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Plasma Retinol, Thyroid Stimulating Hormone and Zinc as Predictors of Bone Mineral Density Status

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Abstract

Background: Conflicting results on the association between serum retinol level and bone mineral density (BMD) have been published. Thyroid hormones are essential for skeletal development and have direct effect on bone formation and resorption. Bone has one of the highest concentrations of zinc of all tissues, and has been shown to release zinc during deficiency for soft tissue metabolism. **Objective:** The objective of this study was to assess the relation between plasma levels of retinol, thyroid stimulating hormone (TSH) & zinc and BMD of Egyptian adolescents and adults. **Method:** The study was a part of a cross sectional national survey conducted by National Nutrition Institute. The sample was a multistage stratified random. Target individuals were classified into two age groups (10- ≤ 18 and 28-≤ 59 years). Bone mineral density and plasma levels of retinol, TSH and zinc were determined. **Results:** Low and high plasma retinol levels were more prevalent among osteoporotic adolescent and adult males respectively than in normal subjects.. The reverse was observed in adult females. Bone mineral density correlated negatively with plasma retinol level in adult males and females and positively in adolescent males, while among females the association was significant (P = 0.030) and stronger. The highest deficiency of TSH was found among adult and adolescent osteoporotic males, followed by osteopenic adult males and adolescent females. Highly statistically significant difference (P < 0.001) existed between osteoporotic and normal adult males concerning TSH deficiency. The prevalence of zinc deficiency ranged from 5.7% to 9.5% for all target individuals. Plasma Zn levels were correlated negatively with bone mineral density in adult males and females. **Conclusion:** The results of this study reflects the controversy on the association of plasma retinol and BMD. However, the predominant finding revealed that both low and high plasna retinol levels compromise bone health. Bone status and thyroid function support the adverse effect of hyperthyroidism upon either bone osteoporosis or osteopenia and subsequently upon fracture risk. Plasma zinc deficiency correlated negatively with BMD in adult osteoporotic men.

Key words: **Human BMD. Osteoporosis. Osteopenia. Fractures Plasma. Retinol. TSH. Zinc**

Introduction

Osteoporosis is a complex, multi-factorial condition characterized by reduced bone mass and impaired micro-architectural structure, leading to an increased susceptibility to fractures. In spite of being a geriatric disease , it has an adolescent onset so nutrition, environment and life style early in life play an important role in the development and maintenance of bone mass as well as prevention and management of osteoporosis (Kawahara *et al*., 2002).

Normal bone growth and development require adequate levels of vitamin A. Both low and high doses of vitamin A may

contribute to the development demineralization of bone and development of osteoporosis. Therefore, for prevention or treatment of osteoporosis, it is best to obtain vitamin A from food sources and not to eat more than the recommended dietary allowance (RDA) (Milstone and Leachman, 2001). Previous epidemiological studies conducted on retinol supplemented subjects showed an association between high serum levels or dietary intake of retinol and risk of osteoporotic hip fracture (Maggio *et al*., 2006). Furthermore, Michaelsson *et al*. (2003) reported a U-shaped curve for blood levels of serum retinol and the risk of fractures. However, Ballew *et al*. (2001) found no relationship between blood levels of vitamin A and BMD.

Thyroid hormones are essential for skeletal development. They exert an important action on bone remodeling and their excess is associated with increased bone turnover, affecting bone resorption more than bone formation with net loss of bone and hence reduction in BMD (Boelaert and Franklyn, 2005). Thyroid stimulating hormone, TSH per se inhibits markers of bone resorption by osteoclast (Sun *et al*., 2006). In hyperthyroidism, bone remodeling is accelerated and activities of bone-forming osteoblasts and boneresorbing osteoclasts are disproportionately increased leading to a net loss of 10% of mineralized bone per remodeling cycle (David *et al*., 2003; Boelaert and Franklgn, 2005). As thyrotoxicosis is one of the established causes of osteoporosis, Subclinical thyrotoxicosis or excessive T_4 replacement therapy for hypothyrodism increases the risk of fracture (Bauer *et al*., 2001).

Zinc (Zn) is an essential trace mineral that is necessary for normal collagen synthesis and mineralization of bones (Wallwork and Sandstead, 1990). Zn deficiency results in impaired DNA synthesis and protein metabolism, which lead to negative effects on bone formation. The role of zinc in bone formation is well documented in animal models (Yamaguchi, 1998) and reduced serum or plasma zinc concentrations and increased urinary zinc excretion have also been reported in women with osteoporosis (Gur *et al*., 2002).

However, after long research, no national data has been found for the association between plasma TSH, retinol or zinc levels and osteoporosis in Egypt. The present study is a cross-sectional statistical one to focus on this relation among Egyptian adolescents and adults for early detection, intervention and prevention of osteoporosis.

Subjects and Methods:

1-Subjects:

Data obtained from the National Survey (cross sectional study) for the Determination of Bone Mass Density among Adolescents and Adults in Egypt conducted by the National Nutrition Institute (2004) were used in the present study.

The sample was a multistage stratified random sample selected from 6 governorates (Cairo, Dakahlia, El-Beheira, Sohag and Red Sea) representing different geographical areas in Egypt. The age of adolescents ranged from 10- ≤ 18 years, while it ranged from 28 - ≤ 59 years for adults.

2-Measument of Bone mineral density (BMD)

Diagnosis of osteoporosis is currently based on the level of target individual's BMD compared with the average peak BMD of young adult Caucasian and expressed as T-score using WHO (1998) criteria, as shown in table (1). Cut off points of peak bone mass were 0.735- 0.740 gm/cm^2 for males and 0.635- 0.640 gm/cm^2 for females.

BMD was measured by dual-energy x-ray absorptiometry (DEXA) bone desitometer (Norland Medical System, Inc,1998), which acts peripherally on calcareous bone.

Osteoporosis and severe osteoporosis cases were considered as one group in the presentation of this study.

As bone mass is in a dynamic states during adolescence and bone formation

reaches its peak bone mass at early adulthood around 20 years. So there is no reference bone density for adolescents. Thus in this study, the reference BMD of adults recommended by WHO (1998) is used under the term relative osteopenia and relative osteoporosis during adolescence.

3-Biochemical measurements:

Sub samples of target individuals separated plasma was stored at -20° C for subsequent analysis. Vitamin A (1122 samples) was measured according to WHO/UNICEF (1994) and Bieri *et al* (1979). TSH (1144 sample) was determined by Elisa using kits EHTSN 969 as descr-ibed by Spencer (1995). Plasma zinc conc-entration (1438samples) was determ-ined by atomic absorption spectrometer (Unicam 929) after protein precipitation with trichloroacetic acid using the method of Smith *et al*. (1979).

Cut off points of the estimated parameters are 20-80 ug/dl; 0.39-6.16 mIU/L and 70-150 ug/dl for retinol, TSH and zinc respectively.

4-Statistical analysis:

It was performed using Chi-Square and pearson correlation according to Kirkood and Sterne (2003).

Results:

Mean plasma levels of retinol of target individuals was shown in Figure 1.

Plasma retinol deficiency Table, 2 was more prevalent among osteoporotic cases than normal subjects, in male adolescents (28.2% versus 23.7%) (χ^2 = 0.511 P >0.05) and male adults (21.9% versus 18.4%) (χ^2 =0.355 P >0.05) respectively. The reverse was observed in females, as plasma retinol deficiency was more prevalent in normal subjects than in osteopenic adolescents and osteoporotic adults (23.9% versus 14.6%)(χ^2 = 1.632 P>0.05) and (19.7% versus 14.3%) (χ^2 =0.689 P> 0.05) with insignificant difference.

High plasma retinol level, (Table 2) was more prevalent among osteoporotic cases than normal subjects (7.7%, 4.5%) and (9.4%, 4.4%) for male adolescents and adults respectively. Concerning female adolescents, the prevalence was higher among osteopenic cases than normal (8.3%, 3.3%) respectively. On the contrary, the lowest prevalence of high retinol level in adult females was found among osteoporotic cases then increased gradually in osteopenic and normal subjects (3.6%, 5.3% and 8.4%)respectively.

Some participants with condition characteristics that might affect the measured items or BMD were excluded from correlations.

BMD correlated negatively with plasma retinol level in adult males (R =-0.013 P= 0.853 N= 195) and females (R = -0.075 P= 0.218 N= 268) and positively in adolescents (R = + 0.007 P= 0.921 N= 215) males, while the association was significant and stronger in females (R = +0.142 P = 0.030 N= 234).

The mean plasma TSH levels of studied individuals was explained in Figure 2.

Table 3 showed that in adolescents males, prevalence of TSH deficiency was 11.8% and 7.1% for osteoporotic and osteopenic males respectively versus 7.6% of normal. Among osteopenic females, TSH deficiency was 9.6% against 6.4% for normal subjects, osteopenic females was only 3 targets so, they can not be statistically evaluated. There was no significant difference between TSH values of male and female adolescents in relation to BMD. For adults, the highest percent of TSH deficiency (13.8% and 9.9%) was found among osteoporotic and osteopenic males respectively. Among females 6.6% and 4.7% of osteopenic and osteoporotic respectively had TSH deficiency, while 8.1% and 6.7% of normal males and females respectively were TSH deficient. There was highly significant difference (χ^2 = 42.4 P < 0.001 df= 1) between osteoporotic and normal adult males. However, no significant differences were observed between other groups.

The mean plasma zinc levels of studied individuals was explained in figure (3).

Table 4 showed that highest prevalence of plasma zinc deficiency was found among osteopenic adult males (9.1%) and adolescent females (8.7%)respectively, followed by percentages of 6.9%, 6.8% and 6.1% for osteopenic & osteoporotic adolescent males and osteopenic adults males respectively. However, no cases of osteoporosis or osteopenia were detected in adult females.

Plasma Zn levels were correlated negatively with bone mineral density in male (R= - 0.023, P= 0.718, N=257) and female (R=-0.058, P=0.318, N=224) adults.

Table (1): World Health Organization criteria for the diagnosis of osteoporosis.

Category	Criteria (expressed as T-score)
Normal	BMD ≤ 1 SD
Osteopenia	> - 1 SD BMD ≥ - 2.5 SD
Osteoporosis	BMD < -2.5 SD
Sever (Established) Osteoporosis	BMD < -2.5 SD In presence of one or more fragility fracture.

Figure (1): Mean plasma retinol levels of target individuals in relation to Bone mineral density (BMD)

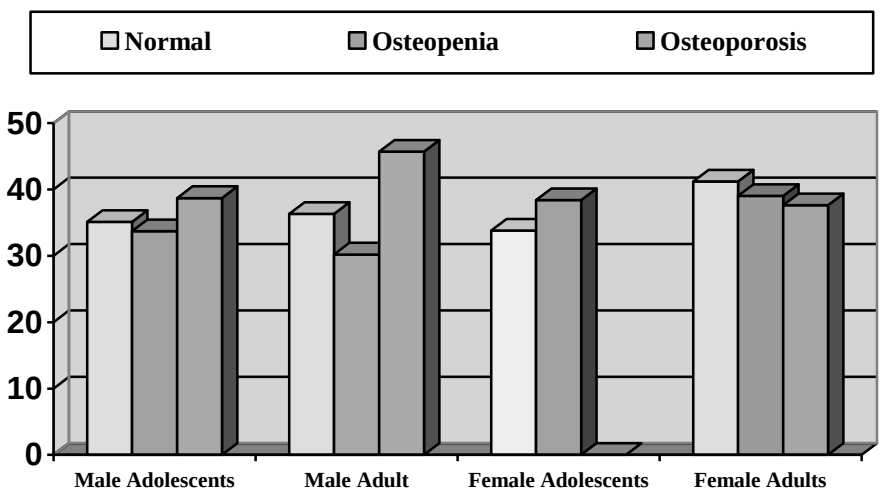

Table (2): Percent Distribution of Plasma retinol Levels of Target Individuals in Relation to Bone Mineral Density in The Total Sample.

Target	Cut off levels (ug/dl)	Normal	Osteopenia	Osteoporosis
Male Adolescents	< 20	23.7	17.8	28.2
	20- 80	71.8	78.1	64.1
	> 80	4.5	4.1	7.7
	Total Number	198	73	39
Male Adults	< 20	18.4	22.2	21.9
	20- 80	77.2	74.1	68.8
	> 80	4.4	3.7	9.4
	Total Number	228	27	32
Female Adolescents	< 20	23.9	14.6	0
	20- 80	72.8	77.1	0
	> 80	3.3	8.3	0
	Total Number	276	48	0
Female Adults	< 20	19.7	21.1	14.3
	20- 80	71.4	73.7	82.1
	> 80	8.4	5.3	3.6
	Total Number	294	19	28

Figure (2): Plasma TSH levels of Target Individuals in Relation to Bone Mineral Density

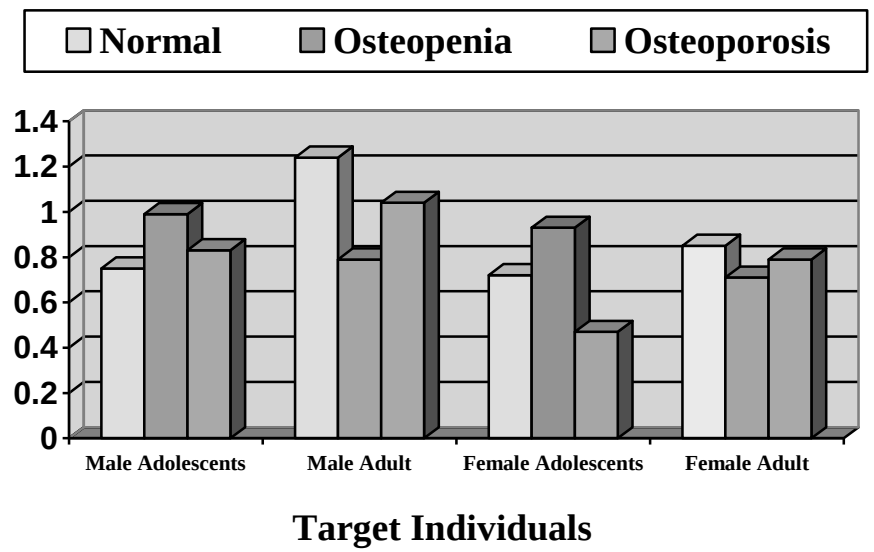

Table (3): Percent Distribution of Plasma TSH Levels of Target Individuals in Relation to Bone Mineral Density in The Total Sample.

Target	Cut off levels (mIU/L)	Normal	Osteopenia	Osteoporosis
Male Adolescents	< 0.39	7.6	7.1	11.8
	0.39- 6.16	92.3	92.5	87.2
	> 6.16	0.2	0.3	1
	Total Number	178	82	27
Male Adults	< 0.39	8.1	9.9	13.8*
	0.39- 6.16	91.3	90.1	85.3
	> 6.16	0.6	0	0.9
	Total Number	220	22	30
Female Adolescents	< 0.39	6.4	9.6	0
	0.39- 6.16	93.3	88	100
	> 6.16	0.3	2.4	0
	Total Number	222	46	3
Female Adults	< 0.39	6.7	6.6	4.7
	0.39- 6.16	92.6	93.4	95.3
	> 6.16	0.7	0	0
	Total Number	265	17	29

*P< 0.001

Figure (3): Plasma Zinc Concentrations of Target Individuals in Relation to Bone Mineral Density

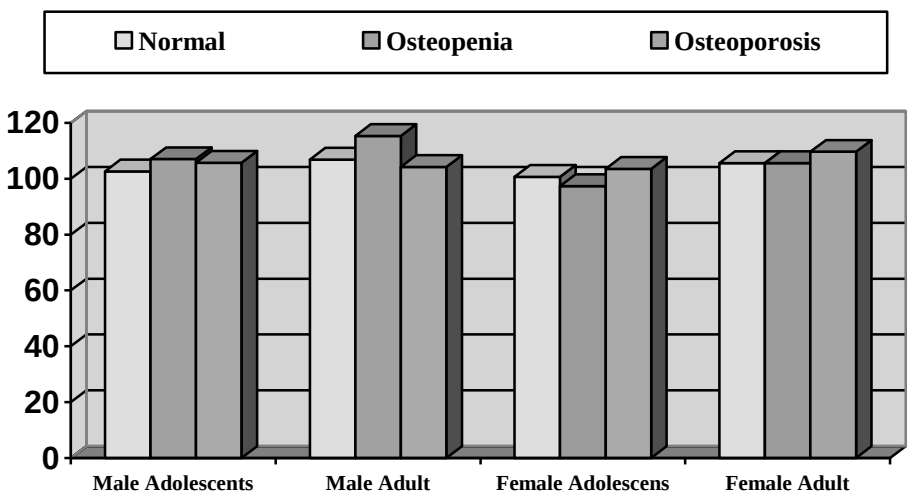

Table (4): Percent Distribution of Plasma Zinc Levels of Target Individuals in Relation to Bone Mineral Density in The Total Sample.

Target	Cut off levels (ug/dl)	Normal	Osteopenia	Osteoporosis
Male Adolescents	< 70	8.3	6.9	6.8
	70-150	90.1	87.8	93.2
	> 150	1.6	5.3	0
	Total Number	192	131	44
Male Adults	< 70	5.7	6.1	9.1
	70-150	90.6	84.8	90.9
	> 150	3.7	9.1	0
	Total Number	297	33	44
Female Adolescents	< 70	9.5	8.7	0
	70-150	87.6	89.9	100
	> 150	2.9	1.4	0
	Total Number	242	69	2
Female Adults	< 70	6.0	0	0
	70-150	89.3	100	95
	> 150	4.8	0	5
	Total Number	336	8	40

Discussion:

The increased prevalence of high retinol levels in normal subjects than osteoporotic and osteopenic cases are in accordance with Maggio *et al*. (2006) as they found that plasma levels of retinol were consistently lower in osteoporotic than in normal women. However, Feskanish and others in 2002 recorded that intake of preformed retinol was associated with the risk of hip fracture among postmenopausal women who were not taking hormone replacement therapy (HRT), while Rejnmark *et al*. (2004) showed no association between intake of vitamin A and BMD of the femoral neck or lumbar spine. Neither did BMD differ between those 5% Danish perimenopausal women who had the highest and those 5% who had the lowest vitamin A intake.

On the other hand, deficiency in males agreed with Michaelsson *et al*. (2003) as they reported a U shaped curve for serum retinol and the risk of fracture in elderly men of Sweden. The risk is primarily associated with the highest quintiles, although to a lesser extent, risk of fracture increased in the lower quintiles of serum retinol as well.

The low prevalence of retinol deficiency in osteoporotic and osteopenic adult and adolescents females as compared with the normal are in accordance with Ballew *et al*. (2001) as they found no relationship between blood levels of vitamin A and BMD in Americans in the Third National Health and Nutrition Examination Survey (NHANES-III). While Maggio *et al*. (2006) mentioned that plasma levels of retinol tested in non-supplemented elderly women with or without severe osteoporosis were consistently lower in osteoporotic than in control women.

The results of high plasma retinol levels in the present study go in line with Penniston and Tanumihardjo (2006) as they mentioned that osteoporosis and hip frac-

ture are associated with preformed vitamin A intakes that are only twice the current recommended dietary allowances (RDA). To control vitamin A deficiency large therapeutic doses are administered in developing countries to women and children who often are undernourished. Nevertheless, little attention has been given to the short term kinetic (i.e, after absorption but before storage) of a large dose of vitamin A or to the short and long term effects of such a dose given to lactating women on serum and breast-milk concentrations of retinol and its metab-olites. Assessing vitamin A status in persons with subtoxicity or toxicity is complicated because serum retinol concentrations are non sensitive indicators in this range of liver vitamin A reserves.

Because BMD is relatively stable between the ages of 20 and 50, there are relatively few studies evaluating the effect of the measured items (of the present study) on bone health during young and middle adulthood.

Overt hyperthyroidism results in reduction in BMD, where bone remodeling is accelerated, leading to a net loss of 10% of mineralized bone per remodeling cycle (David *et al*. 2003).

Our results showed that almost 14% and 12% of osteoporotic adult and adolescent males respectively was deficient in TSH, about 5% of adult females was also deficient. Highly significant difference was found between osteoporotic and normal adult regarding TSH deficiency, this findings agreed with Sun *et al*. (2006), who reported that low TSH levels correlate with increased fracture risk. Increased incidence of fracture femur by 1.8% fold was reported amongst women with previous hyperthyroidism followed a large prospective study (Boelaert and Franklyn, 2005). In accordance with these findings, another study reported increased fracture incidence rate ratio (1.26 - 2.29) around the time of diagnosis of hyperthyroidism (Vestergaard and Mosekilde, 2002) Also, a prospective cohort study, revealed that hyperthyroidism conferred a 2-fold increase in risk of hip fracture (Bauer *et al*., 2001). Although thyroid hormones stimulate both the osteoblastic and osteoclastic activity, their effects are much stronger on osteoclasts leading to osteopenia and the risk of repeated bone fracture in hyperthyroid patients (Zehra *et al*., 2004).

Among studied osteopenic individuals, about 10% & 7% of adult and adolescent males and 10% &6.6% of adolescent and adult females respectively were TSH deficient.

Like overt hyperthyroidism, subclinical hypethyroidism has been implicated in the development of osteoporosis (Boelaert and Franklyn, 2005).

In the present study, detectable percent (7.6% & 8.1%) of adolescents and adults males and (6.4% & 6.7%) of adolescents and adults females respectively were deficient in TSH. This may be because TSH deficiency in this group is not yet associated with bone complication to be manifested.

Decrease in TSH may be due to primary hyperthyroidism or over replacement of thyroid hormone in treatment of hypothyroidism (Fischbach, 2000).

The high prevalence of zinc deficiency in adult males observed in the present study was consistent with Hyun *et al*. (2004) as they found that plasma zinc concentrations were lower in men with osteoporosis than in their matched normal control. Moreover, Li *et al*. (2005) reported that lack of zinc intake levels in the meal of elderly people was correlated with BMD. However, Relea *et al*. (1995) found no significant differenced in zinc concentration of osteoporotic postmenopausal women with osteoporosis than those without osteoporosis.

In animals, Zn deficiency has been associated with abnormalities in bone growth, bone formation, and mineralization. A significant positive correlation between human bone zinc content and bone strength suggests that zinc may play a role in bone health (Yamaguchi, 1998).

Finally it may be concluded that both low and high levels of retinol compromise bone health. Bone status and thyroid status

support the adverse effect of hyperth-yroidism upon bone either osteoporosis or osteopenia. Plasma zinc deficiency correl-ated with BMD to some degree.

Recommendations

Vitamin A must be taken from natural food sources in adequate amount and its supplementation to women and children should be reevaluated. Further studies are needed to evaluate the importance of hyper-thyroidism & sub-clinical hyperthyroidism in terms of osteoporosis risk and to unravel the potential role of zinc in osteoporosis. Furthermore, the next studies should focus on the association between nutrition, environment and osteoporosis.

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محتوى البلازما من فيتامين أ و الهرمون المنشط للغدة الدرقية و الزنك كمؤشرات لكثافة العظام

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المعهد القومي للتغذية.

مقدمة: يؤثر كلا من التركيز المنخفض و المرتفع من فيتامين أ على كثافة العظام كما أن الهرمون المنشط للغدة الدرقية أساسي لعملية بناء الهيكل العظمى و لة تأثير مباشر على تكوين العظام و كذلك تحتوى العظام على أعلى تركيز من الزنك و تقوم عند نقصة بإخراجة إلى الخلايا. **هدف الدراسة**: تهدف هذة الدراسة إلى تقييم العلاقة بين تركيز كلا من فيتامين أ و الهرمون المنشط للغدة الدرقية و الزنك في البلازما و كثافة العظام بين المراهقين و البالغين المصريين.**تصميم التجربة**: يتم استخدام بيانات البحث القومي لتقييم كثافة العظام بين المراهقين و البالغين في مصر لإظهار تأثير تركيز البلازما من القياسات السابقة على كثافة العظام. فسم المشاركين فى الدراسة إلى مجموعتين من حيث العمر (من10 إلى 18 سنة , من 28 إلى 59 سنة). تم قياس كثافة العظام و كذلك مستوى كل من فيتامين أ و الهرمون المنشط للغدة الدرقية و الزنك في البلازما. **النتائج**: كان الانخفاض و الارتفاع في محتوى البلازما من فيتامين أ أكثر ظهورا في المراهقين و البالغين المصابين بالهشاشة على التوالي, بينما ظهر العكس في النساء البالغات. كانت العلاقة بين كثافة العظام و محتوى بلازما الرجال و النساء البالغين من الفيتامين علاقة عكسية, بينما كانت طر دية في المراهقين من الجنسين.

ظهر أعلى مستوى لنقص الهرمون المنشط للغدة الدرقية بين المراهقين و البالغين من الرجال و الهرمون المنشط للغدة الدرقية يليهم في النقص البالغين و المراهقات الذين يعانون من المراحل المبكرة للهشاشة,وقد كان الفرق في نقص الهرمون بين الرجال البالغين المصابين بالهشاشة و غير المصابين فرقا إحصائيا كبيرا.

تراوح النقص في محتوى البلازما من الزنك بين 5,7 و 9,5 لكل العينة المختارة, و كانت العلاقة بين كثافة العظام و تركيز الزنك علاقة عكسية في النساء و الرجال البالغين.

الخلاصة: عكست نتائج هذة الدراسة التضارب بين محتوى البلازما من فيتامين أ و قد ظهر بوضوح أن لكل من التركيز المنخفض و المرتفع علاقة بصحة العظام. وكذلك التأثير العكسي لزيادة نشاط الغدة الدرقية على هشاشة العظام و بالتالي خطر الإصابة بالكسور. كما ظهرت العلاقة العكسية بين نقص الزنك و الهشاشة بين الرجال البالغين.