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Research Article

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Assessment of Dietary Vitamin D, Vitamin K and Calcium Intake of Portuguese Women of Childbearing age living in Switzerland

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Abstract

Background: *The lack of sun plays a major role in vitamin D deficiency, particularly among vulnerable groups, including migrants and women. Vitamin D and calcium are crucial nutrients for optimal bone health, heart function and muscle contraction. Recent studies suggest that the two micronutrients alone do not appear to be as beneficial as when their intake is coupled with vitamin K. Therefore, it is necessary to prevent their deficiencies. To this date, we observe a deficit of culturally sensitive methods for assessing dietary intake. The aim of this study was to estimate the dietary vitamin D, vitamin K and calcium intake among Portuguese women of childbearing age living in Switzerland.*

Methodology: *A food frequency questionnaire was developed and administrated. A comparative method, a 24-hour dietary recall, was applied twice, over two non-consecutive days. The same methodology was applied to and compared with two control groups: Portuguese women living in Portugal and Swiss women living in Switzerland. Data were analysed using Microsoft Excel® version 15.0, 2013, Nutritional. Software® version 1.32.44, 2012 and IBM SPSS®, version 24.0, 2016 for windows.*

Results: *Portuguese in Switzerland had the lowest vitamin D intake (6.04 µg /day) from food. When dietary supplements were considered, significant differences were found between the Swiss (11.1 µg) and Portuguese groups (7.04 µg) ($p = 0.004$). Regarding vitamin K, the focus group had the lowest intake (102.20 µg/day). Concerning calcium, the Swiss group was the only one achieving the daily recommendations (1355.14 mg) and significant differences were found between the Swiss and the Portuguese groups ($p < 0.001$).*

Conclusion: *In the present study the three participant groups had a deficient intake of vitamin D and only the Swiss group had an adequate calcium intake. Vitamin K intake did not seem to be a concern in terms of dietary deficiencies for all the groups of participants.*

Keywords: Vitamin D, Vitamin K, Calcium, FFQ, Dietary, Intake, Migrants, Portugal, Switzerland

Introduction

Vitamin D is a critical nutrient for the overall health and is better synthesized by ultraviolet-B radiation in the human skin [1]. Therefore, especially in Central and Northern European countries, the lack of sun is the major responsible agent for vitamin D deficiency [2]. Even though hypovitaminosis D is a worldwide concern, literature shows that specific sub-groups of the population have higher risks of suffering from both calcium and vitamin D deficiencies [3]. Vitamin D and calcium inadequacies are influenced by some specific personal characteristics such as sex and age. Women are at higher risk of inadequate vitamin D and calcium intake compared to men, specially post-menopausal and pregnant women [4]. In addition to being key nutrients for optimal bone health, vitamin D and calcium are very important for healthy heart function and muscle contraction. During pregnancy, both vitamin D and calcium deficiencies are associated with higher risk of gestational diabetes, preeclampsia, low length and circumference at birth as well as preterm birth. Moreover, their deficiencies may cause reduced bone mineralization in offspring during childhood and adolescence [5]. Therefore it is important to ensure and maintain optimal levels of both – calcium and vitamin D among women in general.

For many years, vitamin D and calcium have been considered the golden nutrients for bone health. Nevertheless, recent literature suggests that vitamin D and calcium alone are less effective than when their intake is simultaneous with the intake of other nutrients such as vitamin K, especially for the purposes of bone health and osteoporosis prevention [6, 7]. The interdependence of these three micronutrients has already been proven for maintaining bone and overall health status, particularly in women [8]. Based on these studies, it is necessary that women of childbearing age include these key micronutrients – calcium, vitamin D and vitamin K – in their daily diet.

Another at-risk subgroup for nutrient inadequacy is the migrant population. Recent studies found that migrants coming from southern to northern European countries are an at-risk group for nutrient inadequacy [9]. Moreover, other research studies showed that immigrants in central and north European countries have a higher risk of vitamin D deficiency in comparison to locals [10]. In Switzerland, the Portuguese make up the third largest foreign community, corresponding to almost 13% of the total foreigners in the country [11]. Since the beginning of the 20th century, and with the continuing unfavourable economic situation, the number of Portuguese migrants in Switzerland has increased exponentially over the years [12]. Currently, however, very little is known about the health and nutritional status of migrants in Switzerland, especially those coming from other developed countries [13]. Due to the high prevalence of migrants in Switzerland, Swiss health authorities are highly interested in a better understanding of their situation in terms of nutrition and health. Such necessity is even more pressing considering that some of the migrant's communities living in Switzerland, such as the Portuguese, are very large. This knowledge would not only improve the quality of life of these people, but could also save healthcare costs, as it would encourage targeted prevention and allow the establishment of specific treatments.

To date, there is a deficit of culturally appropriate methods for assessing dietary intake. In Switzerland, there is very limited qualitative or quantitative data regarding the food intake and dietary habits of the Portuguese population. The development, application and validation of questionnaires and other assessment methods to evaluate the intake of different macro- and micronutrients may be useful to clarify the needs of a target population and suggest effective public health strategies. Despite the existence of a few studies that have assessed dietary calcium, vitamin D and vitamin K intake together, there is not a single report study developed, applied and validated for the Portuguese population, specifically our target group, Portuguese women of childbearing age living in Switzerland. Therefore, the aim of this study was to estimate and evaluate dietary vitamin D, vitamin K and calcium intake among Portuguese women of childbearing age living in Switzerland.

Materials and methods

A culturally sensitive questionnaire, based on a Food Frequency Questionnaire (FFQ), was developed and administrated. The questionnaire was composed of a total of 32 multiple-choice questions in the format of a matrix of dropdown menus and one open-ended question, regarding the intake of water and other liquids. The FFQ was created based on the Swiss and Portuguese food composition databases. In total it was composed of 267 foods and beverages, which were distributed in 14 food groups, according to their nutritional properties: "Cereal products, pulses and potatoes", "Bread and bread products", Fish and fish products", "Meat and meat products", "Egg and egg products", "Milk and dairy products", "Vegetables", "Fruits", "Fats and oils", "Nuts, seeds and oleaginous fruits", "Snacks and prepared dishes", "Sweets", "Water, wine and beer", "Other foods and beverages". To each food/beverage product was given six different portion sizes: "S", "M", "L", "XL", "XXL" and "XXXL", also used by other authors [14-16]. Each portion size corresponded to a specific weight in grams or millilitres, according to the "menuCH©" (National Nutrition Survey) – Swiss food photo book [17] and "Pesos e Porcões de Alimentos© [18] for the Swiss and Portuguese foods and beverages, respectively. In addition to information about food portions, food intake frequencies were also asked with a range of nine options: "Never (<1 per month)", "1-3 per month", "once a week", "1-4 a week", "5-6 a week", "once a day", "2-3 per day", "4-5 per day" and "6 or more per day" [15, 16]. Each questionnaire was applied once, personally (in Switzerland and Portugal) or via video-call, with an average duration of 30 min. A comparative method, a 24-hour dietary recall (24H-R), was applied twice, over two non-consecutive days, with an average duration of 20 minutes. The 24H-R were carried out in parallel with the interview-questionnaires. The same methodology was applied and compared with two control groups: Portuguese women of childbearing age living in Portugal and Swiss women of childbearing age living in Switzerland.

The assessment of vitamin D, vitamin K and calcium from the FFQ was done using the Nutritional. Software® (nut.s) science, version 1.32.44, 2012, for windows. The studied micronutrients were calculated using Excel® file, version 15.0, 2013, for windows, as the following example: a participant self-reported to have eaten a portion "M" of beans, with the frequency "2-3 times in the last month". It can be assumed she ate on average (2+3) /2= 2.5 times a portion "M" of beans in the last 30 days. The portion "M" weights 40 g and the calcium content in 100 g of beans is 36 mg, according to the nut.s software [19]. Therefore, the calcium content was calculated as Ca_ Beans = ((0.4 x 36) x 2.5)/30 = 1.2 mg. Therefore, we may conclude that in the last 30 days the participant had an average daily intake of 1.2 mg of calcium from the eaten beans [20].The supplements were then calculated separately and added to the same document in a different column entitled "Current Supplementation". For the present study, only the current supplementation was considered for dietary supplements intake calculation. Therefore, only participants who were taking any form of supplements (containing vitamin D and/or vitamin K and/or calcium) at the time of the interview-questionnaire application were considered for further supplements calculations.

Afterwards, the data was statistically analysed using IBM SPSS® Statistics software, version 24.0, 2016, for windows. Different statistical methods were used to relate pairs of variables. One-way analysis of variances (ANOVA) test was applied to correlate one categorical variable with more than two levels with a numeric and continue variable. This allowed us to determine if the means of independent variables were statistically significant different between groups. If the p value resulting from the ANOVA test was < 0.05 it meant that at least one of the 3 groups (PortPT, PortCH and SwissCH) is statistically significantly different from the others. Following the one-way ANOVA test, the Fisher's Least Significant Difference (LSD) test was used to explore comparisons and statically significant differences between specific pairs of participant groups: PortCH with PortPT, PortCH with SwissCH and SwissCH with PortPT. This test allowed a better understanding of specific statistical differences with the multiple mean comparisons. The Student's test was applied to numeric and normal distributed variables. It was used to determine if different mean values were statistically significant different from a standard value. The Pearson's correlation was used to assess correlation between a pair of numerical and continues variable. For each test, the null hypothesis was rejected when their probability value was less than 5 % (p value < 0.05).

Results

Socio-demographic information

The participants of the present study were recruited from the northern part of Portugal and the German-speaking part of Switzerland. The main inclusion criteria for participation were being healthy adult

women of childbearing age (20 to 44 years old) [21].

The sample consisted of a total of 63 women: 21 Portuguese women living in Portugal (PortPT), 21 Portuguese women living in Switzerland (PortCH) and 21 Swiss women residing in Switzerland (SwissCH). All 63 women said "yes" regarding the "Participation agreement" (consent form). Portuguese women in Portugal were resident in the cities of Braga (62%) and Porto (38%). Portuguese and Swiss participants in Switzerland were recruited from 13 different cities. However, the majority were residents of Bern and Zurich, 45% and 21% respectively. The women were aged between 20 and 44 years old, with a mean of 33 years and Standard Deviation (s^2) of 1.97 years. When the Portuguese women in Switzerland (focus group) were asked about how long they had been living in Switzerland, 38% reported between 2 and 5 years, almost 29% between 11 and 20 years and 19% between 6 and 10 years. The most common educational background was Bachelor degree (44%), followed by "High school diploma" for 25% of the participants. Body Mass Index (BMI) was normal for most of the women (n = 52). 11 women had BMI values indicating they were overweight (25 to 29.9 kg/m^2) (Table1).

Table 1: Socio-demographic characteristics of respondents

Consent form agreement (Total number of participants)	**(N)**	%
Yes	63	100
Country of residence (N)		
Portuguese in Portugal	21	33
Portuguese in Switzerland	21	33
Swiss in Switzerland	21	34
Years living in Switzerland (PortCH) (N)		
< 2	1	5
2 - 5	8	38
6 - 10	4	19
11 - 20	6	29
>20	2	9
Education background (N)		
9th grade	1	2
High school diploma	16	25
Professional course	9	14
Bachelor	28	44
Master	8	13
Doctoral/Post-doctoral	1	2
BMI (N)		
Normal	52	83
Overweight	11	17
Age range (Mean)	33 (20 to 44),	SD=1.97†

Descriptive statistics; Absolute and relative frequencies
†SD: Standard deviation

Fortified foods and dietary supplements

None of the participants reported having consumed any fortified foods in the last month. Regarding dietary supplements intake (Multivitamins/ multivitamins-mineral or single calcium and/or vitamin D and/or vitamin K) in the last year, 14% of PortPT, 29% of PortCH and 52% of SwissCH reported having taken dietary supplements. At the time of the study, none of the PortPT and only one PortCH were taking dietary supplements, whereas almost half of the SwissCH (48%) were, however all these participants reported an intake of dietary supplements containing only vitamin D (D2 or D3) and none of those were taking supplements containing calcium or vitamin K (Table 2).

Table 2: Consumption of fortified foods and vitamin D dietary supplements by participant groups

	PortPT n (%)	**PortCH n (%)**	**SwissCH n (%)**
Fortified foods intake in the last month (vitamin D, or/ and K, or/ and calcium)			
Yes	0 (0)	0 (0)	0 (0.0)
No	21 (100)	20 (95)	20 (95)
I do not know	0 (0)	1 (5)	1 (5)
Dietary supplements in the last 12 months (vitamin D)			
Yes	3 (14)	6 (29)	11 (52)
No	18 (86)	15 (71)	10 (48)
I do not know	0 (0)	0 (0)	0 (0)
Current dietary supplements intake (vitamin D)			
Yes	0 (0)	1 (5)	10 (48)
No	21 (100)	20 (95)	11 (52)
I do not know	0 (0)	0 (0)	0 (0)

Descriptive statistics; Absolute and relative frequencies

Vitamin D

The means of the total daily dietary vitamin D intake of each participant group, determined from the FFQ alone, were 7.45 µg, 6.04 µg and 6.76 µg respectively for PortPT, PortCH and SwissCH and statistically significant differences were found between the Portuguese groups (p = 0.041). When the current vitamin D dietary supplements were added, the mean intake of PortPT remained the same; however the mean intake of the PortCH increased to 6.64 µg, rendering the differences between Portuguese groups not significant. The Swiss group increased significantly to 11.1µg and therefore, the mean differences between SwissCH and the Portuguese groups became statistically significant ((mean difference PortCH-SwissCH = -4.46 µg (p = 0.002) mean differences SwissCH-PortPT = 3.64 µ (p value = 0.010)). When accounting for the dietary supplements, the SwissCH group's mean daily intake (11.1 µg) was the closest to the DRV of 15 µg.

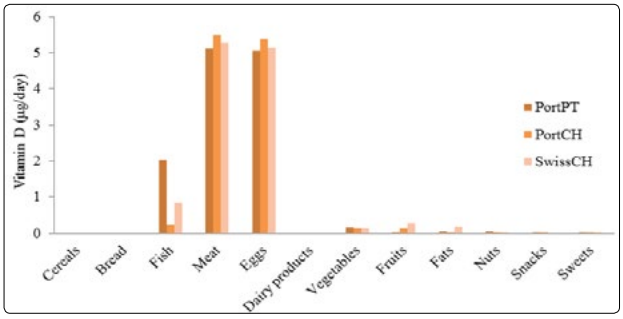

Figure 1: Vitamin D intake between participant groups from the FFQ alone and with the current dietary supplements intake (µg/day)

Figure 2 shows the daily dietary D intake per food group. The vitamin D intake comes mainly from the meat and meat products and eggs and egg products, with no statistical significant differences between participant groups. Differences between participant groups were found for intake from fish and fish products group ($p < 0.001$). The PortPT had the highest intake of vitamin D coming from the fish group (2.04 µg/day), followed by the SwissCH with 0.85 µg/day. The PortCH had the lowest vitamin D coming from this group (0.23 µg/day). Other important statistically significant differences were found in the groups "Fats" and "Snacks".

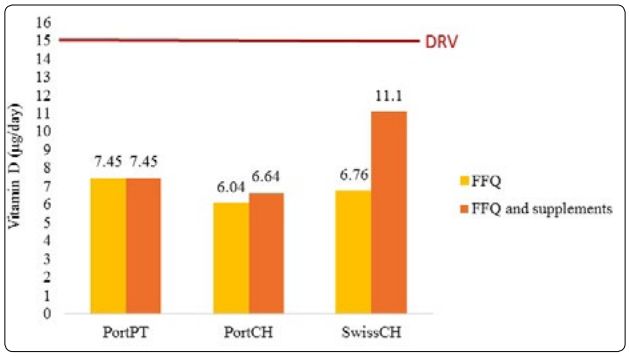

Figure 2: Vitamin D intake by FFQ food groups (µg/day)

Vitamin K

The mean total daily vitamin K for PortCH was statistically significantly lower compared with the control groups ($p = 0.045$). The PortCH had a mean vitamin K intake of 102.2 µg/day, while PortPT and SwissCH had a mean intake of 133.5 µg/day and 136.2 µg/day respectively. PortCH had an average vitamin K intake of 31.22 µg/day lower than the PortPT ($P = 0.038$) and almost 34 µg/day lower than the SwissCH ($p = 0.025$). Regarding DRVs, all the three participants reached the adequate intake of 70 µg/day for female adults.

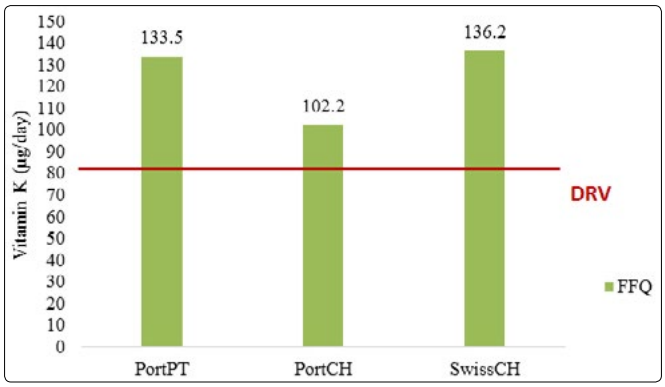

Figure 3: Vitamin K intake between participant groups (µg/day)

Figure 4 show that the "vegetables" group provides the greatest contribution to the vitamin K for all the three groups of participants. The PortPT group had the highest vitamin K intake coming from this food group (105.07 µg/day), followed by the SwissCH with 84.42 µg/day. The PortCH had the lowest vitamin K from the vegetables (66.73 µg/day). Statistical significant differences were found between participant groups ($p = 0.015$).

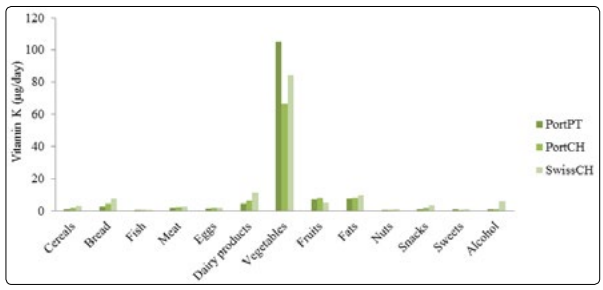

Figure 4: Vitamin K intake by FFQ food groups (µg/day)

Calcium

Regarding the mean total dietary calcium intake from the FFQ, statistically significant differences were found between the SwissCH and the Portuguese groups ($p < 0.001$). The focus group, PortCH, had the lowest mean value (634.1mg/day), followed by the PortPT (703.5 mg/day). The Swiss group had the highest calcium intake (1355.3 mg/day). The DRV of 1000 mg for calcium was not achieved by either of the two Portuguese groups. The Swiss group was the only group reaching the DRV for calcium.

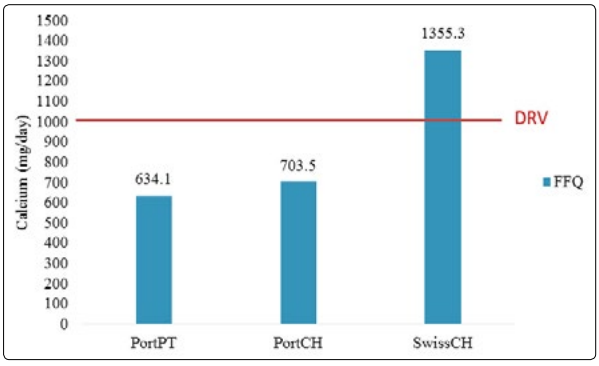

Figure 5: Calcium intake between participant groups (mg/day)

Figure 6 shows the average daily calcium intake from the different food groups. Statistical significant differences between participant groups were found for "dairy products", "vegetables", "Bread", "cereals", "fats", "snacks" and "water". In all participant groups, the food group with the largest contribution to calcium intake was "dairy products". The Swiss group had the highest calcium intake from the dairy (666.49 mg/day), and it was statistically significantly higher when compared to the Portuguese groups ($p < 0.001$). The daily calcium intake for the Portuguese groups coming from the dairy was 339.89 mg and 309.11 mg for PortPT and PortCH, respectively. The "vegetables" food group contributed with 73.21 mg/day (PortPT), 49.74 mg/day (PortCH) and 67.87 mg/day (SwissCH) to daily intake, with a p value of 0.015 for differences between groups.

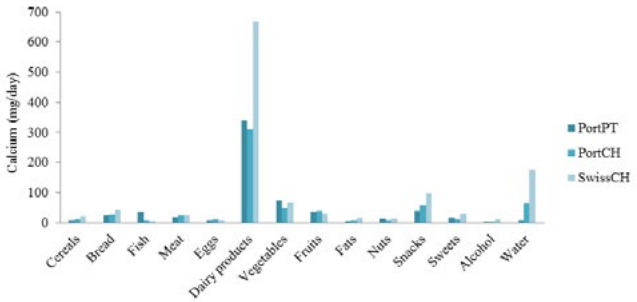

Figure 6: Calcium intake by FFQ food groups (µg/day)

Calcium intake from the water

Table 3 contains the information of calcium content, in milligrams per litre, in all of the mineral/bottled water and tap water consumed by the study participants. The average calcium content in the Swiss bottled water was 54 mg/L, while it was 1.25 mg/L in the Portuguese bottled water. Regarding tap water, in Switzerland, the quantity of calcium in the water varied between 107 mg/L (Aargau) and 8.39 mg/L in Davos (mean = 70.38 mg/L). In Portugal, the tap water in the area of Braga contained approximately 38 mg/L, and 33.40 mg/L in the area of Porto [22, 24].

Table 3: Source of the water intake (tap/bottled) and calcium content per litre of water (including tea and sparkling water)

	Bottled (Brand)*	Calcium(mg)/L	Tap (City)	Calcium(mg)/L
Swiss drinkable water				
	A	100	Aargau	107
	B	80	Valais	91.82
	C	50	Bern	89
	D	30	Fribourg	84
	E	10	Zürich	70.15
	-	-	Glarus	42.3
	-	-	Davos	8.39
Mean	-	54¥		70.38¥
Portuguese drinkable water				
	F	2.7	Braga	37.98
	G	1.5	Porto	33.40
	H	1.1	-	-
	I	1	-	-
	J	0.8	-	-
	K	0.4	-	-
Mean	-	1.25¥	-	35.69¥

¥ Mean of the calcium intake (mg/L)
* A-K Registered brand

Figure 7 shows the relative quantities (percentage) of water consumed by the three participant groups, according to source. The PortPT reported drinking mainly bottled water (95%), and, on average, only 5% of their water intake was from the tap. This scenario was reversed for the SwissCH, with an average intake of 95% of the water coming from tap, and with only 5% of consumption of bottled water. 71% of the PortCH daily water intake was from the tap and 29% from mineral/bottled water.

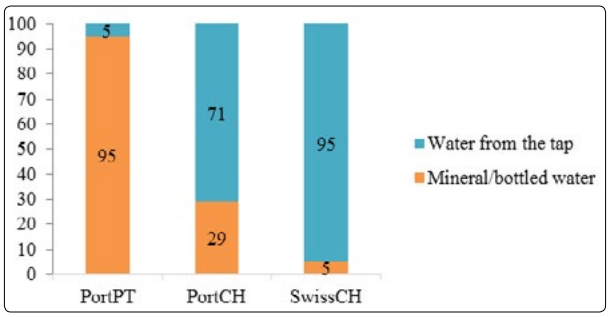

Figure 7: Source of the water intake (%)

Figure 8 shows the average daily calcium intake from water for each participant group. Statistically significant differences were found between all three groups. The PortPT had the lowest intake of calcium from water (7.734 mg/day), followed by the PortCH with 66.364 mg/day. The SwissCH group had the highest daily intake of calcium from water (175.598 mg) ($p < 0.001$).

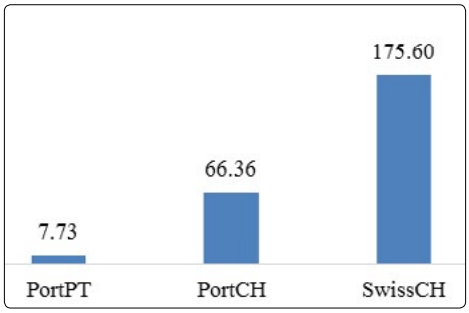

Figure 8: Average calcium intake from water (mg/day)

The quantity of water intake (L/day) was also significantly different between the Swiss and the Portuguese groups. Regarding the daily water consumption, PortCH appeared to be the group with the lowest intake (mean = 1.381 L/day), followed by the PortPT with an average of 1.408 litres of water consumed. The SwissCH had the highest intake, with over 2L/day on average

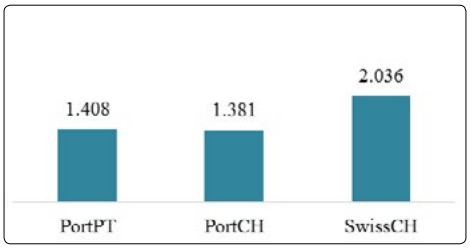

Figure 9: Average water intake (L/day)

Comparison of vitamin D, vitamin K and calcium intake based on the FFQ and the 24H-R

Table 4 hows the Pearson's correlation coefficients between the two methods (FFQ and 24H-R), for the targeted micronutrients, according to participant groups. In the case of the PortPT group, strong positive correlations were found, with coefficients of 0.836, 0.865 and 0.915 for vitamin D, vitamin K and calcium respectively ($p < 0.001$). PortCH had a statistically strong positive correlation between methods for vitamin D ($r = 0.694$; $p < 0.001$), and for calcium ($r = 0.521$; $p = 0.012$). However, a non-statistically significant difference was found ($p = 0.173$) for vitamin K between the two methods, with only a moderate correlation of 0.309. The SwissCH group showed a statistically significant strong positive correlation between methods for the three micronutrients: $r = 0.492$ for vitamin D ($p = 0.023$), $r = 0.635$ ($p = 0.002$) for vitamin K and $r = 0.883$ for calcium ($p < 0.001$).

Table 4: Correlation between vitamin D, vitamin K and calcium intake based on the FFQ and the 24H-R

	N	N	Correlation	P
PortPT	D_FFQ & D_24	21	0.836‡	<0.001*
	K_FFQ & K_24	21	0.865‡	<0.001*
	CA_FFQ & CA_24	21	0.924‡	<0.001*

PortCH	D_FFQ & D_24	21	0.694‡	<0.001*
	K_FFQ & K_24	21	0.309	0.173
	CA_FFQ & CA_24	21	0.537‡	0.012*
SwissCH	D_FFQ & D_24	21	0.492‡	0.023*
	K_FFQ & K_24	21	0.635‡	0.002*
	CA_FFQ & CA_24	21	0.883‡	<0.001*

Pearson's correlation (r)
D/K/Ca_FFQ: Dietary intake of vitamin D/ vitamin K/ Calcium from the FFQ
D/K/Ca_24: dietary intake of vitamin D/ vitamin K/ Calcium from the 24H-R
* Significant differences between FFQ and 24H-R intake, $p < 0.05$.
‡ Significant correlation between FFQ and 24H-R, $p < 0.05$.

Discussion

In the present study, none of the participants reported having consumed fortified foods in the last 30 days. This could be because they are not yet aware of the positive nutritional and health benefits of these specific foods. At the same time, low income may be the main reason for not choosing these specific food items, as well as lack of health and nutritional claims written on the package targeting the focus group of the study. Specifically regarding milk, the health claims written on fortified products target children and the elderly in both Portugal and Switzerland, directly impacting the demographic purchasing these products [25]. Women of childbearing age are not targeted for these enriched milk products. Therefore, the advertisements for these products will not capture their attention. In addition, the general population is still not yet focused on the importance of overall health status to prevent diseases, but mainly interested in rapid treatments to cure and solve specific health problems. The role of public health interventions is to provide the information and awareness about food and nutritional education that could prevent future undesirable outcomes.

Dietary supplements, rather than fortified foods, have emerged in several European countries [26, 27]. The pharmaceutical industry has been developing an enormous number of dietary supplements, either containing a single vitamin or mineral, or combinations of specific micronutrients in formulas. Additionally, there are large advertising campaigns informing the population about the benefits of complementing a healthy diet with supplements [28]. Based on the results of the present study, Switzerland seems to have a strong culture of vitamin D supplementation in comparison to Portugal. This could be the reason why the intake of the supplements differs so much between the participant groups (PortPT, PortCH, and SwissCH). Apparently Swiss women are more aware of the problems resulting from vitamin D deficiency than Portuguese migrants. Half of the Swiss participants were taking vitamin D supplements, whereas only one Portuguese migrant was (at the time of the interview-questionnaire administration). None of the Portuguese women in Portugal were using vitamin D supplementation at the time of the interview. This correlates with the observation that when intake from vitamin D supplements were added to the total vitamin D intake from the FFQ, the mean differences between Swiss and the Portuguese groups became significant. These findings suggested that dietary supplements significantly increase the micronutrients intake in the observed population. Similar conclusions have been found in other studies assessing micronutrient intake from food and supplements. A population-based study in Switzerland concluded that only a small amount of vitamin D is contained in the diet [29]. In the United States, it was found that dietary supplements intake provides an important contribution for the recommended dietary intake of vitamin D and calcium. The authors concluded that around 43% of the overall population, and 70% of women, self-reported the use of calcium supplements [5]. Moreover, supplement intake appeared to be a crucial factor in the vitamin D daily intake among the Swiss in our study, which means that this group is the one closer to achieving the recommended amounts. For the Portuguese migrants, vitamin D intake was incredibly far below the recommended level, with less than the half of the expected DRV (8.3 mcg/day) being consumed.

When evaluating micronutrient intake, it is also important to study to what extent different food groups contribute to the daily intake of specific vitamins and minerals. This may differ between cultures according to their culinary and dietary practices. This study finds that meat and eggs were the two main food groups contributing the most to the daily intake of vitamin D for all three participant groups. The large contribution from the meat group originates from the high consumption of meat and meat products, and not because it constitutes a good source of vitamin D. The large intake from the eggs is due to both high consumption of eggs and eggs products and significant concentration of vitamin D in eggs [30]. It is very common for meat and/or meat products to be found in at least one of the main meals (lunch or dinner) of the Portuguese diet. A Swiss study has found that Portuguese migrants have a higher meat intake when compared to other migrants. However, this study did not find any statistically significant differences in the consumption of meat and egg products between Portuguese and Swiss groups [13], which is in agreement with our results. Moreover, several other research studies around the world have found meat to be the main contributor of vitamin D intake in many different populations. In Ireland, a representative cross-sectional study showed that "meat and meat products" was the biggest contributor to the vitamin D intake (34%) in the Irish diet [31]. Another study that assessed the vitamin D intake in a Serbian region without food fortification concluded that the highest percentage of vitamin D in the diet also comes from the "meat and meat products section" [32]. In Germany, a cohort study reported different results. In this case, fish and fish products were the food group contributing with the highest vitamin D percentage [33]. In the present study, it could be expected that the Portuguese women in Portugal would be the participant group with the greatest vitamin D intake from the fish group. Since Portugal is surrounded by sea and has a culinary culture based on the Mediterranean diet, there is a much higher consumption of fish and fish products when compared to a land-locked country such as Switzerland. Therefore, it is not surprising that the higher contribution to vitamin D intake for the Portuguese in Portugal comes from fish and fish products. Our results agree with a cohort study conducted in Porto, Portugal, assessing calcium and vitamin D intake which found that meat, eggs and fish products contributed most to vitamin D intake among the participants [34]. It could also be expected that the group of Portuguese women living in Switzerland would have a greater intake of fish and fish products than the Swiss. Surprisingly this was not the case and the PortCH was the group with the lowest dietary vitamin D intake coming from the fish and fish food group. This difference can be explained by two factors: 1) the limited variety and freshness of fish available in Switzerland in comparison to a country surrounded by sea, such as Portugal; and 2) fish products in Switzerland are expensive, and migrants often have lower incomes than homeland residents.

As expected, most of the vitamin K intake came from the vegetable group. We fond statistically significant differences in levels of vegetable derived vitamin K between participant groups. This could be the result of two different reasons: the intake of vegetables in terms of quantity/ portion sizes, and the intake of different variety of vegetables (which will differ in vitamin K content) between Portuguese and Swiss women. The Portuguese have the habit of consuming soup before the main dish. Portuguese soups contain a wider range of vegetables, and therefore this could be the major explanation for Portuguese women in Portugal had the highest value of vitamin K coming from the "vegetables" group. Moreover, dark leaved vegetables, such as spinach, tronchuda cabbage and galega kale, are strongly present in the Portuguese diet, particularly in northern cities during the autumn and the winter. These are all good sources of vitamin K. Similar results for the distribution of vegetable-derived vitamin K levels were found in 2013 study from the School of Health Professions, Zurich University of Applied Sciences. That study compared the fruit and vegetable consumption of migrants living in Switzerland, including Portuguese migrants, with their Swiss counterparts and showed that migrants from Portugal had a lower vegetable intake than the Swiss [35]. This result is in accordance with the present study, where Portuguese women in Switzerland are the group with the lowest vitamin K intake from vegetables, which indirectly shows the lower intake of vegetables in the diet in comparison with the control groups [13]. However, all three participant groups attained the DRV for vitamin K, and so vitamin K deficiency does not seem to be a problem for any of the groups.

In contrast to the results for vitamin K, statistically significant differences were found in the calcium intake between the three participant groups, especially in the food groups of "dairy products" and "water". The very high calcium intake coming from dairy products in the Swiss group was expected. The Swiss diet is well known for its wide variety of dairy products and high consumption of milk, cheese and yogurt products, which could easily explain the 666.5 mg of calcium from the "dairy" intake seen in the Swiss women of the present study. At the same time, for reasons of acculturation and adaptation to the Swiss diet, it was expected that the Portuguese living in Switzerland would also present a high value of dairy-derived calcium. However, this was not the case, and the PortCH proved to have less than half of the dairy-derived calcium levels of the SwissCH cohort. Moreover, both Portuguese groups consumed very small amounts of calcium coming from dairy products. Since only 10 % of the PortCH group had been living in Switzerland for more than 20 years, it is possible that most Portuguese immigrants in the study are still following a diet reminiscent of their home culture, and have not started consuming more dairy products. It would be interesting to correlate the "years living in Switzerland" with the calcium intake from the "dairy products" to determine if the diet changes with living time in a new country. No statistically significant temporal trend was observed in this study, possibly due to the limited sample of participants.

Concerning the distribution of calcium intake according to food group, the most diverse results are the ones related to water consumption. In the present study, the calcium intake from both tap and mineral water was vastly different between the three participant groups, being lowest for the Portuguese women in Portugal. Almost all of the interviewed Portuguese women in Portugal (95%) only drank bottled water either because they do not consider tap water to be good for consumption or simply because they do not like the taste of it. This happens despite the proper treatment of tap water in parts of the cities of Braga and Porto [36]. The very low calcium intake from water in the PortPT participants is explained by 1) the fact that both tap and bottled water have very low calcium contents (mean = 1.25 mg/L) and 2) PortPT have a much lower daily intake of water. Throughout Switzerland, water is highly treated and quality controlled, reaching gold standards of quality. Therefore, in contrast to the PortPT, almost all the Swiss participants (95%) reported drinking tap water, even though bottled mineral water was sometimes available. In Europe, the amount of calcium in tap and mineral water varies from 1.5–600 mg/L [37]. In Switzerland, the calcium concentration in tap water is particularly high compared to other countries, including Portugal. In the present study, Swiss tap water consumed by the study participants had an average of 70.4 mg of calcium per litre (Portuguese tap water = 35.7mg/L).

Considering Portuguese and Swiss women living in Switzerland have access to the same water, it was not expected that the water-derived calcium intake would be significantly different between these two groups. However, the observed differences can be explained by both the type and quantity of water drank by the two groups. The PortCH consumed almost half the amount of water the Swiss women did. Addition, 29% of water consumed daily by PortCH was mineral bottled water, which on average contains less calcium that tap water. The Centre of Bone Diseases from the University Hospital of Lausanne in Switzerland conducted a study that compared the mineral concentrations in 150 different waters across Europe. It concluded that calcium rich waters have a positive effect on bone metabolism and provide a good alternative to dairy products. As well as the large quantity of calcium, there are other components in the water that influence calcium bioavailability. The same study found that high levels of calcium in the water correlate with higher levels of SO4, which may increase the calcium excretion [37]. Therefore, further study is needed to a better understanding of the inaccessibility and bioavailability of calcium from different waters [38].

Our results for the vitamin D, K and calcium intake from the FFQ showed good agreement with those from the 24H-R. The overall strong correlation between methods confirms the validity of the FFQ developed for the target group (PortCH). The weakest correlation was observed for vitamin K in the PortPT group ($r = 0.309$). Similar weak correlations between dietary assessments methods for vitamin K were found in other studies. Particularly, a study validating an FFQ against a three-time 24H-R for bone health in pregnant women also found only a moderate correlation between methods for vitamin K intake ($r = 0.41$) [8]. In Canada, a pilot study for the validation of an FFQ for the intake of vitamin D, K and calcium daily intake had similar results to our study, with $r = 0.63$, 0.89 and 0.54 for calcium, vitamin D and vitamin K, respectively [39]. To summarise, the present study provides preliminary scientific evidence for the validation of an FFQ focusing on the bone micronutrients (vitamin D, vitamin K and calcium) in Portuguese women of childbearing age living in Switzerland.

Conclusions

Our goal was to study the intake of vitamin D, vitamin K and calcium in migrant Portuguese women of childbearing age living in Switzerland. We not only collected data on the dietary intake of this group, but also carried out a comparative study between the Portuguese women living in Switzerland and both their Swiss

counterparts and Portuguese women living in Portugal. To our knowledge, this is the first study comparing bone micronutrients between migrants and native residents.

We conclude that Portuguese women living in Switzerland are at a higher risk of inadequate vitamin D and calcium intake than Swiss women. Additionally, vitamin K intake was significantly lower compared to both control groups (Portuguese women living in Portugal and Swiss women living in Switzerland). However, the average vitamin K intake was adequate in all participating groups. In addition, Swiss women had the highest average intake of all three micronutrients: vitamin D, vitamin K and calcium.

It can also be concluded that the methods used in this study (FFQ and 24h-R) are useful tools for the quantification of the targeted micronutrients.

Recommendations

Further studies do need to further validate the FFQ as a tool to analyse the vitamin D, vitamin K and calcium in the diet of Portuguese immigrants living in Switzerland. In the future, this tool may be adapted to study other age groups of Portuguese living in Switzerland, such as children and the elderly, and could even be used as a reference for the development and application of similar tools to other minority groups which may be at risk of dietary deficiencies or insufficiencies.

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