Bachelor's Degree Final Project

STUDY OF PREVALENCE OF HYPOVITAMINOSIS D IN ADULT POPULATION IN TARRAGONA

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Abstract

The determination of vitamin D in population has increased dramatically in recent years, not only because of its essential role in bone metabolism but also due to the extra-skeletal effects it carries out. Hypovitaminosis D has a high prevalence worldwide, even in sunny countries such as Spain. The present study carried out in the area of Tarragona aims to argue whether solar radiation and Mediterranean diet are enough to obtain optimal concentrations of vitamin D.

The diagnosis of vitamin D concentration was made through a competitive immunoassay based on chemiluminescent detection; a clinical laboratory analyser automatically measures the concentration of 25(OH)vitamin D of each sample. 300 adult samples were analysed, 150 women and 150 males, establishing different age ranges: young (between 18 and 65 years old), middle-age (between 66 and 79 years old) and elders (over 80 years old). The values obtained from each patient were classified as insufficient (concentration of 25(OH)vitamin D in serum <20 ng/ml), deficient (concentration of 25(OH)vitamin D in serum greater than 20 ng/ml and less than 30 ng/ml) or sufficient (concentration of 25(OH)vitamin D in serum greater than 30 ng/ml). With the obtained results a dispersion graph and a box and whisker plot were created, the prevalence of hypovitaminosis is high in Tarragona, being slightly higher in women and becoming a risky factor the age. To face these results, population should increase exposure to solar radiation in order to ensure sufficient skin synthesis of vitamin D and adapt a proper diet including food which is rich in this vitamin. Vitamin D supplementation should also be implemented, under medical supervision, and food should be fortified with vitamin D to ensure optimal concentrations of it in adults.

Introduction

Over the last few years, interest in vitamin D has increased, not only because its essential role in bone metabolism, but also because of its extra-skeletal effects, making it a significant and important element.

Nearly 50% of the world's population suffer from vitamin D deficiency [(5)], that represents around 1 billion people of different ages and ethnic groups; these data have improved concern and put this deficiency in the spotlight of scientists, considering hypovitaminosis D a global pandemic.

Vitamin D is a fat-soluble vitamin which has a steroid molecular structure, it is derived from cholesterol and capable of intervening in the regulation of the activity of multiple cell lines. The main function of this vitamin its important role in bone metabolism, playing a key role in maintaining the homeostasis of calcium, ensuring the correct physiological absorption of it by the intestine and becoming necessary in the formation and maintenance of strong and healthy bones. It stimulates the intestine calcium absorption by a saturable transcellular pathway, as well as increases the reabsorption of calcium in the renal tube and also the expression of the membrane transporter.

The nuclear vitamin D receptor (VDR) is found in almost every cell of the body, such as vascular, myocardial and immune cells. This fact suggests that vitamin D is not only involved in the main system (musculoskeletal), but also in many others. Apart from VDR receptors, most tissues and cells, whether they are normal or neoplastic, have the enzymes needed for vitamin D metabolism.

The main biochemical forms of vitamin D are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). To date, only few studies have been performed comparing both types of vitamins [(1)]. However, the evidence indicates that vitamin D3 seems to be more effective than vitamin D2.

In humans, the contribution of this vitamin comes from the skin's transformation of 7-dehydrocholesterol into cholecalciferol by the action of UV rays from sunlight. Apart from UVB radiation, vitamin D can also be obtained from food, it can become from animals (cholecalciferol) or plants (ergocalciferol). Even so, this concentration is much lower than the one coming from solar source.

Once in the human body, either vitamin D3 or D2, is transported by the blood to the liver where it is hydroxylated. First hydroxylation is made in the 25th position of the molecule, by a 25-hydroxylase, leading to 25-hydroxy-colecalciferol (25(OH)D), which is also called calcifediol or calcidiol. Later, in renal tubular cells occurs another hydroxylation made by 1-α-hydroxylase, generating 1,25-dihydroxy-vitamin D (1-25$(OH)_2$D) or calcitriol, which is the active form of vitamin D, in fact, the most active metabolite of this endocrine system.

Calcitriol has a wide range of biological actions, the main one is to increase calcium reabsorption and renal phosphate excretion. It increases serum calcium concentrations due to the induction of its transport by the intestine and increasing bone resorption. 1-25$(OH)_2$D has also other functions, such as inhibition of cell proliferation, renin production or angiogenesis. Moreover, it inducts terminal differentiation and stimulates insulin and cathelicid production, so it can be stated that this metabolite regulates the transcription of approximately 3% of the human genome, up to 200 genes, intervening in the regulation of differentiation and cell growth.

Endocrine system is implicated in level regulation, the renal hydroxylation is activated by parathyroid hormone (PTH) and calcitonin, and is inhibited by calcium, phosphorus and 1-25$(OH)_2$D itself found in plasma.

Vitamin D metabolites are bound to proteins. Approximately 85% of 1-25$(OH)_2$D is bound to vitamin D binding protein (VDBP) or transcalciferin and 15% to albumin, which transport it through the plasma to different tissues and distributes it throughout the body.

The most reliable clinical indicator of vitamin D status is 25(OH)D, as it reflects the body's vitamin D storage levels and correlates with the clinical symptoms of this hormone deficiency. The high prevalence of vitamin D deficiency is a major health problem as well as hypovitaminosis D is a risk factor for mortality and morbidity in population. Several studies [2–6] have been determined how vitamin D is a factor involved in the pathogenesis of various skeletal and non-skeletal diseases, such as infections, psychiatric disorders, different types of cancer, autoimmune diseases, and cardiovascular risk factors such as hypertension, dyslipidemia, type 2 diabetes mellitus, obesity and atherosclerosis. These data have made those nowadays clinical guidelines of different diseases have included the measurement of this parameter and its complementation in case of deficit, in order to reduce mortality. In addition, its deficiency is the result of different situations, insufficient sun exposure, inadequate nutrition, decreased absorption, abnormal metabolism or even vitamin D resistance.

Various scientific societies, for example NICE, US Preventive Service Task Force (USPSTF), Spanish Society of Endocrinology and Nutrition and American Family Physician (AFP) recommend that health professionals do not routinely request vitamin D testing unless the patient has symptoms or a very high risk of vitamin D deficiency, also when there is a clinical reason to perform it. In Catalonia, the determination of serum vitamin D levels is not routinely recommended in general population, even so, the measurement of this parameter is under continuous review and in scientific dissertations. Depending on the methods used to determine it, results are heterogeneous (15-20%), creating a debate on the validity of the test [7–9], also it is the subject of scientific discussion about which cut-off points are considered clinically relevant.

The latest recommendations of the Catalan Government Health Department [7] show that the evidence of the benefits of measuring the concentration of Vitamin D in asymptomatic population are insufficient and that it is only justified in risk population, institutionalized people, those who take medications that interfere with the normal metabolism of vitamin D, patients who suffer from poorly absorbing diseases that interfere with their metabolism, individual with osteoporotic disease and people who have very limited exposure to sun, among others. Although, in the last years the tendency to determinate vitamin D and the supplementation of it has increased exponentially, suggesting that the identification and correction of the deficiency of this metabolite would have an improvement in health and would fight against the possibility to suffer certain pathologies, especially from osteoporosis or bone fractures.

There is an argument of supplementing the general population to correct the situation of hypovitaminosis around the world, the dose and dosage period must be correctly determined to avoid hypervitaminosis D and its adverse effects that can be lethal. However, differing in many countries, most vitamin D supplementation recommendations stipulate a range between 400 and 2000 IU daily [1].

Spain is a low latitude country and typically sunny, but the reality is that due to the distance to the equator, the exposure to solar light is not enough to synthesize the basic daily requirements of this vitamin, especially in winter and autumn months. As observed from previous studies [8,10], the percentage of Spanish population with vitamin D deficiency is high.

Objective

To analyse whether solar radiation and Mediterranean diet are enough to obtain optimal concentrations of vitamin D.

Methods

High-pressure liquid chromatography, mass spectrometry, enzyme immunoassays, competitive protein binding assays, radioimmunoassays, automated chemiluminescence protein-binding assays and chemiluminescent immunoassays are commercially methods used for the determination of vitamin D status, they all are used in clinical and research settings.

In our study for the determination of vitamin D status in patients a clinical laboratory analyser called *Atellica* was used, from the commercial house SIEMENS, which automatically measures a specific metabolite. It is a competitive immunoassay based on chemiluminescence detection, it uses an anti-fluorescein mouse monoclonal antibody covalently bound to paramagnetic particles (PMP), an anti-25(OH)vitamin D mouse monoclonal antibody labelled with acridinium ester (AE) responsible of producing chemiluminescence and used to extrapolate the concentration of the analyte of interest, and a vitamin D analogue labelled with fluorescein to differ it from the vitamin D present in the patient's sample. The *Atellica* Vitamin D Total Immunoassay is for in vitro diagnostic use in the quantitative determination of total 25(OH)vitamin D in human serum using the *Atellica* IM Analyzer, this assay is intended as an aid in the determination of vitamin D sufficiency. Serum was the sample type used for this assay, although plasma (EDTA, lithium heparin, and sodium heparin) samples could be used too. In addition, in *Sant Pau i Santa Tecla*'s hospital, where this study was performed, *Atellica* is used to perform another analyte's apart from 25(OH)vitamin D.

The system automatically performs the process. Firstly, it dispenses patient's sample into a cuvette and dispenses Ancillary Reagent which contains vitamin D analogue conjugated to fluorescein, then incubates for 1 minute at 37°C.

Secondly, it dispenses Lite Reagent which contains mouse monoclonal anti-25(OH)vitamin D antibody labelled with acridinium ester in buffer and incubates for 5 minutes at 37°C. This antibody will join to all vitamin D, to the analogue and to the serum one.

Thirdly, it dispenses Solid Phase which contains mouse monoclonal anti-fluorescein coated with paramagnetic particles in buffer and again Ancillary Well Reagent with vitamin D-analogue conjugated to fluorescein, then incubates for 5 minutes at 37°C. In this case, the fluorescein is only present in the analogue vitamin D, therefore the vitamin D present in serum will be free from this antibody. Moreover, parametric particles tend to attach to a magnet located under the cuvette.

Next, it separates and washes the cuvette with *Atellica* IM Wash. It will aspirate everything that is not attached to the PMP which retains vitamin D at the end of the cuvette, therefore only the analogue will not be removed. Finally, it dispenses *Atellica* IM Acid and *Atellica* IM Base in order to produce a change in pH to initiate the chemiluminescent reaction which will report final results, an inverse relationship exists between the amount of vitamin D present in the patient sample and the amount of relative light units (RLUs) detected by the system.

All reagents are liquid and ready to use, workers only have to ensure that the system has sufficient loaded reagent packs in the reagent compartment and it automatically does all the process.

Routinely, before introducing new samples, calibration and control check must be performed, creating a calibration curve. That means that a previously known concentration is introduced in the machine, the chemiluminescence produced (RLUs) and the concentration of the calibration samples are connected and related, resulting in this calibration curve.

For calibration of the *Atellica* Vitamin D Immunoassay, calibrators provided with each kit are used. A Low (L) calibrator and a High (H) calibrator are measured in duplicate. However, they have to be prepared adding special reagent water into each vial, letting the vials stand at room temperature to allow the lyophilized material to dissolve. Finally, they must be mixed before introducing them to *Atellica* to ensure the material is homogeneous.

There is an example below of the calibrator made on February 1, 2021.

Table 1. Signals obtained from each analyte, comparison of theoretical and experimentally observed values.

	Low (L)		**High (H)**	
	Analyte	Signal	Analyte	Signal
Duplicate 1	18.59 ng/mL	1289625.00 RLUs	113.28 ng/mL	608062.00 RLUs
Duplicate 2	18.87 ng/mL	1285228.00 RLUs	114.29 ng/mL	603969.00 RLUs
Average observed	18.73 ng/mL	1287426.50 RLUs	113.79 ng/mL	606015.50 RLUs
Theoretical concentration	17.70 ng/mL		114.00 ng/mL	
% CV observed	1.06	0.24	0.63	0.48

Table 2. Results obtained from calibration curve created from calibrators, including different criteria in order to accept or discard the calibration curve.

Criteria	Result	Value	Expected
Slope of the line	Approved	1.03	0.33 - 4.07
Signal proportion	Approved	1.12	0.018 - 3.582
Low level drift allowed	Approved	-0.01	-1.17 – 3.582
High level drift allowed	Approved	0.00	-1.17 – 0.57
Minimal duplicates	Approved	2.00	2.0 – 99.0
% CV Low level	Approved	0.24	0.0 – 5.3
%CV High level	Approved	0.48	0.0 – 5.5

Table 3. Theoretical and experimentally observed values from control samples.

CC	CC limits	CC Observed
Level 1	10.5 – 28.7 ng/mL	15.55 ng/mL
Level 2	78.0 – 122.0 ng/mL	94.52 ng/mL

Once *Atellica* receives the calibrator, it analyses the data from the calibrator and the data from the master curve (real calibration curve been determined under specific conditions for this reagent and analyser from Siemens Germany). When the calibration is made, the analyser measures the value of RLUs and if it is within the expected range, thus ensuring two levels and averaging, in this case the low level gives a signal of 1285228.00 RLUs and the high level of 1289625.00 RLUs. Through the master curve and the RLUs obtained from the samples of the calibrators, the concentration of the analyte for each duplicate is obtained, in this case the observed average is 18.73 ng / mL and the theoretical one is 17.70 ng / ml. In this instance the result is within the expected range and it accepts it directly.

Although the calibration curve created is accepted ($y = 1.03x$), as a last step to know if patients' serum samples can be introduced to *Atellica* in order to determine their vitamin D concentrations correctly, two control samples must be analysed.

Through the calibration curve obtained from the calibrators and the URLs released by the controls, the concentration of both levels is calculated. It is observed that they both are placed within the accepted limits by the controls so it can be stated that calibration curve is correct and be used to calculate the concentration of vitamin D of the patient's samples.

Results

The sample obtained consists of 300 individuals pertinent to Tarragona, Catalonia (Spain), who were asked to determine their vitamin D levels in blood. The determination of the concentration of Vitamin D was carried out at *Sant Pau i Santa Tecla* Hospital, located in the city of Tarragona. This determination could be requested by doctors to patients treated in outpatient clinics, hospitalized ones or those who went to medical emergency. These data were obtained between January 1 and February 28, 2021.

It consists of a heterogeneous group, from hospitalized people who were tested to evaluate pathologies or to observe the state of the vitamin, to patients who were part of risk population and to whom doctors asked to perform the test in case of suspected deficiency. On the other hand, we found cases in which it was asked to assess the nutritional status of the person and patients with unified medical history without clinical pathologies identified as a method of prevention.

The determination of the values from which it is considered insufficiency, deficit or sufficiency is based on the recommendations of the International Osteoporosis Foundation (IOF), the National Osteoporosis Foundation (NOF) and according to the guidance of *Atellica*, based on available literature, which consider that the level of 25(OH)vitamin D is sufficient (**S**) when it is greater than 30 ng/ml (75 nmol/l). Below these optimal levels, insufficiency (**I**) is considered to exist when levels are between 20 ng/ml (50 nmol/l) and 29 ng/ml (74 nmol/l) and deficit (**D**) values below 20 ng/ml (50 nmol/l). Regarding the obtaining of values higher than the sufficiency range, no toxicity has been demonstrated in patients with levels of 25(OH)vitamin D below 100 ng/ml, which is why a minimum toxicity threshold has been proposed for above 150 ng/ml (375 nmol/l) [9].

The samples obtained were classified according to age and sex of the patients, taking into account only adult samples, rejecting paediatric ones. The different age groups were set according to the classification of the World Health Organization (WHO), people between 18 and 65 years as young people, people between 66 and 79 years as middle age and individuals between 80 and 99 years as elders.

Table 4. Classification of population according to sex and age, classifying sufficiency, insufficiency and deficiency groups depending on their values of 25(OH)vitamin D.

WOMEN'S SAMPLES	AGE	VALUE (ng/ml)	CLASSIFICATION	MAN'S SAMPLES	AGE	VALUE (ng/ml)	CLASSIFICATION
1	19	30.1	S	**1**	20	31.96	S
2	21	16.22	I	**2**	20	14.56	I
3	23	43.93	S	**3**	21	7.03	I
4	23	13.99	I	**4**	25	41.96	S
5	24	8.17	I	**5**	26	31.64	S
6	25	11.05	I	**6**	27	8.48	I
7	27	34.47	S	**7**	28	10.19	I
8	28	9.44	I	**8**	29	8.87	I
9	29	16.06	I	**9**	30	16.61	I
10	30	9.03	I	**10**	32	30.48	S
11	30	13.43	I	**11**	35	18.99	I
12	32	13.71	I	**12**	36	29.56	D
13	33	15.09	I	**13**	38	19.02	I
14	35	32.68	S	**14**	38	25.5	D
15	36	10.32	I	**15**	39	43.65	S
16	37	7.07	I	**16**	41	10.61	I
17	38	14.67	I	**17**	42	24.86	D
18	39	19.35	I	**18**	44	8.8	I
19	40	39.62	S	**19**	44	9.57	I
20	40	27.81	D	**20**	45	20.39	I
21	41	10.52	I	**21**	46	14.38	I
22	42	9.53	I	**22**	47	29.52	I
23	43	20.8	D	**23**	48	10	I
24	44	45.98	S	**24**	49	14.28	I
25	45	16.54	I	**25**	50	22.34	D
26	46	6.59	I	**26**	50	17.45	I
27	46	21.03	D	**27**	51	26.17	D
28	47	35.42	S	**28**	52	17.07	I
29	48	12.42	I	**29**	53	28.62	D

30	49	14.31	I	**30**	54	18.53	I
31	50	26.62	D	**31**	55	12.2	I
32	50	9.39	I	**32**	55	16.01	I
33	51	23.02	D	**33**	56	14.19	I
34	52	16.04	I	**34**	57	9.35	I
35	53	18.26	I	**35**	58	10.78	I
36	53	14.01	I	**36**	58	52.52	S
37	55	21.22	D	**37**	58	28.2	D
38	57	7.52	I	**38**	59	8.22	I
39	58	5.97	I	**39**	59	38.93	S
40	59	24.97	D	**40**	60	13.5	I
41	60	10.29	I	**41**	60	8.79	I
42	60	26.67	D	**42**	60	20.88	D
43	61	29.86	D	**43**	61	10.87	I
44	62	17.53	I	**44**	62	12.88	I
45	63	16.38	I	**45**	63	51.07	S
46	63	14.98	I	**46**	64	20.71	D
47	64	13.49	I	**47**	65	20.45	D
48	64	21.43	D	**48**	65	11.65	I
49	65	14.82	I	**49**	65	37.78	S
50	65	24.45	D	**50**	65	30.52	S
Average Youth			16.05	**Average Youth**			17.99
Standard Deviation Youth			9.78	**Standard Deviation Youth**			11.53
51	66	10.08	I	**51**	66	15.07	I
52	66	6.45	I	**52**	66	20.72	D
53	66	16.83	I	**53**	66	25.5	D
54	66	33.07	S	**54**	67	11.12	I
55	67	9.34	I	**55**	67	22.2	D
56	67	22.68	D	**56**	67	21.19	D
57	68	10.02	I	**57**	68	18.5	I
58	68	22.51	D	**58**	68	17.12	I
59	68	11.93	I	**59**	68	10.79	I
60	69	12.3	I	**60**	69	28.77	D
61	69	12.06	I	**61**	69	24.53	D

62	69	22.67	D	**62**	69	18.84	I
63	69	8.05	I	**63**	70	9.41	I
64	70	10.83	I	**64**	70	7.13	I
65	70	12.8	I	**65**	70	22.1	D
66	70	12.7	I	**66**	70	45.1	S
67	70	18.15	I	**67**	71	19.58	I
68	70	36.98	S	**68**	71	10.87	I
69	71	15.91	I	**69**	71	17.22	I
70	71	20.05	D	**70**	71	24.05	D
71	71	14.88	I	**71**	72	37.09	S
72	71	16.65	I	**72**	72	9.09	I
73	71	30.84	S	**73**	72	10.27	I
74	72	10.49	I	**74**	72	26.13	D
75	73	23.44	D	**75**	72	6.92	I
76	74	18.78	I	**76**	73	11.6	I
77	75	18.83	I	**77**	73	14.88	I
78	75	29.52	D	**78**	73	44.73	S
79	75	25.39	D	**79**	74	10.99	I
80	76	10.4	I	**80**	75	32.16	S
81	76	14.26	I	**81**	75	11.59	I
82	77	17.66	I	**82**	75	6.85	I
83	77	32.94	S	**83**	75	6.76	I
84	77	10.16	I	**84**	76	9.48	I
85	77	14.29	I	**85**	76	31.21	S
86	77	14.02	I	**86**	76	14.06	I
87	77	13.26	I	**87**	76	15.2	I
88	77	15.45	I	**88**	76	35.77	S
89	78	28.54	D	**89**	77	12.4	I
90	78	38.12	S	**90**	77	32.86	S
91	78	15.36	I	**91**	77	54.4	S
92	78	18.37	I	**92**	77	25.29	D
93	78	10.97	I	**93**	78	19.62	I
94	78	8.14	I	**94**	78	19.62	I
95	78	11.61	I	**95**	78	9.16	I

96	79	24.83	D	**96**	78	12.31	I
97	79	18.77	I	**97**	79	20.28	D
98	79	24.45	D	**98**	79	15.8	I
99	79	17.9	I	**99**	79	8.92	I
100	79	11.55	I	**100**	79	11.75	I
Average Middle Age			15.68	**Average Middle Age**			17.17
Standard Deviation Middle Age			7.83	**Standard Deviation Middle Age**			10.85
101	80	17.26	I	**101**	80	36.04	S
102	80	9.35	I	**102**	80	12.94	I
103	80	11.88	I	**103**	80	15.14	I
104	81	35.92	S	**104**	80	27.28	D
105	81	25.01	D	**105**	81	23.64	D
106	81	12.89	I	**106**	81	26.26	D
107	81	11.18	I	**107**	82	8.12	I
108	82	22.28	D	**108**	82	15.53	I
109	82	39.32	S	**109**	82	15.61	I
110	82	6.88	I	**110**	83	12.21	I
111	82	14.63	I	**111**	83	15.09	I
112	82	17.38	I	**112**	83	12.56	I
113	83	11.56	I	**113**	84	24.28	D
114	83	33.6	S	**114**	84	8.22	I
115	84	21.88	D	**115**	84	8.28	I
116	84	15.55	I	**116**	84	17.17	I
117	84	7.91	I	**117**	84	22.82	D
118	84	12.01	I	**118**	84	13.83	I
119	85	11.61	I	**119**	84	8.95	I
120	85	8.13	I	**120**	85	35.83	S
121	85	8.3	I	**121**	85	9.63	I
122	86	18.15	I	**122**	85	29.38	D
123	86	8.78	I	**123**	85	22.3	D
124	87	24.38	D	**124**	85	7.81	I
125	87	23.2	D	**125**	86	21.6	D
126	87	28.2	D	**126**	86	11.5	I
127	88	8.8	I	**127**	86	13.87	I

128	88	11.3	I	**128**	86	15.35	I
129	89	9.67	I	**129**	86	35.72	S
130	89	12.46	I	**130**	87	12.55	I
131	89	12.17	I	**131**	87	8.87	I
132	90	11.03	I	**132**	87	11.07	I
133	90	28.34	D	**133**	87	12.03	I
134	90	10.84	I	**134**	88	11.58	I
135	90	7.13	I	**135**	88	26.96	D
136	91	14.87	I	**136**	88	10.87	I
137	92	6.31	I	**137**	89	14.44	I
138	93	5.5	I	**138**	89	33.4	S
139	94	28.52	D	**139**	89	33.68	S
140	94	9.24	I	**140**	89	27.21	D
141	95	10.29	I	**141**	89	9.01	I
142	95	22.03	D	**142**	89	7.96	I
143	96	9.3	I	**143**	90	19.95	I
144	96	10.28	I	**144**	90	11.22	I
145	96	9.56	I	**145**	91	7.27	I
146	97	20.51	D	**146**	92	20.36	D
147	97	8.12	I	**147**	93	12.64	I
148	97	18.74	I	**148**	95	11.86	I
149	98	11.59	I	**149**	97	11.84	I
150	99	23.69	D	**150**	97	8.75	I
Average Elders			11.98	**Average Elders**			13.85
Standard Deviation Elders			8.29	**Standard Deviation Elders**			8.52

Regarding women, 3 groups can be identified according to the age of the patients: young women (50) who represent 33.33% of the total women, middle age women (50) who represent 33.33% of the total of women and elder women (50) representing 33.33% of the total number of women.

From the 50 tests performed on the total number of young women, the average for young women is 16.05 ng/ml (SD = 9.78); 32 of these women show an insufficient concentration of vitamin D, which means 64% of them have obtained an insufficient concentration of vitamin D, 22% (11 women) have obtained a concentration with deficiency and only 14% (7 women) have obtained an optimal and sufficient concentration of the analyte analysed.

Regarding middle age women represented by 50 people they show an average of 15.68 ng/ml (SD = 7.84). 70% (35 women) have insufficient concentrations of vitamin D, 20% (12 women) are deficient and only 10% (5 women) get sufficient concentrations of it.

Regarding the last group of 50 elder women, they show an average of 11.98 ng/ml (SD = 8.29). 72% (30 women) get insufficient concentrations of vitamin D, 22% (11 women) are deficient and only 6% (3 women) have sufficient concentrations.

According to men, 3 groups can be identified according to the age of the patients: young men (50) which represent 33.33% of the total number of men, middle age men (50) which represent 33.33% of the total number of men and elder group (50) representing 33.33% of the total number of men.

Regarding 50 young men, they show an average of 17.99 ng/ml (SD = 11.53). 60% (30 men) of the total number of young men have insufficient concentrations of vitamin D, 20% (10 men) obtain deficient concentrations and 20% (10 men) obtain sufficient concentrations of it.

Otherwise, average obtained from the group identified by 50 middle age men is 17.17 ng/ml (SD = 10.85). 62% (31 men) of the total middle age men have insufficient concentrations of vitamin D, 22% (11 men) are deficient and 16% (8 men) obtain sufficient concentrations.

Of the 50 tests performed on the total number of elder men, the average for older men was 13.85 ng/ml (SD = 8.52); 34 of these men show an insufficient concentration of vitamin D, which means 68% of them have obtained an insufficient concentration of vitamin D, 22% (11 men) have obtained a concentration with deficiency and only 10% (5 men) have obtained an optimal and sufficient concentration of the analyte analysed.

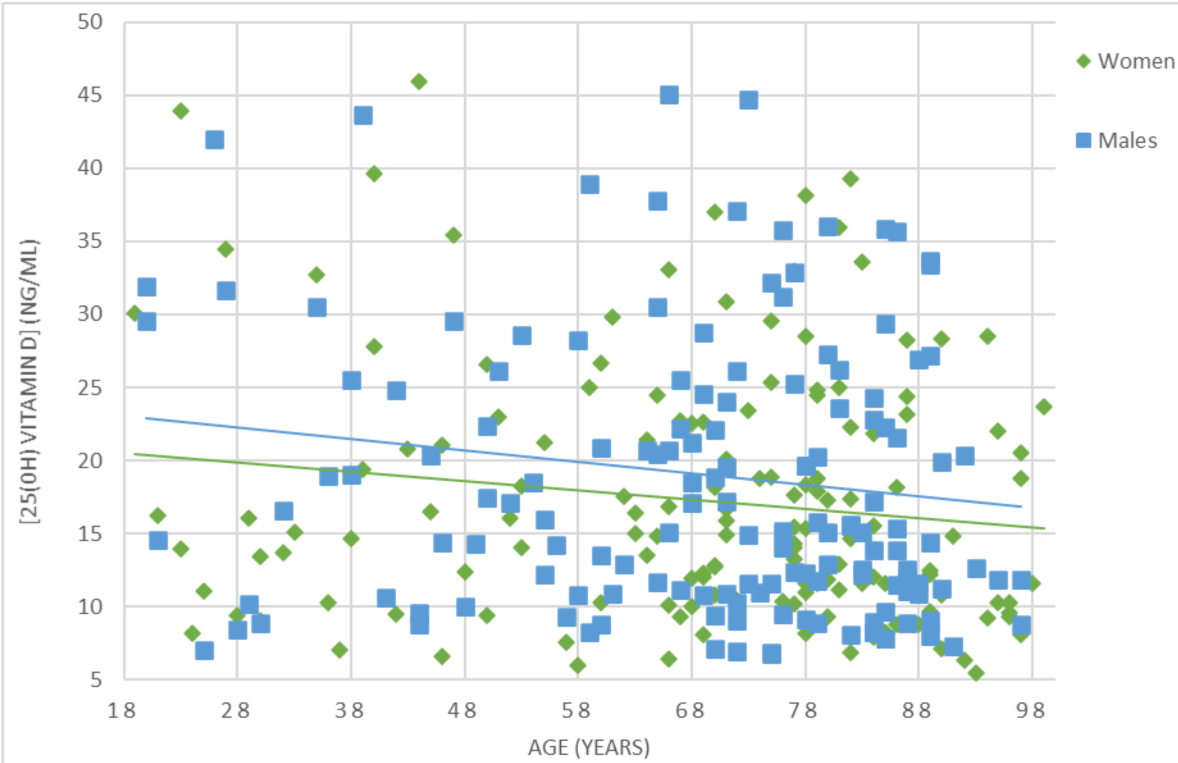
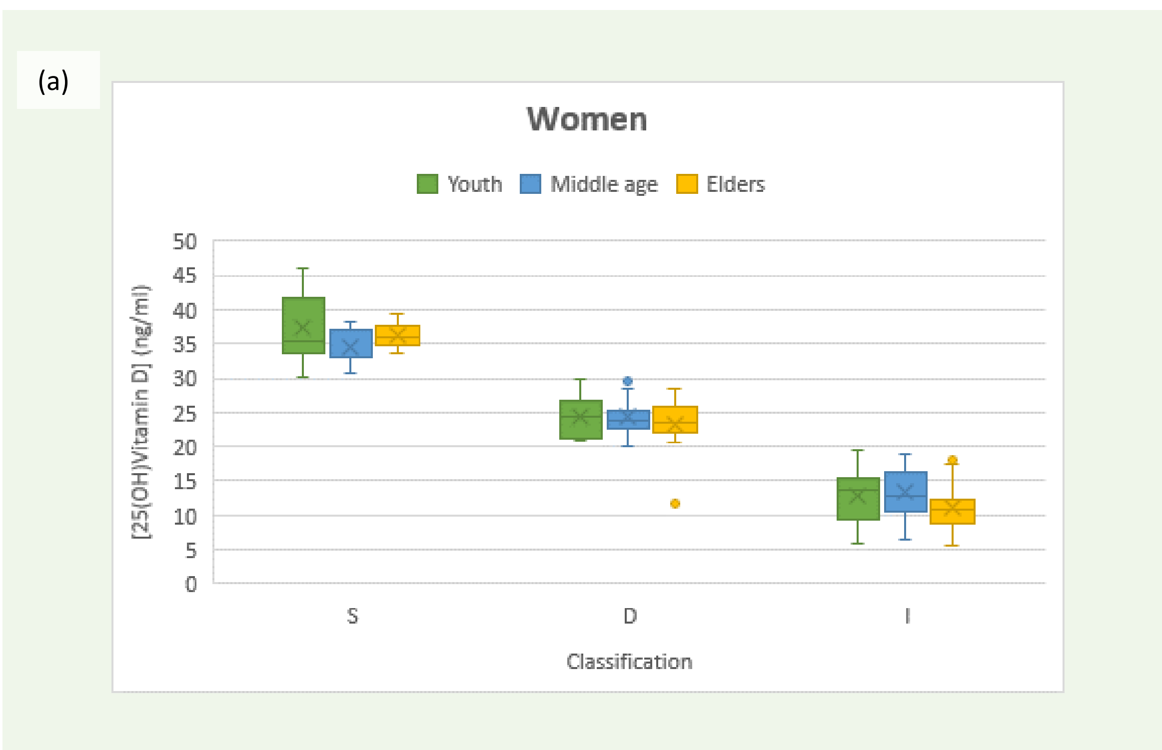


Figure 1. Scatter plot of 25(OH)vitamin D according to age divided by sex. Solid line: trend line of 25(OH)vitamin D values to women and males.



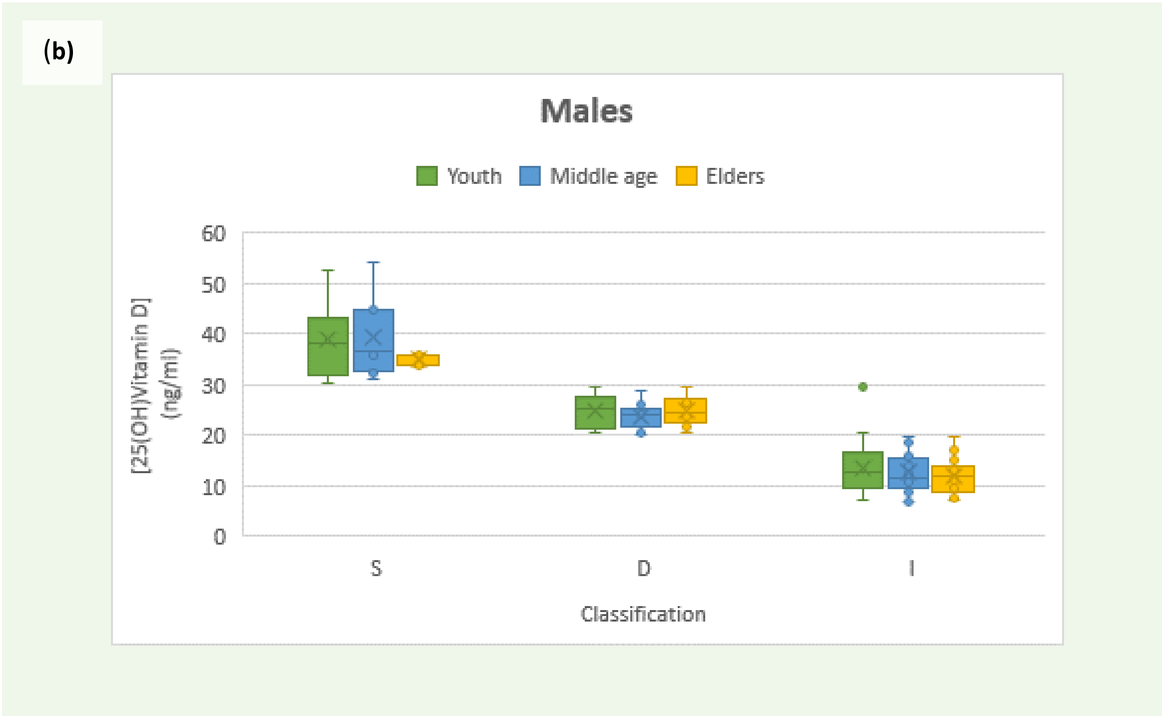


Figure 2. Box-and-whisker plot showing the serum levels of **(a)** 25(OH)vitamin D in different age groups in women, **(b)** levels of 25(OH)vitamin D in different age groups in males

Young women show an average of sufficiency values of 35.42 ng/ml, an average of deficiency values of 24.45 ng/ml and average of insufficiency values of 13.6 ng/ml. Middle age women show an average of sufficiency values of 34.39 ng/ml, an average of deficiency values of 24.41 ng/ml and an average of insufficiency values of 13.41 ng/ml. Elder women show an average of sufficiency values of 36.28 ng/ml, an average of deficiency values of 23.3 ng/ml and an average of insufficiency values of 11.16 ng/ml.

Young males show an average of sufficiency values of 39.05 ng/ml, an average of deficiency values of 25.18 ng/ml and average of insufficiency values of 12.54 ng/ml. Middle age males show an average of sufficiency values of 36.43 ng/ml, an average of deficiency values of 24.05 ng/ml and an average of insufficiency values of 12.67 ng/ml. Elder males show an average of sufficiency values of 35.72 ng/ml, an average of deficiency values of 24.28 ng/ml and an average of insufficiency values of 11.85 ng/ml.

Discussion

Although the Spanish climate is conducive to synthesize through sunlight sufficient vitamin D concentrations, the level of this hormone in general population is low, even lower than the average concentration obtained in previous studies made in Central Europe or Scandinavia [(9)]. This can be explained because, although solar radiation in these countries is low, many of them traditionally use dairy products enriched with vitamin D and the population tend to take supplements of it to maintain optimal physiological concentrations. In addition, in countries such as Norway or Sweden there is a high intake of fatty fish and cod liver oil, food that contain high vitamin D concentrations.

As shown in *Table 1* and *Figure 1*, the percentage of population with vitamin D deficiency and insufficiency is extremely high. It can also be noticed that it is slightly higher in women than in men and increases exponentially with age. This is also related to the number of test determinations required by doctors in each group of population, thus having a very high number of requests in elders, also in females the request for the determination of this parameter is more frequent than in males.

In box-and-whisker plot, *Figure 2,* samples of women and males are evenly distributed by age in order to avoid including variability by it and extracting whether age is an interfering factor regarding deficiency.

In women's box-and-whisker plot, *Figure 2 (a)*, focusing on sufficiency the average of the values is higher in elders and young patients, moreover the average of the values of middle-aged women is lower. The average of the deficiency values is quite similar in all age ranges, but shows a lower average in case of elder women. The average insufficiency is marked by a low average in elders and a higher average in middle-aged and adult women. With these results can be concluded that the group of elder women is the group with lower values, on the other hand, the values of sufficiency are slightly higher in elder women, probably due to oral vitamin D supplementation as these patients are more closely monitored by their specialist physicians and general doctors in the primary care centre. Given this particular exception, it can be seen in the graph that as patients get older, their averages are lower.

In males' box-and-whisker plot, *Figure 2 (b)*, regarding sufficiency the average of the values is higher in the case of young patients, being lower the average of middle-aged men and even inferior the average of elders. The average values of deficiency are higher in young men, and lower and quite similar in middle-aged and elderly patients. The average of insufficiency is more marked by a low average in elders, being lower than the average of middle-aged and young men. With these results can be concluded that the group of elder men is the one with the lowest values in general, so it also confirms that when age increases the average of patient's values decreases.

In box-and-whisker plot, *Figure 2,* if the average of deficit, insufficiency and deficiency of women are compared to the average of deficit, insufficiency and deficiency of males, no clear differences are observed, this might lead us think that the ranks of normality for men and women according to these data are equal. Subsequently, new studies could be conducted to study a larger number of patients and see if statistically there would be differences between both sexes, if so, it would be interesting to establish different ranges of normality according to each sex.

The hypovitaminosis D observed in the region of Tarragona, and which can also be observed in many areas of Spain [6,8] is due to the low intake of vitamin D in the Mediterranean diet, and despite the solar radiation is high, skin synthesis is not enough to compensate the poor dietary intake. Although Spain has abundant sunlight, most of Iberian Peninsula, as is the case of Tarragona, is located above the parallel 35º N, so the inclination of the sun's rays reduces the possibility of synthesizing this vitamin in winter months. Furthermore, this study was performed in January and February.

Another possible factor to consider is that the closer we get to the equator, darker is the coloration of skin, that is why Spanish population tends to have darker skin than Nordic population, which makes skin synthesis difficult as the abundant amount of melanin they have on their skin prevents the penetration of sunlight, as well as the use of sunscreens, which in recent years has increased considerably in order to prevent skin cancer. There is no doubt that there is an important link between solar radiation and skin cancer, on the one hand you need to protect yourself from excessive sun radiation by using sunscreen, but on the other hand these sun protection factors decrease dramatically cutaneous synthesis of vitamin D making it difficult to obtain optimal concentrations of it.

As seen in *Table 1*, *Figure 1* and *Figure 2*, older people have lower concentrations of vitamin D, this could be due to sun exposure, as elders do not usually go out on the street as much as young people. In winter many of them tend to stay home and avoid being exposed to low temperatures and bad weather in order to avoid getting colds. In addition, the type of clothes they wear tend to be dense which minimizes skin exposure to sun, this makes skin synthesis even more difficult. Moreover, many of them are institutionalized in residences, so they generally do not go out on the street and the sun exposure is even lower. When temperature is hot and solar radiation is strong, elders avoid being in the sun and prefer being indoors, furthermore many of them are aware of the high risk of suffering from skin cancer they have and try to avoid the sun, that makes it difficult to old people to compensate the hypovitaminosis caused in the cold months in the sunny ones.

In addition, a critical factor for elders is the reduction in the concentration of 7-dehydrocholesterol in the skin over the years, which significantly decreases the ability to produce cutaneous vitamin D through ultraviolet radiation. Therefore, due to all these factors, exposure to the same solar dose, an elder and a young person would produce different concentrations of vitamin D, so an important risk factor in hypovitaminosis is the age. In addition, with aging, the risk of suffering from dysphagia increases, so they generally tend to eat less as it takes a lot of effort and their diet is different, prioritizing foods and textures that are easy to swallow. In addition, older people have a high risk of developing hypochlorhydria, which leads to a decrease in the absorption of vitamin D. Otherwise, the liver and kidneys of elders are less effective, so they are less able to convert vitamin D into its active form.

A higher prevalence of vitamin D deficiency and insufficiency has also been observed in women than in males. People with osteoporosis have a high risk of hypovitaminosis D, it has been noticed that this disease affects more women than men, as oestrogen levels drop sharply after menopause, without them female bones are no longer protected, as a result there is a progressive loss of bone mass and deterioration of bone quality, greatly increasing the risk of osteoporosis. Approximately one-third of women between the ages of 60 and 70 suffer from osteoporosis and 2-thirds of women aged 80 or older [(11)]. Other studies [(9)] have also shown that people who have suffered from osteoporotic fractures have a 100% prevalence of hypovitaminosis D.

Several studies [(2)] in recent years have demonstrated that women have a high risk to suffer from vitamin D deficiency and this is associated with adverse outcomes that occur during pregnancy, including preeclampsia and gestational diabetes.

This is currently evolving, but women generally have worse socioeconomic status than men, performing indoor jobs such as housewives or cleaning, unlike men who have jobs where they receive high solar radiation, such as masonry work. In addition, currently in Spain there is a high number of people of Moroccan origin, from adolescence, women use traditional clothes and *hijab*, leaving little skin exposed to solar radiation and thus making it difficult to synthesis cutaneous vitamin D.

Ultimately to achieve optimal levels of vitamin D there are different actions to take, such as increase sun exposure and avoid abusing the sunscreen. Food also needs to be controlled, but there are few foods that contain this vitamin naturally in extremely high concentrations, although in some foods such as fatty fish, egg, meat, dairy foods and derivatives contain vitamin D concentrations. Lastly, after analysing the results obtained in the present study, it would be recommended to supplement the population in general, since although the prevalence of vitamin D deficiency predominates in elders, young people also have worrying concentrations of it, so it has been concluded that solar radiation and Mediterranean diet are not enough to obtain optimal concentrations of vitamin D. Physicians when recommending supplements must take into account several factors, which is why pharmacological supplementation recommended is divided into age ranges, but in patients who have associated comorbidities should be individually and customizing the doses to keep it suitable for the whole population. In addition, it would help people get optimal concentrations of vitamin D supplementing foods with vitamin D as it actually occurs in other countries.

It is important to maintain sufficient concentrations of vitamin D as its insufficiency could cause secondary hyperparathyroidism, increased bone turnover and loss of bone mass, muscle weakness, cataracts and increased risk of fracture. On the other hand, current studies state that hypovitaminosis D can have health consequences at skeletal level [(11)], it has been associated with different types of cancers such as colon, prostate and breast and with a higher mortality of these cancers, also increasing autoimmune diseases such as type 1 diabetes mellitus, multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis. Otherwise, it could also increase the risk of suffering metabolic syndrome, high blood pressure, peripheral arterial disease, myocardial infarction, and cardiovascular mortality.

It would be interesting to repeat this study at different times of the year, so it could be observed if in other seasons apart from winter the status of 25(OH)vitamin D in patients increases. Theoretically in sunny months concentrations should increase, although it could be studied if it increases to the point of obtaining sufficient values of it. Moreover, it would be interesting to repeat it in another year once the global pandemic due to Sars-CoV-19 has ended, as in this last year people have not been able to leave home too much due to the security measures implemented by the government, this is why solar radiation received by the population of Tarragona has been lower than in other years, as many people have studied online and workers did telework.

Conclusions

The prevalence of hypovitaminosis in population is high becoming a national health problem. The low levels of 25(OH)vitamin D obtained from the population study carried out in Tarragona, during winter months, confirm the high deficiency and insufficiency of vitamin D in the general population. It has been observed that age is a risk factor when suffering from hypovitaminosis, so elders have a higher deficit rate than young people. There are also small differences in sex where the prevalence of hypovitaminosis is slightly higher in women. It should be noted that in the year 2021 in which this study was done, due to the global pandemic caused by Sars-CoV-19, the time spent at home was longer than other years, so it can also influence the low concentrations of 25(OH)vitamin D.

Therefore, hypovitaminosis D is not a myth but a reality, which can lead to bone and health problems. After observing the population concentrations of 25(OH)vitamin D, it is necessary to make patients aware of the important role it plays in the body and increase sun exposure, always under medical supervision, as the recommendations regarding sun exposure must avoid contradictory messages. It is necessary to strike a balance in order to prevent skin cancer but obtaining sufficient concentrations of vitamin D. Adapting a proper diet with foods rich in natural vitamin D is important to ensure optimal intake and obtain sufficient plasma concentrations of this hormone.

It has been concluded that although Spain is a sunny country, according to the values of 25(OH)vitamin D obtained in this study, the sun exposure and the Mediterranean diet are not enough to obtain optimal concentrations of vitamin D, so that it would be interesting to implement supplementation to ensure optimal concentrations in general population. Furthermore, adapting the dose to age and individually to each patient according to associated comorbidities. However, further studies should be performed to study which vitamin D concentrations would be the most suitable in the population of Tarragona. It would also be interesting to supplement foods with vitamin D as it actually occurs in the Nordic countries, according to the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) the addition of micronutrients to processed foods is a strategy that can lead to relatively rapid improvements in nutritional status but this fortified food needs to be consumed in adequate amounts.

Therefore, implementing these measures would make it possible to obtain sufficient concentrations of vitamin D and eradicate the severe hypovitaminosis in Tarragona, improving the health of population and reducing the risk of serious problems derived from the deficiency of vitamin D.

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