Vitamin d is a fat soluble vitamin health essay

Health & Medicine

1. Abstract

Vitamin D is a fat soluble vitamin that plays a role in regulating the levels of calcium in the body. Recent studies suggest that vitamin D deficiency maybe associated with diseases such as cardiovascular diseases, osteoporosis and cancer are prevalent in Singapore. The Recommended Daily Allowance (RDA) of vitamin D is 100 IU. Currently, there is no validated Food Frequency Questionnaire (FFQ) designed to measure the dietary vitamin D intake in Singapore. Thus, the objective of our study is to validate a newly designed 32-food item FFQ and its ability to measure the dietary vitamin D intake of young adults in Singapore. For this study, 30 subjects were recruited, all fulfilling a set criteria (BMI 18-23kg/m2 and aged 21-35 years), to complete a FFQ and a 3-day 24 Hour Recall assessing their dietary vitamin D intake. Dietary vitamin D intake as recorded in the FFQ and 24 hour recall were analyzed via Foodworks to investigate the agreement between the two dietary assessment methods. The Bland Altman test shows a statistical agreement between the 2 methods and the values are within the two standard deviations. However, given that the RDA is 100 IU and the two standard deviations are -227. 8 to +247. 4, the FFQ has limited clinical applicability. Nevertheless, Kappa (0. 286) suggests that the FFQ may be used as a screening tool for inadequate vitamin D intake. In conclusion, the FFQ may be used as a screening tool for inadequate vitamin D intake, however, is not accurate enough to measure the true value of the dietary vitamin D intake of subjects.

2. Introduction

2. 1 Background Information on Vitamin DVitamin D is a fat soluble steroid hormone precursor found in the body of humans and animals that plays a role in the regulation of calcium levels in the body. There are two forms of vitamin D: Vitamin D3 (Cholecalciferol) and Vitamin D2 (Ergocalciferol). Both vitamin D3 and vitamin D2 can be found naturally in certain foods. Vitamin D3 is a very unique vitamin as humans are able to self-synthesize vitamin D3 in the skin upon sufficient exposure to ultraviolet radiation. Due to this self-synthesizing characteristic, vitamin D3 is technically not a vitamin (Herrmann & Obeid, 2011). Based on the Singapore's Health Promotion Board, inadequate vitamin D intake is defined as <100 IU per day. 2. 2 Sources of Vitamin DThere are two major sources of vitamin D that can be obtained. The most abundant source is via the Ultra Violet rays. Found in our skin is a high concentration of a sterol cholesterol compound. This sterol cholesterol undergoes conversion by an enzyme into the sterol 7-dehydrocholesterol. When we are exposed to the solar ultraviolet B (UVB) radiation of the sun, this sterol 7-dehydrocholesterol is converted into a pre-vitamin D3, a precursor of vitamin D3 (Herrmann & Obeid, 2011). The other source is through dietary intake of vitamin D either from food or supplements. All sources of vitamin D undergo two hydroxylation processes in the body for activation. The first hydroxylation occurs in the liver which converts vitamin D to 25-hydroxyvitamin D (25(OH)D), also known as calcidiol. The second hydroxylation occurs mainly in the kidney which converts 25(OH) D to its active form of vitamin D in the body, 1, 25-dihydroxyvitamin D (1, 25(OH) $_2$D), also known as cacitriol. http://www. nimr. mrc. ac. uk/images/multimedia/mill_hill_essays/vitd-fig1-large. gifVitamin D

Synthesis in the body. Obtained from The National Institute for Medical ResearchDietary intake of vitamin D is naturally present in a selected number of foods, such as fish liver oils and egg yolks, and is also available in fortified vitamin D foods such as breakfast cereals, milk, fruit juices and margarines. Vitamin D in the form of dietary supplements is also commercially available (Office of Dietary Supplements, 2011). 2. 3 Functions and Importance of Vitamin DOne of the functions of Vitamin D is to aid calcium absorption in the body, which is important for good bone health. However, the functions of vitamin D extend far beyond maintaining calcium homeostasis. Vitamin D may also play a role in decreasing the risk of some chronic diseases, including some cancers, autoimmune diseases and cardiovascular diseases (Grant & William B, 2008). This unique vitamin acts upon the whole body to regulate cell growth and delay the formation and development of certain immune diseases and even cancer (Rachel & M. D, 2009). However, recent studies show that, as compared to westerners, Asians are said to have low peak bone mass in relation to the insufficient intake of vitamin D, despite the constant availability of sunlight (Goswami, R; Mishra, S K & Kochupillai N, 2008) (Masud F, 2008). Therefore it can be assumed that the levels of vitamin D in Singaporeans would be at a constant level or roughly maintained. In contrast to other countries such as Europe or America whereby the 4 seasons are experienced, the levels of vitamin D would fluctuate according to the seasons. Despite the presence of abundant sunlight, vitamin D deficiency has been reported in all age groups, especially in the urbanized areas, from toddlers to the elderly (A H Zargar et al. 2007). This is most likely due to the fast-paced sedentary lifestyles urbanites have. Urbanites usually spend their days indoors and in air-conditioned

environments. This would lead to insufficient exposure time to the sun. On top of insufficient sun exposure, other factors that may cause the lack of vitamin D include type of clothing, dressing styles, cultural and personal habits. It is known that vitamin D absorption from the sun is also related to individual’s skin pigmentation. People with darker skin would require sun exposure time of up to 5 times in order to absorb the same amount of vitamin D. Also, in fast-paced urbanized areas, bad dietary habits are common, which may also be a contributing factor to vitamin D deficiency due to inadequate consumption of vitamin D rich foods. An experiment conducted in Singapore showed that 95% of the 40 subjects had serum 25(OH)D levels below the sufficient value of <20µg/L, and with 5%of 21-302µg/L. (J Gwee, Na Alshaikh & S Lo, 2011). It is thought of that our young Singaporeans aged 21 to 35 are the most active age group outdoors, thus having the most amount of time spent in the sun. This awareness has led to an increased interest to conduct studies of dietary intake of vitamin D, especially amongst young adult Singaporeans. Thus, we are trying to find out whether young adults in Singapore are still deficient of Vitamin D under such circumstances. The main sources of vitamin D are generally from ultra violet rays. It is impractical to suggest for individual to be exposed to the sun in view of the risk of skin cancer. Thus, it is safer to obtain vitamin D from the diet with the RDA of 100 IU. 2. 4 Measuring Vitamin D LevelsMost studies that validate the accuracy and the validity of a dietary tool are done via comparing the measure dietary amount to the serum levels of the vitamin or mineral in question. In the context of vitamin D, 25(OH)D is used as a biomarker to define sufficiency, insufficiency and deficiency. 25(OH)D is used because of the long half-life it possesses as opposed to 1, 25(OH)D, which

has a short half-life. However in our study, biomarkers of vitamin D was not used as a gold standard reference, mainly because 25(OH)D comes from both dietary sources and exposure to the sun. In view of this, we would have to use another dietary measuring tool, in our case, the 24 hour recall to validate the FFQ, which will be discussed in later sections.

3. Objective of Project

This study aims to validate the use of a Food Frequency Questionnaire's to assess the dietary vitamin D intake of young adults in Singapore.

4. Review of Literature

4. 1 Food Frequency Questionnaire and 24-hour RecallThe Food Frequency Questionnaire (FFQ) is often used to assess habitual dietary intake in epidemiological studies on diet and chronic diseases. FFQs are not only used as a food record, but are also used to characterize the dietary habits of each individual, without the reliance on multiple day assessments. Its ability to assess the estimation of an individual's usual food consumption over a defined period of time might be sufficient for the estimation of relative risks to certain nutrient deficiencies. Since the principle of FFQ is to tap on an individual's average long-term diet and consumption pattern over a period of time, it is theoretically a better tool to determine chronic illnesses as compared to an assessment method which measures the intake for a few days. In this case, a FFQ would be the most appropriate tool for the indication of Vitamin D deficiency and its related diseases. However, detection of chronic diseases is not in the context of our study. The main reason why FFQ was chosen is that relative to other dietary assessments, such as 24 hour recall (retrospective) or food record (perspective), the FFQ is

lower in both cost and subject burden. (Pedro Marques-Vidal et al, 2011 & Hongyu Wu et al, 2009)Based on the American Society for Clinical Nutrition (1999), it is common for FFQs to be used with the 24-hour diet recall to assess dietary intake. (Anja Kroke et al, 1999) Since the use of FFQ is aimed to assess an individual's usual long-term dietary intake, the relative validity of FFQ will be assessed by comparing the data collected against a reference, in this case, the 24 hour diet recall. Also, most validation studies conducted have reported a strong correlation between estimates from both methods used together. Therefore, to determine the ability of an FFQ to measure dietary vitamin D intakes of individuals of 21 to 35 years old, we used the data obtained from the 24 hour recall as a ' gold standard' to validate the FFQ. 4. 2 Bland-Altman Statistical AnalysisThe Bland-Altman Statistical Analysis is used to compare two different kinds of measurement methods by comparing the mean difference of the data obtained from the two methods, usually at a 95% limit of agreement. The Bland-Altman is used to find the agreement between continuous variables. This method of analysis has been used in over 11500 similar cases, evidencing its accuracy and usefulness (British Journal of Anaesthesia, 2007). In the study, the 2 methods of measurement being compared are an invalidated FFQ and a 3-day 24 hour recall. The data which was compared is the amount of dietary vitamin D intake from 30 subjects who had participated in both the FFQ and 24 hour recall. Statistical Package for the Social Sciences SPSS version 20 was used to analyze the 2 sets of data. The data obtained from both methods of measurement is then plotted on a Bland-Altman Plot, which is visually compared to evaluate how well the 2 methods of measurement agree to each other. The 2 things that need to be visually evaluated is the number of

plots falling within the 95% confidence level and how close the plots are to the mean difference line. The more plots within the confidence level and the nearer the plots are to the mean confidence line, the stronger the agreement. 4. 3 Cohen’s KappaMeasured Vitamin D from the FFQ and 24 hour recall would be categorized in accordance to inadequate or inadequate intake, which is defined as <100 IU and ≥100 IU respectively. In view that these are categorical data, the Cohen Kappa statistic would be used to measure the degree of freedom between the two methods. The Kappa statistic is intended to give a quantitative measurement of the magnitude of agreement between different methods of assessment (Viera & Garrett, 2005). In this study, the Kappa statistic will be used to evaluate the agreement between a newly created FFQ and a 3-day 24 hour recall, both designed to evaluate the dietary intake of vitamin D in the same target population. The Kappa value (K) is obtained by calculating the difference between the amount of agreement observed (Po) to the amount of agreement expected through chance (Pe) (Viera et al, 2005). Po was obtained by calculating how many times a subject has the same results for his FFQ and 24 hour recall. After the Po value was obtained, the Pe value can then be calculated. The difference between the Po and Pe value is then used to calculate the K value. The K value obtained will then be standardized on a -1 to 1 scale and then interpreted according to Figure 1. The closer the K value is to 1, the stronger the agreement. In this study, in order to calculate the Po, Pe and K value, categorical data obtained from the FFQ and 3-day 24 hour recall is analyzed via the SPSS program version 20.

Poor

Slight

Fair

Moderate

Substantial

Almost Perfect

Kappa

0. 00. 20. 40. 60. 81. 0KappaAgreement <0Less than Chance Agreement0. 01-0. 20Slight Agreement0. 21-0. 40Fair Agreement0. 41-0. 60Moderate Agreement0. 61-0. 80Substantial Agreement0. 81-1. 00Almost Perfect Agreement(Figure 1: Interpretation of Kappa Value. Table and interpretations were obtained from the Family Medicine Journal, Understanding Interobserver Agreement: The Kappa Statistic)4. 4 Paired t-testA paired sample t-test is done when a subject undergoes two different types of treatments. The main function of the paired t-test is used to analyze the differences between each set of variables. In this study, the two treatments which a subject undergoes is the FFQ and 24 hour recall. The purpose of the paired t-test is to find out what is the chance that the difference between the dietary vitamin D value obtained through the FFQ and 24 hour recall is 0. (Armitage and Berry, 1994 & Altman, 1991)

5. Materials and Methods

5. 1 Recruitment of SubjectsIn total, 30 subjects were recruited for this study. The Principle Investigator (PI) has chosen the following as a selection criterion. The BMI of each subject must fall between 18-23 kg/m2 and has to

be 21 to 35 years of age. Subjects must also not be suffering from any illnesses and cannot be under any medication. Subjects should also not be taking vitamin D supplements. Recruitment was done by sending mass emails to individuals who fall under the criteria. 5. 2 Data CollectionThe Food Frequency Questionnaire (FFQ) used in this study was designed by the PI (Appendix 1) and will be validated against a 3-day 24 Hour Recall (Appendix 2) which was deemed as a gold standard for this study. To reduce interviewer bias, both FFQ and 24 hour recall was administered by the same investigator. The FFQ contained 32 food items that are rich in vitamin D and was administered to the subjects. Subjects completed the FFQ according to the frequency of the food they consumed throughout a month. Powerpoint slides containing pictures of the foods in the FFQ was used to facilitate the completion of the FFQ and to aid the subject's understanding of the foods found in the questionnaire (Appendix 3). All subjects also completed a 3-day 24 hour recall assessment. The recalls consisted of the diets of each subject of 2 weekdays and 1 weekend. This is to ensure that the values obtained by the assessment were from their regular eating pattern. In order to set the standards for the serving sizes, tools, such as cups and plates, were used during the recall interview to obtain uniform and accurate results. (Appendix 4) The duration of each FFQ was approximately 20 minutes long, and approximately 30 minutes for the 24 hour recall. 5. 3 Statistical Analysis5. 3. 1 FoodWorks 2009To reduce analytical bias, FoodWorks 2009 was used for the analysis of both FFQ and 24 hour recall. The data obtained from both FFQ and 24 hour recall was entered into FoodWorks 2009 to determine the dietary vitamin D intake. When comparing the dietary vitamin D intake between the two assessment methods, the average intake for one day was

compared. For example, in the FFQ, if a food was consumed once a month, the quantity of dietary vitamin D it contained was divided by 30. For the 3-day 24 hour recall, the value of each food was divided by 3. 5. 3. 2 Bland Altman's TestTo determine the strength of agreement between the FFQ and 24 hour recall, the vitamin D values obtained from both dietary assessment methods were plotted using the Bland Altman's test. The Bland Altman's plot was then visually evaluated to analyze the results obtained. 5. 3. 3 Cohen's Kappa TestThe values of the vitamin D of the FFQ and the 24 hour recall were then compared against the Singapore's Recommended Daily Allowance (RDA) of vitamin D. Based on Singapore's Health Promotion Board; the value of the vitamin D RDA is 100 IU. We have categorized the vitamin D value of both assessments into ' Sufficient' or ' Insufficient', by using RDA as a reference. The Kappa test was then done to obtain a Kappa value (K) which was evaluated to interpret the degree of agreement. 5. 4 Possible Errors InvestigationAny disagreements between the two dietary assessment methods were investigated by assessing the quality and quantity measurements of the two methods. Quality was measured by ranking the food found in the 24 hour recall according to their vitamin D contribution in terms of percentage. This is to investigate whether there are any foods outside the FFQ that significantly contribute dietary vitamin D. Quantity was measured via paired sample t-test. This is to investigate if over or under reporting had occurred during either of the dietary assessment methods. 5. 5 Measuring Quality of Vitamin D Rich Foods in the FFQThe quality of food found in the FFQ was investigated by finding out which foods contributed the most dietary vitamin D in each of the subject's diet. The 24 hour recall vitamin D data collected from FOODWORKS 2009 was converted into

percentages to evaluate which foods contributed the most vitamin D in their daily diet. (Appendix 5) This was done to check whether any foods that were low in Vitamin D was found in 24 hour recall and not in the FFQ, was consumed in such a high frequency that it contributed a significant amount of dietary vitamin D. Thus, the FFQ was incapable in assessing dietary vitamin D contributed by these low in vitamin D sources. 5. 6 Investigating Over or Under Reporting via Paired Sample t-testA paired sample t-test was done to investigate whether over or under reporting had occurred during either the 24 hour recall or FFQ. A paired t-test was chosen as each subject had undergone 2 different treatments, which are the FFQ and 24 hour recall. For each subject, the difference between their dietary vitamin D value obtained from the FFQ and 24 hour recall was calculated as well as the mean of the 2 assessment methods.

6 Results

6. 1 Result of Bland Altman plot(Figure 2: Difference of FFQ against mean of 24 hour recall and FFQ in IU)Figure 2 was obtained by comparing the data collected from the FFQ and the 24 hour recall by all 30 subjects. The y axis represents the difference between the data collected from the FFQ and 24 hour recall and the x axis represents the mean value of both methods of assessment. Each dot represents data collected from one subject. The range within the dotted lines represents the 95% confidence interval, ranging from +247. 4IU to -227. 8IU. The blue line in the middle represents mean difference of the 2 measurements, with a value of 9. 8. It was also observed that 28 out of the 30 plots fall within the 95% confidence interval with only two outliers. 6. 2 Result of Cohen’s Kappa Test

Food_Recall * FFQ Crosstabulation

FFQTotalInsufficientSufficientFood_RecallInsufficientCount426Expected Count2. 23. 86. 0sufficientCount71724Expected Count8. 815. 224. 0TotalCount111930Expected Count11. 019. 030. 0

Symmetric Measures

ValueAsymp. Std. ErroraApprox. TbApprox. Sig. Measure of AgreementKappa. 286. 1741. 705. 088N of Valid Cases30a. Not assuming the null hypothesis. b. Using the asymptotic standard error assuming the null hypothesis.(Figure 3: Measure of agreement between FFQ and 24 Hour Recall)The K value, 0. 286, was obtained by entering the categorical data of the vitamin D value (sufficient, insufficient) from all 30 subjects and then running a Cohen's Kappa test. The K value represents the agreement between the FFQ and the 24 hour recall. It is then interpreted by comparing it to Figure 1. After interpretation, the FFQ fairly agrees to the 24 hour recall. 6. 3 Results of Measuring Vitamin D ContributionStatisticStd. ErrorMean Percentage of 24-hour recall vitamin D food found in FFQMean81. 36672. 3116395% Confidence Interval for MeanLower Bound76. 6388Upper Bound86. 09455% Trimmed Mean81. 8148Median83. 5000Variance160. 309Std. Deviation12. 66133Minimum54. 00Maximum99. 00Range45. 00Interquartile Range19. 75Skewness-. 467. 427Kurtosis-. 806. 833(Figure 4: Mean of Vitamin D intake contributed from Foods found in the FFQ in terms of percentage)Figure 4 was obtained by calculating the total amount vitamin D intake of the subjects through foods found in the designed FFQ and then averaging the value. (Appendix 6) The mean of the values obtained is 84. 6%

which has a 95% confidence interval range of 76. 64 to 86. 09. 6. 4 Results of Paired Sample t-test

Paired Samples t- test

Paired DifferencesTDfSig. (2-tailed)MeanStd. DeviationStd. Error Mean95% Confidence Interval of the DifferenceLowerUpperPair 1ffq - recall-9. 80933121. 2263322. 13280-55. 0759935. 45732-. 44329. 661(Figure 5: Chances of over or under reporting of 24 hour recall)Figure 5 was obtained by inputting the vitamin D intake values from both the FFQ and 24 hour recall of every subject and then running a paired sample t-test which calculates the difference between the two methods. The significant 2 tailed value (p value) is the calculated chance that the difference in values obtained from both methods is 0. The mean obtained is -9. 81 with a 95% confidence interval range of -55. 08 to + 35. 46 and a p value of 0. 661.

7. Discussion

Results from the Bland Altman test show that 93% of the values obtained are within the 95% confidence interval. The Bland Altman test shows a statistical agreement between the two methods with 28 out of the 30 values within the two standard deviations. However, given that the RDA is 100 IU and the two standard deviations are -227. 8 to +247. 4, the FFQ has limited clinical applicability. For example, if the FFQ obtains a value of 100 IU after assessing a subject’s intake, the true value of his intake can range anywhere from -127. 8IU to 347. 4IU. Given that the RDA of vitamin D is 100 IU, this margin of error is too large and is not acceptable in a clinical setting. In order to find out the possible causes of error that could have resulted in the large value range of the 95% confidence interval, the quality of the FFQ was

assessed by investigating if the 32 food items were truly contributing to the significant amount of vitamin D consumed by the subjects. Possible over and under reporting was also investigated. After analyzing the specific food items and finding out which contributes the most to the subjects' dietary vitamin D intake, we have discovered that on average, 84. 6% of their vitamin D intake is contributed by foods already present in the FFQ. These foods are mainly, milk, whole grain breakfast cereal, egg and fatty fish. (appendix 5) Foods that could have potentially significantly impacted vitamin D intake, such as chicken or pork, contained lesser vitamin D relative to the foods present in the FFQ. Thus, they did not have a significant impact on vitamin D intake even though they were eaten in high frequencies. Therefore, from this we can say that the FFQ is comprehensive enough in terms of the type of food it contains because it covers the foods that mainly contribute to one's dietary vitamin D intake. An investigation to find out whether over or under reporting had occurred during either methods of assessment was then done. This resulted in a Paired Samples Test with a 95% confidence interval range of -55. 1 to +35. 5 IU and a significant value of 0. 661. This means that there is a 66% chance that the difference in values obtained from the FFQ and the 24 hour recall is 0. For example, if a FFQ and a 24 hour recall were completed by the same subject, there is a 66% chance that both assessment methods will give the same results. The paired t-test showed that there is no evidence of over or under reporting of the FFQ and 24 hour recall (p= 0. 661). However, given that the range of the confidence interval is large (-55. 08 to +35. 46), this suggests that the sample size may be too small to detect a statistical difference. After investigation of both possible causes of error, we have discovered that the FFQ was comprehensive enough in terms of

variety of vitamin D rich foods and that there is no evidence that over or under reporting during the FFQ or 24 hour recall had occurred. After these findings, we have identified one more possible cause of error which is not having a large enough sample size. As the sample size increases, the range of the confidence interval narrows. However this does not mean that by doubling the sample size, the range of the confidence level will be halved. As our confidence interval range is too large, in theory, by increasing the sample size by recruiting more subjects to complete both dietary assessment methods, our confidence interval of both the Bland Altman and Paired t-test will reduce, thus obtaining more accurate results. Although the Bland Altman test shows limited applicability of the FFQ, nevertheless, the Cohen Kappa test shows that the FFQ may be used as a screening tool for inadequate vitamin D intake. After interpreting the results of the Kappa test, we have discovered there is fair agreement (K= 0. 286) between the FFQ and 24 hour recall and that there is a trend towards significance (p= 0. 088). This shows that it is not statistically significant and this could be due to an inadequate sample size. Therefore, with the current results, the FFQ cannot be used to accurately assess the true value of dietary vitamin D intake of subjects. However, the FFQ may be used as a screening tool to determine the subjects' vitamin D status because of the results obtained from the Cohen's Kappa test as it is trending towards significance (p= 0. 088).

8. Conclusion

Our studies show that the FFQ cannot be used to replace the 24 hour recall in measuring the true dietary vitamin D due to a wide interval level caused by a small sample size. However, it may still be effective in being used as a

screening tool to determine the vitamin D status of a large population. We have also found out that the FFQ is comprehensive enough as it covers most of the dietary sources of vitamin D in Singapore and that there is no evidence which supports that under or over reporting in either method had occurred.

9. Recommendations

For future studies, it is recommended to increase the sample size as it will result in a decrease in the 95% confidence interval. In the context of this study, the sample size will help to confirm the Cohen Kappa as it has just missed the significance by 0. 038. However, it is unlikely that increasing the sample size will significantly affect the paired sample t-test. To further facilitate the understanding of the subjects during the FFQ interview process, a more comprehensive tool, such as a flipchart containing more pictures and in multiple languages can be used. (Appendix 7)