

Vitamin D Status among First Grade University Female Students

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ABSTRACT

The present work was carried out to study the vitamin D status among first grade university female students. 125 First grade university apparently healthy female youth who were attending Helwan University were conveniently selected. A written consent was obtained. They were subjected to: anthropometric measurements, Dietary assessment by 24 hours dietary recall, frequency food sheet, and laboratory evaluation of vitamin D, parathyroid hormone, calcium, phosphorus and alkaline phosphatase. The energy and nutrients' content were analyzed using the food composition table of the National Nutrition Institute. Food intake was also compared to the recommended dietary allowances by WHO/FAO. This result revealed that 53.0% of the participants were interviewed in autumn; of them 26.0% had normal serum vitamin D level, 62.0% had insufficient values and 12.0% had their results in the deficient range. 40.0% of participants were interviewed in spring and their vitamin D results had nearly the same distribution. In addition to there was a significant correlation between vitamin D metabolic state and season of the year. This study concluded that there was a high prevalence of low vitamin D levels among a group of apparently healthy university undergraduate in Helwan University in Cairo, Egypt. There is an urgent need for public education about the vital role of vitamin D to minimize the complications of its deficiency. This study emphasized the need for further vitamin D assessment and interventions targeted at all people.

Key words: Vitamin D, Adolescent, University female students.

INTRODUCTION

Vitamins D are a group of fat-soluble vitamins. Vitamin D₂ is naturally present in very few foods, added to others, and available as a dietary supplement. Vitamin D₃ is produced endogenously when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis. Vitamins D when adequate prevent rickets in children and osteomalacia in adults (**Institute of Medicine, Food and Nutrition Board, 2010**).

Moreover, it has been widely identified as a neurosteroid with multiple actions in the brain during the past 20 years. It has long been recognized that out of the many areas in the body, the brain can also produce 1,25dihydroxy vitamin D (**Isaac, et al., 2019**).

Worldwide including the Arab states of the Persian Gulf, vitamin D deficiency has been documented as a frequent problem in studies of young adults, elderly persons, and children (**Mitchell, et al.,**

2012). Female youth in developing countries constitute one of the vulnerable groups that are likely to suffer health, social and economic problems. Reaching these young females early, for the promotion of their health, can prevent negative health outcomes and consequently preserve scarce resources. In addition, working with this age group can positively shape and ease their transition to their future motherhood role (**WHO/UNFPA/UNICEF, 1999**).

In Egypt, young adolescent females constitute a sizable sector of the population (12.5% i.e. around 8.75 million), most of whom need care and protection (**CAPMAS, 2002**).

As a setting, University hostel provide unique advantages of having thousands of female youth that have completed their secondary level education, from all Egypt governorates with variable social, environmental and behavioral characteristics (**Abou Zeina,**

2002).

SUBJECTS AND METHODS

Subjects:

A convenient sample of 125 first grade university apparently healthy female was selected among those who were living in the university dormitories. Any medical condition that influence vitamin D status and could affect results was excluded from the survey.

Methods:

Written consents were obtained from participants and a face-to-face interview took place using pre-structured medical and dietary questionnaires. Weight and height were measured and BMI was calculated using Quetelet equation. Natural waist was measured and blood pressure evaluation was done with a standard clinical sphygmomanometer.

Laboratory indicators

Laboratory indicators were measured using (Stanbio Total Calcium Liquicolor,

Procedure No 0150) for calcium (Ca) (**Sarkar and Chauhan, 1967**), (BioMed – phosphorous (PH123100)) for phosphorus (Pi) (**Vassault, et al., 1989**), and (DRG-DEA) Kinetic method for alkaline phosphatase (ALP) (**Tiets, 1995**). All previous tests were done using spectrometric device (Kenza, France). Serum 25-OH vitamin D (VD) was done by DRG ELISA. (**Houghton and Vieth, 2006**) LOT: 80k035 Cat .Nr:EIA5396` and Serum Parathyroid Hormone (PTH) was done by immune-enzymatic assay (hPTH-ASIA) Cat NO.:kAP1481 (**Martin, et al, 1979**). Blood samples were analyzed at the National Nutrition Institute (NNI).

Dietary Questionnaires

Dietary Questionnaires used were; a 24 hours recall questionnaire, a food frequency sheet and dietary practices and food pattern questionnaire with special emphasis on topics related to vitamin D status particularly milk and dairy products, cola, and tea consumptions. The energy and

nutrient content of the 24 hours were analyzed based on food composition table of the *NNI (2006)*. The nutritional value of foods and beverages consumed were compared to the recommended dietary allowances "RDAs" of *FAO/WHO (2004)*. Frequency consumption per week (<3 or >=3) was used as a reliable indicator of consumption.

Statistical analysis:

Data processing and analysis were done using SPSS program (IBM SPSS Statistics, SPSS Inc., Chicago, IL (**Sabine and Brian, 2000**)).

Ethical considerations:

Privacy of participants as well as confidentiality of data collected was ensured by giving each participant a serial number. Collected data was used only for the stated research purpose.

Results:

Table (1) showed that 53.0% of the participants were interviewed in autumn; of them 26.0% had normal serum

vitamin D level, 62.0% had insufficient values and 12.0% had their results in the deficient range. Those interviewed in spring constituted 40.0% and their vitamin D results had nearly the same distribution as those tested in autumn with 2 students who were categorized in the deficient group. The winter sample formed 7.0% and out of 9 students 7 had insufficient levels and 2 had deficient values.

According to *DRG ELISA (Houghton and Vieth 2006)* 75% of participants in spring and autumn seasons had their serum level of vitamin D just at or below 30.0 ng/ml; mainly in the insufficient range and those having their serum level in the normal range constituted the remaining 25%. Results in winter was significantly different and 75% of values were more to the deficient range (≤ 20 ng/ml). Calcium values were just close to the lower normal limit value (9.2) in 50% of participants regardless of season. In contrast to calcium, phosphorus values were at the

upper normal limit value (4.5) in 50% of samples and other half exceeded even the normal limit. Calcium phosphorus levels had the least values in spring and the highest in autumn for calcium while phosphorus in winter. Serum alkaline phosphatase levels were in the normal range with no special trend. PTH values were in the normal range in 75% of sample regardless of season and the remaining 25% could have values in the high range.

Based on Cut-offs of VD, PTH, and Calcium Simultaneously (Metabolic State); 6 (14.3%) of participants; in the spring sample, were considered deficient in their VD and 15 (35.7%) were considered as having normal levels. As for the autumn sample, 5 (8.3%) were deficient and 43 (71.7%) had normal VD values. Comparing this classification with that based on VD cut-offs alone (table 2) denoted the importance of using cut-offs of vitamin D and related-lab indicators; PTH, phosphorus, and calcium, for the proper

evaluation of serum vitamin D values.

Table (4) showed no significant statistical differences were observed in anthropometric parameters

Although no significant statistical differences were elicited in relation to VD metabolic status, yet there were several alarming findings concerning dietary intake among college females. The average number of meals taken per day by the VDD/VDI and hypocalcaemia groups was only 2 compared to 3 meals reported by the VD sufficient group. Also the average frequency of dairy intake was once per day even by the sufficient group. Energy derived from total protein was nearly 15% and the average calcium intake ranged from 400 mg by the deficient group to 500 mg by the sufficient group, and up to 600 mg by the hypocalcaemia group. Regardless of vitamin D groups, level of adequacy of calcium in diet was unsafe (less than 50% RDA). Calcium to P ratio was reversed (0.5 instead of the normal 2 to

1)(Hawley, 2005).

General speaking and apart from fruits, frequency consumption of healthy food items was less than 3 times per week particularly milk and fish. In contrast, consumption of unhealthy food items was higher particularly tea, trans-fat, cola, and chipsy. Nearly all student females; 90%, consumed milk just once per day and two thirds considered milk among un-favored food items while one third considered milk among costly food items.

DISCUSSION:

The selection of 25[OH] D instead of calcitriol as a marker for vitamin D status has been listed by **Brandi, (2010)**. First, 25[OH]D₃ is the highest in concentration of all vitamins D. Second, it remains stable for almost two weeks, and lastly, it is responsible for vitamin D-related toxicity instead of calcitriol. According to 25[OH] D blood level, 9.6% of participated

student females were categorized as having a deficient level (less than 10 ng/ml), 64.8% of them had an insufficient level (between 10 and 29 ng/ml), and 25.6% were sufficient (VD level was \geq 30 ng/ml). However, using cut-offs of VD alone and in combination with cut-offs of other VD- related indicators resulted in a significantly different classification (table 3) which denoted the importance of using cut-offs of vitamin D and related-lab indicators; PTH, phosphorus, and calcium, for the proper evaluation of vitamin D actual state. In children and adolescents 25 (OH) vitamin D deficiency is more important because the peak bone mass is attained in these critical ages and any adverse effects due to low 25 (OH) vitamin D and calcium levels will negatively influence their later lifelong (**Greer and Krebs, 2006**) ; (**Rizzoli, et al., 2010**). It is well known that prolonged deficiency of 25 (OH) vitamin D (concentrations $<10-25$ nmol/L) can lead to rickets in infancy and early childhood

and osteomalacia in adults. It is evidenced that low 25 (OH) vitamin D level increases parathyroid hormone (PTH) concentration in serum, and this later causes bone turnover and bone loss, defects mineralization, and increases risk of fractures of bones in old people (**Lips, 2001**). But the effect of increasing PTH in children and adolescents could be explained differently because of continued growth (**Cashman, et al., 2008**). For example, high serum PTH concentration is a normal finding during adolescence (**Krabbe, et al., 1982**) (**Cadogan, et al., 1998**). Results of this study showed that PTH values were in the normal range in 75% of sample regardless of season and the remaining 25% had values in the high range (table 2). The “spread” of vitamin D level as measured by the inter-quartile range (IQR) appeared to vary with season with lower values during winter and higher values during spring as shown in table (1). This went with what was reported by **Quadri, et al., (2016)** who found a

pronounced fluctuation of vitamin D values according to season in Swiss athletes. Lower values were reported during winter and higher values during summer. This finding shows how easy it is to present inadequate vitamin D levels during 3/4 of the year, even for athletes in Switzerland, where outdoor winter sports are extremely popular (**Al-Agha, et al., 2016**).

There is a great debate on whether the vitamin D deficiency is a consequence or a factor predisposing to obesity. Obese individuals seem to be more at risk of developing vitamin D deficiency due to vitamin D sequestration in adipose tissue and limited physical activity which consequently causes limited sun exposure (**Salehpour, et al., 2012**). Another study that assessed the effect of vitamin D3 supplementation on body fat mass in healthy overweight and obese women found that increasing 25 (OH) D concentrations by vitamin D3 supplementation led to body

fat mass reduction (**Harkness and Bonny, 2005**). However, results of this work did not show any statistical significant difference based on BMI or waist circumference and vitamin D level.

Natural dietary sources of 25 (OH) vitamin D are very few and foods that are fortified with 25 (OH) vitamin D are often inadequate to satisfy either a child's or an adult's 25 (OH) vitamin D requirement. On the other hand, adolescence is critical ages to skeletal growth and reach to optimal peak bone mass. The enough intake of calcium and 25(OH) vitamin D from daily diet, and the achievement of normal serum range of calcium and 25 (OH) vitamin D, have positive effects on bone in adolescents. For example, milk consumption positively correlates with bone mineral density of the total body, spine and radius in adolescent girls (**Chan, et al., 1995**); (**Teegarden, et al., 1999**) and (**Peters, et al., 2012**). Results of this study although was not statistically significant, yet it

showed a positive trend between frequent fish consumption and VD serum level. In theory, consuming calcium-rich foods such as bones, fermented dairy (e.g., unsweetened yogurt, kefir, and cheese), leafy greens, almonds, and chia seeds may be an effective strategy for improving both calcium intake and long-term health. Considering the above-mentioned facts, increasing vitamin D fortification of dairy products can be recommended as a population-wide public health strategy to fight 25 (OH) vitamin D deficiencies especially in adolescents.

Nakamura et al. (2000) reported significantly higher mean serum 25(OH) D levels in older women consuming fish more than 4 times/wk relative to those who ate fish less frequently or not at all. Frequent fish eaters were able to maintain desired serum 25(OH)D levels even during the winter (**Attila, et al., 2002**). General speaking and apart from fruits, frequency of consumption of healthy food items by college females in

this study was less than 3 times per week particularly milk and fish. Other natural vitamin D-rich foods include organ meats, such as liver, and wild mushrooms that naturally contain small amounts of vitamin D₂, but all edible mushrooms make abundant amounts of ergosterol, which, when irradiated with sunlight is converted to vitamin D₂ (**Jasinghe and Perera, 2005**). Mushrooms are important alternatives for fatty fish or other natural food sources of vitamin D. They are of particular importance to vegans and vegetarians whose diet is otherwise extremely limited in vitamin D (**Attila, et al., 2002**). Unfortunately, these food items were not among favored food items for college females participated in this study.

The cost to fortify food with vitamin D or to increase supplement potency is relatively inexpensive compared with the cost of developing drug treatments for the many chronic diseases strongly associated with vitamin D insufficiency.

CONCLUSIONS

This study showed that there was a high prevalence of low vitamin D levels among a group of apparently healthy university undergraduate in Helwan University in Cairo, Egypt. The results of this evaluation highlighted the importance of a structured approach and screening for vitamin D levels in the university community.

RECOMMENDATIONS

The finding emphasized the need for further assessment and interventions targeted at all patients. This study raised a health concern for the Egyptian population in general.

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Table (1): Percent Frequency Distribution of University Students per Season based on DRG ELISA (Houghton and Vieth 2006):

Season	Vitamin D DRG ELISA (Houghton, 2006)						Total (125)	
	Deficient <10 ng/ml		Insufficient 10 – 29 ng/ml		Sufficient 30 – 100 ng/ml			
	No	%	No	%	No	%	No	%
Winter	2 (9)	22.2	7 (9)	77.8	0 (9)	0.0	9	7.2
	8 (66)	12.1	41 (66)	62.1	17 (66)	25.8	66	52.8
	2 (50)	4.0	33 (50)	66.0	15 (50)	30.0	50	40.0
Autumn	$X^2 = 8.858$ $p = 0.065$							
Spring								

Table (2): Descriptive of Vitamin D and related Laboratory Indicators presented as Median (IQR*) (Min-Max) per Season:

Vitamin D related Indicators	Winter (No=9)	Autumn (No=66)	Spring (No=50)
PTH (N=16-46 pg/ml) Median (IQR*) Min - Max	31.8 (22.2 – 40.8) 6.6 – 113.0	30.6 (21.6 – 38.9) 8.2 – 77.7	39.1(30.2 – 49.2) 11.9 – 68.7
Vitamin D (N=30 – 100 ng/ml) Median (IQR*) Min - Max	13.8 (11.2 – 20.2) 6.9 – 23.1	18.5 (14.1 – 30.6) 5.5 – 75.7	26.2 (21.3 – 30.2) 6.8 – 86.0
Alkaline Phosphatase (N =< 270 U/L) Median (IQR*) Min - Max	135.0 (112.0 – 140.0) 98.0 – 156.0	119.5 (93.0 – 157.0) 64.0 – 269.0	123.0 (111.0 – 146.0) 70.0 – 235.0
Calcium (Ca) (9.2-11.0 mg/dl) Median (IQR*) Min - Max	9.3 (8.6 – 9.7) 7.5 – 10.0	9.8 (9.0 – 10.5) 7.5 – 12.2	8.8 (8.2 – 9.3) 7.5 – 12.0
Phosphorus (P0) (N= 2.7-4.5 mg/dl) Median (IQR*) Min - Max	4.9 (4.7 – 5.2) 2.9 – 6.6	4.7 (4.0 – 5.2) 2.8 – 6.8	4.2 (3.9 – 4.6) 3.1 – 6.2
Ca*Pi (=< 70 mg ² /dL ²) Median (IQR*) Min - Max	42.7 (37.6 –50.4) 27.8 – 63.4	45.3 (38.0 –53.5) 24.3 – 64.8	37.0 (33.1 – 41.9) 23.3 – 55.1

IQR = Interquartile range (2nd and 3rd quartile including median)(>= 25 % to =<75% of results)*

Table (3): Percent Frequency Distribution of Participant Females per Season based on Cut-offs of VD*, PTH*, and Calcium Simultaneously (Metabolic State):

Season	Cut-offs of VD, PTH, and Calcium Simultaneously (Metabolic State)**								Total (110) (Column %)	
	VD Deficient (PTH high, and VD and Ca are low)		VD Insufficient (PTH high, regardless of VD level)		Hypocalcemia (low Ca with normal PTH and VD)		VD Sufficient (PTH, VD, and Ca are normal)			
	No=14	row %	No=8	row %	No=26	row %	No=62	row %	No	%
Winter**	3	37.5	0	0.0	1	12.5	4	50.0	8	7.3
*	5	8.3	3	5.0	9	15.0	43	71.7	60	54.5
Autumn	6	14.3	5	11.9	16	38.1	15	35.7	42	38.2
Spring	$X^2 = 18.14$ $p = 0.006$									

* VD=vitamin D, PTH=parathyroid hormone

** Vitamin D Metabolic Status is the re-classification of laboratory results based on the normal physiological PTH-VD axis using cut-offs of vitamin D, calcium, and phosphorus in relation to cut-offs of PTH.

*** As winter sample was small, it was not be included in further

Table (4): Summary of Clinical Data in relation to Vitamin D Metabolic Status presented by mean (\pm sd)

Clinical Data	Vitamin D Metabolic Status*								ANOVA
	VDD (PTH high, and VD and Ca are low)		VDI (PTH high, regardless of VD level)		Hypo-Ca. (PTH and VD are normal)		VDN (PTH, VD, and Ca are normal)		
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	
Age	18.79	.58	18.75	.71	18.69	.79	18.69	.86	NS
BMI wt/ht² %	24.24	5.57	25.37	4.35	24.92	4.70	24.59	4.52	NS
Waist (cm)	76.36	7.95	81.75	10.8	76.96	8.82	76.82	8.75	NS
WC to Ht(cm)	.48	.05	.50	.05	.48	.05	.48	.05	NS
sBPr mmHg	106	10.6	106	10.6	100	10.7	103	11.7	NS
dBPr mmHg	70	9.3	72	6.5	66	6.9	67	9.3	NS

* Vitamin D Metabolic Status is the re-classification of laboratory results based on the normal physiological PTH-VD axis using cut-offs of vitamin D, calcium, and phosphorus in relation to cut-offs of PTH. VDD=vitamin D deficient, VDI=vitamin D insufficient, Hypo-Ca=hypocalcaemia, and VDN= vitamin D sufficient sBPr =systolic blood pressure dBPr= diastolic blood pressure

Table (5): Summary of Dietary Data in relation to Vitamin D Metabolic Status* presented by median (Interquartile range):

Dietary Data	Vitamin D Metabolic Status			Kruskal-Wallis Test
	VDD / VDI	Hypo-Ca	VDS	
	Median (IQR)	Median (IQR)	Median (IQR)	
Meal No.	2 (2 to 3)	2 (2 to 3)	3 (2 to 3)	NS
Dairy Intake (freq./d)	1 (1 to 1)	1 (1 to 1)	1 (1 to 1)	NS
Protein energy (%)	15.97 (12.6 to 18.98)	16.1 (14.1 to 19.2)	15.1 (13.5 to 17.2)	NS
Ca. in diet (mg) (N=1200 mg/day)	423.0 (384.0 to 799.0)	611.8 (385.0 to 771.2)	472.2 (345.8 to 724.0)	NS
Ca to P ratio in diet	0.48 (.44 to .63)	0.54 (.38 to .84)	0.55 (.43 to .69)	NS
Ca. adequacy in diet (% RDA)**	42.3 (38.4 to 79.9) %	61.2 (38.5 to 77.1) %	47.2 (34.6 to 72.4) %	NS
P adequacy in diet (% RDA)**	140.6 (123.3 to 158.8)	141.9 (94.1 to 180.9)	124.9 (89.6 to 151.7)	NS

* Vitamin D Metabolic Status is the re-classification of laboratory results based on the normal physiological PTH-VD axis using cut-offs of vitamin D, calcium, and phosphorus in relation to cut-offs of PTH.RDA=recommended daily intake for particular age and sex, and %RDA=actual intake/RDA * 100

VDD=vitamin D deficient, VDI=vitamin D insufficient, Hypo-Ca=hypocalcaemia, and VDS= vitamin D sufficient

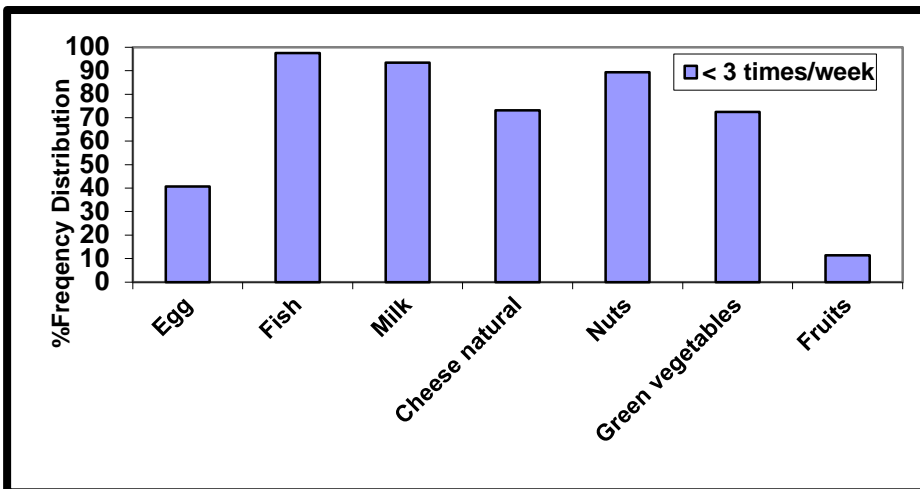


Figure (1): Dietary Consumption of healthy Food Items among University Students (less than 3 times per week)

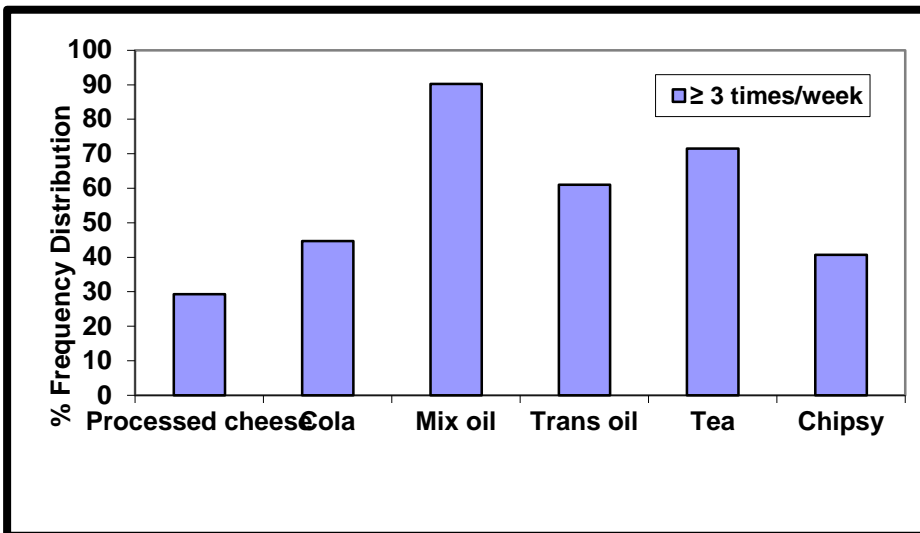


Figure (2): Dietary Consumption of unhealthy Food Items among University Students (3 times or more per week)

تقييم الحالة الغذائية لفيتامين د لدي طالبات الصف الأول الجامعي

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^٣ ماجستير في التغذية / قسم التغذية وعلوم الأطعمة/ كلية الإقتصاد المنزلي /جامعة حلوان

المستخلص العربي

يستهدف هذا البحث طالبات الصف الأول الجامعي لدراسة مستوى فيتامين د لديهن. تم إختيار ١٢٥ طالبة بالصف الأول الجامعي من بين طالبات جامعة حلوان بشكل إختياري وتم الحصول على موافقة كتابية منهن وتسجيل بياناتهن الشخصية لبدء الدراسة البحثية التي تضمنت ما يلي: تقييم المؤشرات الجسمية (الأشروبومترية)، التقييم الغذائي: باستخدام استمارة استرجاع غذاء ٢٤ ساعة- عمل إستبيان لتقييم النمط الغذائي وكذلك التاريخ الغذائي. الاختبارات المعملية: تم جمع عينات الدم من الطالبات وتم تقدير مستوى فيتامين د والكالسيوم والفوسفور والفسفاتيز القلوية في الدم وهرمون الغدة الجار درقية. وتم التحليل بمعمل قسم التغذية العلاجية بالمعهد القومي للتغذية - القاهرة) نتائج البحث: ٥٣,٠٪ من الطالبات تمت مناظرتهم في الخريف ؛ وبلغ مستوى فيتامين د الحد الطبيعي في ٢٦,٠٪ منهن، وكان مستوى فيتامين د غير كافي في ٦٢,٠٪ و ١٢,٠٪ كان لديهن نقص في مستوى فيتامين د. هذا وقد تم مناظرة ٤٠,٠٪ من الطالبات في الربيع وكانت نتائج فيتامين د (د) كما كانت بفصل الخريف، كان هناك ارتباط كبير بين منسوب فيتامين د (د) بالدم وموسم السنة. ٧٥٪ من الطالبات في فصلي الربيع والخريف لديهم مستوى فيتامين د (د) في أو أقل من ٣٠,٠ نانوغرام / مل؛ وبصفة أساسية في النطاق غير الكافي، وكان أولئك الذين لديهم مستوى فيتامين د في المعدل الطبيعي يمثلون النسبة المتبقية ٢٥٪. كانت قيم الكالسيوم قريبة من الحد الأدنى لقيمة الحد الطبيعي (٩,٢) في ٥٠٪ من الطالبات بغض النظر عن الموسم. اما مستويات الفوسفاتيز القلوية في الدم كانت في المعدل الطبيعي. قيم PTH هرمون الغدة الجار درقية كانت في المعدل الطبيعي في ٧٥٪ من العينة بغض النظر عن الموسم ، كانت نسبة ٢٥٪ المتبقية في المدى العالي. أظهرت نتائج استمارة التكرار الغذائي أن ٦٥,٠٪ و ٩٤,٠٪ و ٨٦,٠٪ من الطالبات يتناولون مصادر فيتامين "د" مثل الخضروات الطازجة والخضراء الطازجة والحليب والأسماك والمكسرات أقل من ٣ مرات في الأسبوع على التوالي لم تكن هناك فروق ذات دلالة إحصائية في قياسات مؤشر كتلة الجسم والطول والوزن والخصر عند الطالبات استناداً إلى اختبار T المستقل للعينة. الاستنتاج: بناءً على نتائج الدراسة الحالية ، أكدت الدراسة وجود معدل إنتشار لنقص فيتامين د (د) بين مجموعة من طالبات الجامعة في جامعة حلوان في القاهرة ، مصر. وأن هناك حاجة ملحة لزيادة وعي العامة من الناس والشباب والشابات علي وجه الخصوص بالدور الحيوي لفيتامين د لتقليل مضاعفات نقصه. التوصيات: أكدت هذه الدراسة على الحاجة إلى مزيد من تقييم فيتامين د (د) والتدخلات التي تستهدف جميع الناس.

الكلمات المفتاحية: فيتامين د ، المراهقة ، طالبات جامعية