw = times per week; all fishes = salmon + sardine + saury + tuna + swordfish + flatfish + grunt

Table II.
Mean and standard deviation of the skin color spectrum, sun exposure and vitamin D higher resource food intake frequency in relation to 25 (OH) D levels

Variables	Sufficiency	Insufficiency	Deficiency	*p*-value
Total vitamin D intake (UI per day)	435.9 ± 389.72	405.1 ± 253.08	363.2 ± 178.02	0.574
Food intake (UI per day)				
Egg	37.7 ± 15.99	36.5 ± 20.77	38.5 ± 17.14	0.874
Egg yolk	25.3 ± 13.58	24.2 ± 16.05	27.8 ± 11.87	0.522
Mushroom	29.2 ± 58.34	21.4 ± 28.21	27.8 ± 34.60	0.568
Salmon	198.3 ± 181.76	193.2 ± 180.05	156.0 ± 135.10	0.523
Sardine	51.9 ± 184.84	20.8 ± 27.08	26.2 ± 34.41	0.294
Saury	13.3 ± 18.08	13.3 ± 18.12	8.6 ± 8.69	0.357
Tuna	25.9 ± 59.80	23.7 ± 38.11	26.6 ± 31.82	0.941
Swordfish	21.3 ± 37.40	23.6 ± 38.23	20.8 ± 36.55	0.924
Flatfish cutlass	0.03 ± 0.0438	0.02 ± 0.0412	0.01 ± 0.0269	0.257
Oyster	5.5 ± 9.95	12.4 ± 39.52	7.3 ± 13.64	0.511
Eel	2.5 ± 3.94	2.5 ± 4.87	1.3 ± 3.31	0.403
Grunt	0.7 ± 3.97	3.1 ± 10.57	1.2 ± 7.46	0.392
All fishes	24.4 ± 18.31	22.6 ± 19.2	17.5 ± 16.59	0.292

Notes: Sufficiency 25 (OH) D levels: >30 ng/mL; insufficiency 25 (OH) D levels: between 20 and 30 ng/mL; deficiency 25 (OH) D levels: <20 ng/mL; *p*-value, significance levels than 5% ($p < 0.05$) are considered significant and levels between 5 and 10% are considered indicative of significance; UI = international unit = 0.025 μg; all fishes = salmon + sardine + saury+ tuna + swordfish + flatfish +grunt

Table III.
Mean and standard deviation of vitamin D contents intake by food sources in relation to 25 (OH) D levels

Significant independent variables in the linear regression univariate models were included in a complete model (Table IV). Least significant independent models were excluded from the linear regression model. Remained in the final model as positive predictor fish intake and as negative predictors glove use and the value of Blue from spectrums (Table V). Sex remained significant in the models, while soft lean mass and fat free mass were present. After removing those two anthropometric parameters, sex became not significant and was removed.

Variables	Univariate models Coefficient	*p*-value	R^2
Sex (female reference)			
Male	3.995	0.001	0.08
Age group (<70 reference)			
70-75	−0.953	0.551	0.02
75-79	−2.487	0.146	
80+	−1.736	0.345	
Sun exposition			
Higher UV radiation	0.006	0.190	0.01
Lower UV radiation	0.001	0.779	0.00
Sun protection habits			
Cloves use (yes vs no)	−3.529	0.005	0.06
Hat use (yes vs no)	−0.864	0.486	0.00
Sun protection on arm (yes vs no)	−1.658	0.372	0.01
Sun protection on face (yes vs no)	−1.185	0.380	0.01
Skin color			
Value of blue from spectrums	−0.050	<0.001	0.12
Value of green from spectrums	−0.028	0.005	0.06
Value of red from spectrums	−0.036	<0.001	0.10
Body composition			
Body fat mass	0.093	0.371	0.01
Fat free mass	0.202	0.008	0.05
Percent body fat	−0.036	0.658	0.00
Soft lean mass	0.213	0.008	0.05
Vitamin D intake			
Egg yolk (Vitamin D per day)	−0.012	0.774	0.00
Egg (Vitamin D per day)	0.002	0.939	0.00
Eel (Vitamin D per day)	0.107	0.449	0.00
Fish (Vitamin D per day)	0.057	0.086	0.02
Grunt (Vitamin D per day)	−0.102	0.141	0.02
Mushroom (Vitamin D per day)	−0.010	0.518	0.00
Oister (Vitamin D per day)	−0.005	0.792	0.00
Salmon (Vitamin D per day)	0.005	0.139	0.02
Sardine (Vitamin D per day)	0.003	0.674	0.00
Saury (Vitamin D per day)	0.025	0.505	0.00
Swordfish (Vitamin D per day)	0.002	0.895	0.00
Tuna (Vitamin D per day)	−0.004	0.762	0.00

Table IV.
Results of linear regression univariate models predicting serum levels of 25OH D

Notes: *Linear regression univariate model; lower UV radiation= sun exposure before 10 a.m. and after 4 p.m.; higher UV radiation = sun exposure between 10 a.m. and after 4 p.m.

Variables	Complete model Coefficient	*p*-value	Final model Coefficient	*p*-value
Sex				
(Male/Female)	4.393	0.046		
Sun protection habits				
Cloves use (yes vs no)	−2.733	0.030	−3.578	0.002
Skin color				
Value of blue from spectrums	−0.133	0.004	−0.049	<0.001
Value of green from spectrums	0.019	0.406		
Value of red from spectrums	0.072	0.096		
Body composition				
Fat free mass	4.784	0.304		
Soft lean mass	−5.115	0.301		
Vitamin D intake				
Fish (Vitamin D per day)	0.062	0.043	0.067	0.027
R^2	0.24		0.20	

Notes: *Complete and final regression model; lower UV radiation = sun exposure before 10 a.m. and after 4 p.m.; higher UV radiation = sun exposure between 10 a.m. and after 4 p.m.

Table V. Complete and final linear regression models for predicting serum levels of 25OH D

4. Discussion

This study aimed to explore what influence on serum levels of vitamin D among Japanese older adults. Between these factors, gender, age, skin color, fish consumption, vitamin D food intake and manners of avoid the sun were important in terms of higher or lower 25(OH) D.

The prevalence of vitamin D insufficiency and deficiency was high as well as many studies that reported higher vitamin D insufficiency in Japanese population (Yoshimura *et al.*, 2013; Nakamura *et al.*, 2015; Okazaki *et al.*, 2011; Nanri *et al.*, 2011).

Elderly population is particularly at risk for low 25(OH) D levels (Lips *et al.*, 2014). With increasing age, solar exposure is usually limited because of changes in lifestyle factors, such as clothing and less outdoor activity and diet may also become with lower natural Vitamin D content (Hossein-Nezhad and Holick, 2013). For that reason, a higher proportion of 25(OH) D deficiency among elderly is expected. Even though age group was not statistically significantly associated to serum 25(OH) D levels, elderly people aged over 80 years showed most prevalence deficiency in vitamin D. Similar in another Japanese research, the prevalence of vitamin D sufficiency showed positive associations to older age also in summer season (Nakamura *et al.*, 2015). On the other hand, research conducted recently has reported age was not associated to serum 25(OH) D levels among older adults in London (Jolliffe *et al.*, 2016).

Gender is another factor that may exert influence on 25(OH) D levels. Women are reported to have lower 25(OH) D levels. These differences occur especially due to clothing and sun protection behavior in women (Nimitphong and Holick, 2013), and it makes cutaneous vitamin D synthesis less efficient. This sex difference has been observed in this study, in a Japanese elderly (Suzuki *et al.*, 2008) and adults (Nanri *et al.*, 2011) researches and in a middle aged and elderly population in China (Zhen *et al.*, 2015). In this work, we could demonstrate that differences in serum levels of 25 (OH)D were dependent of anthropometric factors.

The cutaneous vitamin D is synthetized from the activation of 7-dehydrocholesterol through exposure of the skin by ultraviolet B (UVB) radiation (Burgaz *et al.*, 2007). The sun

exposure during the summer months and spring supply about 80 per cent of the annual need for vitamin D (Macdonald *et al.*, 2011). Furthermore, the synthesis of this vitamin in geographic regions closer to the equator (below 37°) is more efficient (Holick, 2004), and in winter, at different latitudes of 33°, little or no Vitamin D_3 is synthesized on the skin (Holick, 2012). Although this research has been carried out in the summer and favorable geographic coordinates, we found low prevalence of sufficient level of 25 (OH) D (>30 ng/mL).

In addition to the external factors that control the amount of available UV radiation, the individual's personal factors also exert influence on the cutaneous synthesis of vitamin D. Among these are the seasonality of sun exposure, skin pigmentation, genetic factors, sunscreen use and outdoor activities (Holick, 2012). Although being from same ethnic group, the participants of this study have shown variation in skin pigmentation according to digital color analyzer. Those with lower values of R, G and B, and darker skin pigmentation showed sufficient serum levels of 25 (OH) D. This paper is the forerunner to associate the Blue spectrum of skin to this association. Unlike other populations previously referenced, lower serum 25(OH) D was associated to lighter skin or non-white ethnicity (Gill and Kalia, 2015; Jolliffe *et al.*, 2016). This is due to the fact that melanin hinders the absorption of solar radiation, reducing the production capacity of vitamin D on darker skinned individuals who need sunlight lasting longer than those with lighter skin (Holick, 2013).

Another factor that hinders the cutaneous synthesis of vitamin D is the sunscreen, which properly applied with a sun protection factor (SPF) 30 can reduce 95 to 99 per cent the skin's ability to produce vitamin D (Hossein-Nezhad and Holick, 2013). In our study, the use of gloves was independently associated with lower levels of 25 (OH) D. This finding is similar to those reported in another Japanese study where no sunscreen use was significant associated to higher levels of vitamin D (Nakamura *et al.*, 2015).

A useful gain in 25(OH) D was seen in people of Asian ethnicity living at latitudes distant from the equator, in a sun exposure three times per week between 30 and 50 min, with casual summer clothing at noontime (Farrar *et al.*, 2013). Individuals with higher exposure most often presented the amounts of sun exposure sufficient to maintain adequate serum levels of 25 (OH) D, around 36 min a day. Similar to this, we found another study that evaluated the feasibility of sunlight to supply vitamin D recommendation levels and found that white people are able to acquire the recommended levels of vitamin D in less than 30 min in summer (Gill and Kalia, 2015). Even though exposure to sunlight is one of the risk factors for development of skin cancer, especially in younger people and lighter skin colors (Bouillon *et al.*, 2013), recent researches showed increase in levels of 25 (OH) D with sunlight exposure (Osmancevic *et al.*, 2015). In addition, exposure to sunlight and the consumption of milk are shown as deficiency protectors of vitamin D. This suggests that the intake of food sources of vitamin D is also effective for improving concentrations of 25 (OH) D (Zhen *et al.*, 2015). However, vitamin D only from exogenous sources (D2 and D3) is insufficient to supply human needs (Hirani *et al.*, 2013).

Unfortunately, vitamin D foods fortification is not available in Japan (Nakamura *et al.*, 2002), so the major dietary sources of the vitamin D are fatty fishes and sundried mushrooms (Nimitphong and Holick, 2013). Japanese are among the largest fish consumers in the world (Organization of the United Nations Food and Agriculture, 1996). Japanese population has the habit of consuming different kinds of fish, among these, raw fish (Japan: Resources Council, Science and Technology Agency, 1993). Fish is the major source of vitamin D in the Japanese diet; salmon is most frequently consumed fish, followed by flat fish. Other frequently consumed fish included baby sardines and mackerel (Nakamura *et al.*, 2002).

In our study, we observed that older adults in Japan generally prefer fish to meat, and these results are similar to presented by Nakamura and colleagues. (Nakamura *et al.*, 2002).

Therefore, of the dietary factors, fish seems to be a major source of this vitamin. As shown in a Japanese population, higher salmon consumption was significantly associated to vitamin D sufficiency (Nakamura *et al.*, 2015). Moreover, consuming fatty fishes and sundried mushrooms might help maintain proper vitamin D status in the winter (Nimitphong and Holick, 2013). Nevertheless, we also found that egg and egg yolk are also important source of vitamin D, in the studied group.

In general, Japanese population Vitamin D deficiency was significantly characterized by poor daily vitamin D intake (Yoshimura *et al.*, 2013). Elderly people are required to have an 800 IU/day intake of vitamin D per day (Holick *et al.*, 2011). Nakamura *et al.* reported an overall average vitamin D intake of 284 IU/day in Japanese elderly (Nakamura *et al.*, 2002). Our study observed a much higher intake (436 IU/day), which still corresponds to 54.5 per cent of requirement.

In terms of body composition and 25(OH) D levels, a number of reports have shown an inverse association between serum 25(OH) D and BMI levels (Yoshimura *et al.*, 2013; Nakamura *et al.*, 2015; Mansuri *et al.*, 2016). It is assumed that people with lower BMI would be less likely to present deficient levels of vitamin D. It is also assumed that the body fat hides lipid-soluble vitamin D in those with higher BMI (Lagunova *et al.*, 2009). However, the results of the present study demonstrated that participants with sufficient 25(OH) D demonstrated bigger mean values of BMI, fat free mass and body fat mass. Those body components lost their significance after being adjusted by other factors.

Our study has as its main strengths the investigation of a wide range of environmental determinants and lifestyle and vitamin D status in Japanese elderly of a restricted geographic localization and including, for the first time in the literature an objective measurement of skin pigmentation using a colorimeter. However, its cross-sectional design brings considerable limitations. First, the potential for measurement error in the recorded subjective variables. There is a memory bias in self-reported sun exposure and food consumption; however, in the multivariate analysis, it was demonstrated a significant association. Second, although we have sought to evaluate a number of variables (skin type, amount and quality of exposed skin and use and type of sun protection) that could affect the skin's ability to synthesize vitamin D, there are many other factors that may affect this ability that could not be accounted for. Another limitation was the assessment of self-reported ultraviolet exposure data rather than direct measurement of exposure. Moreover, most of the questionnaires available in the literature are for short-term exposure to sunlight, so these factors would influence our results.

5. Conclusion

We perceived that sun protection, skin color and fatty fish consumption were significant independent factors associated to serum levels of 25 (OH) D. In other words, a lifestyle modification may represent a possibility to improve levels of 25 (OH) D. In addition to fatty fish intake, an increase of sun exposure is a simple and inexpensive way to prevent vitamin D deficiency and its health consequences. But it is required a sufficient scientific knowledge to create guidelines with appropriate recommendations regarding sun exposure, seeking the cutaneous synthesis of vitamin D, for older people. This report was the first to analyze a skin color components associated to vitamin D levels, finding that skin Blue spectrum was significant. The clinical implication of this find is yet to understand. We also concluded that darker skin color (a surrogate of longer-term sun exposure) participants had a lower prevalence of vitamin D insufficiency, in this ethnic homogeneous population. When accessing patients' skin color, the clinician must account for his or her ethnicity. In addition, governments should regulate supplementation or food fortification with vitamin D, with

special focus in countries with geographical location of insufficient solar radiation for skin synthesis of this vitamin. With this, it becomes a priority that a safe sun exposure ensures the sufficient serum levels of 25 (OH) D without the use of supplements.

References

Bikle, D. (2014), "Vitamin D: production, metabolism, and mechanisms of action", *Chemistry & Biology*, Vol. 21 No. 3, pp. 319-329.

Bouillon, R., Van Schoor, N.M., Gielen, E., Boonen, S., Mathieu, C., Vanderschueren, D. and Lips, P. (2013), "Optimal vitamin D status: a critical analysis on the basis of evidence-based medicine", *The Journal of Clinical and Endocrinology Metabolism*, Vol. 98, pp. E1283-E1304.

Burgaz, A., Akesson, A., Oster, A., Michaëlsson, K. and Wolk, A. (2007), "Associations of diet, supplement use, and ultraviolet B radiation exposure with vitamin D status in Swedish women during winter", *American Journal of Clinical Nutrition*, Vol. 86, pp. 1399-1404.

Brouwer-Brolsma, E.M., Bischoff-Ferrari, H.A., Bouillon, R., Feskens, E.J.M., Gallagher, C.J., Hypponen, E., Llewellyn, D.J., Stoecklin, E., Dierkes, J., Kies, A.K., Kok, F.J., Lamberg-Allardt, C., Moser, U., Pilz, S., Saris, W.H., Van Schoor, N.M., Weber, P., Witkamp, R., Zittermann, A. and de Groot, L.C. P.G.M. (2013), "Vitamin D: do we get enough? A discussion between vitamin D experts in order to make a step towards the harmonization of dietary reference intakes for vitamin D across Europe", *Osteoporosis International*, Vol. 24, pp. 1567-1577.

DiaSorin, (2007), *Manual for the Quantitative Determination of 25(OH) D and Other Hydroxylated Metabolites in Serum or Plasma*, 25-Hydroxivitamin D 125I RIA Kit, Minnesota.

Farrar, M.D., Webb, A.R., Kift, M.R., Durkin, M.T., Allan, D., Herbert, A., Berry, J.L. and Rhodes, L.E. (2013), "Efficacy of a dose range of simulated sunlight exposures in raising vitamin D status in south Asian adults: implications for targeted guidance on sun exposure", *American Journal of Clinical Nutrition*, Vol. 97, pp. 1210-1216.

Gibson, A.L., Holmes, J.C., Desautels, R.L., Edmonds, L.B. and Nuudi, L. (2008), "Ability of new octapolar bioimpedance spectroscopy analyzers to predict 4-component-model percentage body fat in Hispanic, black, and white adults", *American Journal of Clinical Nutrition*, Vol. 87, pp. 332-338.

Gill, P. and Kalia, S. (2015), "Assessment of the feasibility of using sunlight exposure to obtain the recommended level of vitamin D in Canada", *Canadian Medical Association Journal*, Vol. 3 No. 3, pp. E258-E263.

Girgis, C.M., Clifton-Bligh, R.J., Hamrick, M.W., Holick, M.F. and Gunton, J.E. (2013), "The roles of vitamin D in skeletal muscle: form, function, and metabolism", *Endocrinology Review*, Vol. 34, pp. 33-83.

Hanwell, H.E.C., Vieth, R., Cole, D.E.C., Scillitani, A., Modoni, S., Frusciante, V., Ritrovato, G., Chiodini, I., Minisola, S. and Carnevale, V. (2010), "Sun exposure questionnaire predicts circulating 25-hydroxyvitamin D concentrations in Caucasian hospital workers in southern Italy", *Journal of Steroid Biochemistry & Molecular Biology*, Vol. 121, pp. 334-337.

Hirani, V., Cumming, R.G., Blyth, F.M., Naganathan, V., Le Couteur, D.G., Handelsman, D.J., Waite, L.M. and Seibel, M.J. (2013), "Vitamin D status among older community dwelling men living in a sunny country and associations with lifestyle factors: the concord health and ageing in men project, Sydney, Australia", *Journal Nutrition Health and Aging*, Vol. 17 No. 7, pp. 587-593.

Holick, M.F. (2004), "Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease", *American Journal of Clinical Nutrition*, Vol. 80, pp. 1678S-16788S.

Holick, M.F. (2012), "Vitamin D: extraskeletal health", *Rheumatic Disease Clinics of North America*, Vol. 38, pp. 141-160.

Holick, M.F. (2013), "Vitamin D: a d-lightful solution for health", *Journal of Investigative Medicine*, Vol. 59 No. 6, pp. 872-880.

Holick, M.F. and Chen, T.C. (2008), "Vitamin D deficiency: a worldwide problem with health consequences", *American Journal of Clinical Nutrition*, Vol. 87, pp. 1080S-1086S.

Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H. and Weaver, C.M. (2011), "Evaluation, treatment, and prevention of vitamin d deficiency: an endocrine society clinical practice guideline", *Journal of Clinical Endocrinal and Metabolism*, Vol. 96, pp. 1911-1930.

Holmes, E.W., Garbincius, J. and McKenna, K.M. (2013), "Analytical variability among methods for the measurement of 25-hydroxyvitamin D", *American Journal of Clinical Nutrition*, Vol. 140, pp. 550-560.

Hossein-Nezhad, A. and Holick, M.F. (2013), "Vitamin D for health: a global perspective", *Mayo Clinic Proceedings*, Vol. 88, pp. 720-755.

Ishihara, J., Inoue, M., Kobayashi, M., Tanaka, S., Yamamoto, S., Iso, H. and Tsugane, S. (2006), "Impact of the revision of a nutrient database on the validity of a self-administered food frequency questionnaire (FFQ)", *Journal of Epidemiology*, Vol. 16 No. 3, pp. 107-116.

Japan: Resources Council, Science and Technology Agency (1993), *Standard Tables of Food Composition in Japan: Vitamin D*, Japan: Resources Council, Science and Technology Agency, p. 195.

Jolliffe, D.A., Hanifa, Y., Witt, K.D., Venton, T.R., Rowe, M., Timms, P.M., Hyppönen, E., Walton, R.T., Griffiths, C.J. and Martineau, A.R. (2016), "Environmental and genetic determinants of vitamin D status among older adults in London, UK", *The Journal of Steroid Biochemistry and Molecular Biology*, Vol. 164, pp. 30-35.

Kennedy, J.W., Carey, R.N., Coolen, R.B., Garber, C.C., Lee, H.T., Levine, J.B. and Osberg, I.M. (1999), *Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline: EP5-A*, NCCLS.

Kotodama, Y. (2015), "Sun protection the Japanese way", available at: wawaza.com/blogs/protect-skin-sun-damage-japanese-way (accessed 14 October 2016).

Lagunova, Z., Porojnicu, A.C., Lindberg, F., Hexeberg, S. and Moan, J. (2009), "The dependency of vitamin D status on body mass index, gender, age and season", *Anticancer Research*, Vol. 29, pp. 3713-3720.

Lips, P., Van Schoor, N.M. and de Jongh, R.T. (2014), "Diet, sun, and lifestyle as determinants of vitamin D status", *Annals of the New York Academy of Sciences*, Vol. 1317, pp. 92-98.

MacDonald, H.M., Mavroeidi, A., Fraser, W.D., Darling, A.L., Black, A.J. and Aucott, L. (2011), "Sunlight and dietary contributions to the seasonal vitamin D status of cohorts of healthy postmenopausal women living at northerly latitudes: a major cause for concern?", *Osteoporosis International*, Vol. 22, pp. 2461-2472.

MacLaughlin, J. and Holick, M.F. (1985), "Aging decreases the capacity of human skin to produce vitamin D3", *Journal Clinical Investigation*, Vol. 76, pp. 1536-1538.

Mansuri, S., Badawi, A., Kayaniyil, S., Cole, D.E., Harris, S.B., Mamakeesick, M., Wolever, T., Gittelsohn, J., Maguire, J.L., Connelly, P.W., Zinman, B. and Hanley, A.J. (2016), "Traditional foods and 25(OH) D concentrations in a subarctic first nations community", *International Journal Circumpolar Health*, Vol. 75.

Nakamura, K. (2006), "Vitamin D insufficiency in Japanese populations: from the viewpoint of the prevention of osteoporosis", *Journal of Bone and Mineral Metabolism*, Vol. 24, pp. 1-6.

Nakamura, K., Nashimoto, M., Okuda, Y., Ota, T. and Yamamoto, M. (2002), "Fish as a major source of vitamin D in the Japanese diet", *Nutrition*, Vol. 18 No. 5, pp. 415-416.

Nakamura, K., Kitamura, K., Takachi, R., Saito, T., Kobayashi, R., Oshiki, R., Watanabe, Y., Tsugane, S., Sasaki, A. and Yamazaki, O. (2015), "Impact of demographic, environmental, and lifestyle factors on vitamin D sufficiency in 9084 Japanese adults", *Bone*, Vol. 74, pp. 10-17.

Nanri, A., Foo, L.H., Nakamura, K., Hori, A., Poudel-Tandukar, K., Matsushi, Y. and Mizoue, T. (2011), "Serum25-hydroxyvitamin D concentrations and season-specific correlates in Japanese adults", *Journal of Epidemiology*, Vol. 21 No. 5, pp. 346-353.

Nimitphong, H. and Holick, M.F. (2013), "Vitamin D status and sun exposure in southeast Asia", *Dermato-Endocrinology*, Vol. 5, pp. 34-37.

Okazaki, R., Sugimoto, T., Kaji, H., Fujii, Y., Shiraki, M., Inoue, D., Endo, I., Okano, T., Hirota, T., Kurahashi, I. and Matsumoto, T. (2011), "Vitamin D insufficiency defined by serum 25-hydroxyvitamin D and parathyroid hormone before and after oral vitamin D_3 load in Japanese subjects", *Journal of Bone Mineral Metabolism*, Vol. 29, pp. 103-110.

Organization of the United Nations Food and Agriculture (1996), *FAO Yearbook: Fishery Statistics Commodities*, Organization of the United Nations Food and Agriculture, Rome.

Orimo, H., Nakamura, T., Hosoi, T., Iki, M., Uenishi, K., Endo, N., Ohta, H., Shiraki, M., Sugimoto, T., Suzuki, T., Soen, S., Nishizawa, Y., Hagino, H., Fukunaga, M. and Fujiwara, S. (2012), "Japanese 2011 guidelines for prevention and treatment of osteoporosis—executive summary", *Achieves of Osteoporosis*, Vol. 7, pp. 3-20.

Osmancevic, A., Gillstedt, M., Landin-Wilhelmsen, K., Olle Larkö, A.W., Holick, M.F. and Krogstad, A. (2015), "Size of the exposed body surface area, skin erythema and body mass index predict skin production of vitamin D", *Journal of Photochemistry and Photobiology B: Biology*, Vol. 149, pp. 224-229.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J. and Shapses, S.A. (2011), "The 2011 reporton dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what dietetic practitioners need to know", *The Journal of Clinical Endocrinology & Metabolism*, Vol. 96 No. 1, pp. 53-58.

Sasaki, S., Takahashi, T., Iitoi, Y., Iwase, Y., Kobayashi, M., Ishihara, J., Akabane, M. and Tsuganem, S. (2003), "Food and nutrient intakes assessed with dietary records for the validation study of a self-administered food frequency questionnaire in JPHC study cohort", *Journal of Epidemiology*, Vol. 13 No. 1, pp. S23-S50.

Suzuki, H.S., Hammerschmidt, M., Kakizaki, P. and Mukai, M.M. (2011), "Phototype comparison between Caucasian and Asian skin types", *Surgeon and Cosmetic Dermatology*, Vol. 3 No. 3, pp. 193-196.

Suzuki, T., Kwon, J., Kim, H., Shimada, H., Yoshida, Y., Iwasa, H. and Yoshida, H. (2008), "Low serum 25-hydroxyvitamin d levels associated with falls among Japanese community-dwelling elderly", *Journal of Bone And Mineral Research*, Vol. 23 No. 8, pp. 1309-1317.

Yoshimura, N., Muraki, S., Oka, M., Morita, M., Yamada, H., Tanaka, S., Kawaguchi, H. and Nakamura, K. (2013), "Profiles of vitamin D insufficiency and deficiency in Japanese men and women: association with biological, environmental and nutritional factors and coexisting disorders: the ROAD study", *Osteoporosis International*, Vol. 24, pp. 2775-2787.

Zhen, D., Liu, L., Guan, C., Zhao, N. and Tang, X. (2015), "High prevalence of vitamin D deficiency among middle-aged and elderly individuals in northwestern China: its relationship to osteoporosis and lifestyle factors", *Bone*, Vol. 71, pp. 1-6.

Corresponding author
Maria Marina Cabral can be contacted at: mmserraocabral@hotmail.com