Significance of Vitamin A (Retinol) in Ageing

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Summary: In recent years, studies involving animal models, case reports, and some epidemiological studies have indicated that an accumulation of vitamin A in the liver is linked to low bone mineral content, fracture risk, and hence osteoporosis. While vitamin A in its physiological level acts as an inducer of bone matrix protein (osteocalcin) and matrix GLA protein in the osteoblast cells (bone formation), excess reinoic acid (a metabolic product of vitamin A) supresses osteoblastic activity and stimulates osteoclast formation (bone degradation). There also appear to have an antagonistic or synergistic interactions between vitamins A and D, influencing bone metabolosm; such effects are dose related. These effects could be of concern, especially for the older persons, because of the age-associated cumulation of vitamin A in the liver. The vitamin A status in elderly population, however, has not been as well documented as in an earlier age. In effect, the Food and Nutrition Board of National Academy's Institute of Medicine has not made any differenciation in it's recommendation for the Dietary Reference Intake (DRI) of vitamin A between 14 years and >70 years old individuals. This report will delineate the ageing differences in metabolic availability of vitamin A and that such differences are independent of its intake. The report will also attempt to formulate a hypothesis that in old age, deficiency of vitamin A is not a concern but its potential toxicity involving bone.

Keywords: Vitamin A, ageing; toxicity, osteoporosis, vitamin D metabolism, oxidative stress.

INTRODUCTION

Vitamin A (retinol) plays a major role in vision, cell growth and maintenance of the integrity of epithelial cells. The role of this vitamin in the promotion of growth and differentiation of epithelial tissues makes it an important nutrient during development of growth, reproduction, bone development, and immunity. Vitamin A deficiency and its consequences in early life have thus been well documented. The vitamin A status in elderly population, however, has not been as well documented as in earlier age. In effect, the Food and Nutrition Board of National Academy's Institute of Medicine has not made any differenciation in it's recommendation for the Dietary Reference Intake (DRI) of vitamin A between 14 years and >70 years old individuals [1]; this is true for both males and females. This report will delineate the ageing differences in metabolic availability of vitamin A and that such differences are independent of its intake. The report will also attempt to formulate a hypothesis that in old age, deficiency of vitamin A is not a concern but its potential toxicity involving bone.

VITAMIN A METABOLISM

Pre-formed vitamin A (retinol) is present in food essentially as long-chain fatty acid esters (e.g., retinyl palmitate), which are exclusively of animal origin. The retinyl esters are hydrolyzed to retinol in the intestine before absorption [2]. In the enterocytes, retinol is reesterified and incorporated into chylomicrons together with triglycerides. The chylomicrons carry retinyl esters to the liver, where it is stored predominantly in the stellate cells. Prior to transport of retinol to the target tissues, the hepatic retinyl esters are hydrolyzed to retinol, which then binds to its carrier, retinol-binding protein (RBP), which is a zinc containing protein, synthesized in the liver [3]. The retinol-RBP complex is then released into the circulation, where it forms a complex with another protein, transthyretin (TTR), as a 1:1:1 molar ratioo. At target tissues, TTR is released and RBP facilitates the uptake of retinol by RBP receptors. Retinol binds with cellular retinol-binding protein (cRBP), which facilitates the transport of retinol. Retinol may be esterified and stored or may be converted to more active metabolites, such as retinoic acid.

AGE- ASSOCIATED CHANGES IN PLASMA AND LIVER LEVELS OF VITAMIN A

Using male normal rats of different ages, plasma levels of retinol have been reported to be significantly decreased, while its hepatic concentrations are increased in 32 – 35 months old compared with those of the 4-19 months old rats [4]. An evaluation of the hepatic store of vitamin A in humans is difficult since the only way to obtain such data is from liver samples collected during autopsy. There has been, however, an early study [5], which reported vitamin A concentrations in various human tissues, including liver, that were collected at autopsy from five geographic areas in the United States (California, Iowa, Missouri, Ohio and Texas). This report provided liver vitamin A concentrations by age groups but without any comments on the age-associated changes. Re-examination of these data has clearly shown

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that the hepatic concentrations of vitamin A are increased with increasing age (Table **1**), agreeing the experimental results [4].

Age (Year)	No. of Subjects	Vitamin A, ug/g (mean ± SD)
11-30	35	96 ± 35
31-50	70	110 ± 28
51-70	136	140 ± 20
71-90	79	202 ± 18

*modified from Rer. [5].

Plasma (or serum) retinol, because of its easy accessibility, is the most commonly used biochemical index for the vitamin status in humans. The plasma values, however, do not reflect body stores of vitamin A because the plasma contains only 1% of the total body reserve and the values are homeostatatically controlled. Plasma vitamin A is changed only when the hepatic store is severely depleted or excessively high. In addition, factors such as low protein status, acute catabolic status, and decreased hepatic function are all known to affect the RBP production and therefore the metabolic availability of vitamin A from its hepatic stores [6]. Plasma vitamin A levels alone do not reveal if there is an impairment in its metabolic availability. These values can be interpreted better, however, if they are measured along with RBP [7]. This is because the transport of retinol from the liver to the target issues is dependent upon the carrier protein. Thus in vitamin A deficient rats, the serum concentration of RBP is reduced while it's level is increased in the hepatic tissue, and that this scinario is riversed when vitamin A is administered to this deficient rats [8]. Normally, such effects do not appear to be caused by synthesis of RBP, rather the availability of vitamin A in concommitant with the need for vitamin A to be transported to the blood as a function of homeostatic regulation of circulatory level of vitamin A. In old age, however, this effect does not appear to be primarily caused by it's synthesis since the RBP concentration in the hepatic tissue is considerably high, compared with that of the younger age. Furthermore, when vitamin A is administered to rats deficient in the vitamin, the RBP concentration in the liver decreases concomitantly with an increase in serum concentration. The underlying mechanism for homeostasis of plasma retinol is essentially mediated through synthesis and secretion of RBP. However when there is an excess intake of the vitamin for a long period of time the hepatic storage capacity and the retinol-binding capacity of RBP are exceeded to their limits.

Using experimental old rats the hepatic store of vitamin A has been shown to be negatively associated with it's carrier protein [9]. Thus the hepatic storage of retinol concentration is considerably higher, while RBP mRNA is lower in 18-20 months old than in 2-10 months old rats (Table **2**). Wheather this associationship in old age is counterbalanced by an increased turnover rate of RBP or whether this reflects a physiologically diminished demand for vitamin A, cannot be ascertained at this point. Dawson and his associates [9] have, however, shown that unlike the RBP expression the cRBP expression remains unaffected in old age, reflecting a normal transport of vitamin A to its target cells.

The overall evidence clearly suggests that vitamin A, as a nutrient, is not a concern for the older population. Indeed in North American diets there is an abundance of pre-formed vitamin A containing foods including organ meat, beef, eggs, fish oil as well as fortified foods like margarine, non fat milk, breakfast cereal, and some snack foods. In addition, the use of multivitamins/mineral supplements, usually providing 5000 to 10,000 IU (1500-3000 micrograms vitamin A) as retinol or retinyl palmitate (1) is increasingly becoming popular among older population because of their claims being beneficial for many age-related conditions, such as eye health and reduced risk for bladder cancer [10], cataract [11], inflammatory conditions [12, 13], immunity [14] and skin health [15]. In view of the fact that the turnover rate of vitamin A is slow due to its lipid solubility, detary and supplemental abundance in North American diet and possibly its diminished physiological requirements in old age, may account for its accumulation in the liver over a period of one's life time.

TOXICOLOGICAL IMPLICATIONS OF ELEVATED HEPATIC STORAGE OF VITAMIN A

The underlying mechanism by which excess vitamin A exerts its toxic effect is not fully understood. It is, however, thought that some of the effects of hypervitaminosis A may be due to labilization of lysosomal membranes [14]. This may occur through a toxic effect on cell membranes, particularly lysosomal membranes. Normally, vitamin A circulates in blood predominantly as free retinol bound to RBP, while in conditions of excessive intake of vitamin A, it is transported in esterified form in association with plasma lipoproteins but not bound to RBP. Vitamin A toxicity appears to occur when the capacity of the protein to transport free retinol is exceeded, so that the vitamin in its esterified form is

Table 2. **Age-Associated Changes in Hepatic Total Retinol and Relative Expressions of RBP and cRBP in Male Rats***

Age (month)	Retinol (umol/g)	RBP mRNA[1]	cRBP mRNA[1]
2-3	362 ± 25[a]	1.0 ± 0.02[a]	1.0 ± 0.03[a]
8-10	1037 ± 54[b]	1.0 ± 0.06[a]	0.95 ± 0.04[a]
18-20	2134 ± 37[c]	0.69 ± 0.04[b]	1.15 ± 0.05[a]

*modified from Ref. [9]; each value is the mean of six rats ± SEM.
[1]for RBPmRNA and cRBPmRNA, the value for 2-3 months old rats was set to 1.0 and other values were expressed relative to this group.
a,b,c, letters not commonly shared show significance (p< 0.05).

carried to the cell membranes by lipoproteins in large quantities. Retinyl esters, because of their amphipathic characteristics, are believed to be more injurious to the membranes *via* detergent disruption than retinol bound to RBP [15]. It is generally thought that liver vitamin A measure is an indicative of vitamin A toxicity. Since the hepatic store of the vitamin appears to be appreciably high in association with ageing, the possibility of vitamin A toxicity in old age cannot be ignored. It is also noteworthy that in parallel with an age-associated increase in hepatic storage of vitamin A, a progressive increase in the level of this vitamin has been found to occur with ageing of the aortic vascular wall in rats [4]. This age-related changes of vitamin A status is also accompanied by increased productions of a variety of reactive oxygen species in vascular wall [16]. Thus the formations of nitric oxide (NO$^\bullet$), superoxide (O$_2^{\bullet-}$) and peroxynitrite (ONOO$^\bullet$) are associated with vascular ageing. It is of further importance that despite an increased NO production, indicated by an increased nitric oxide synthase activity as well as its expression level, free NO release is markedly decreased in aged aorta, when compared with that of the younger rats. This has been suggested to be a consequence of age-associated O$_2^{\bullet-}$ production with concomitant quenching of NO by the formation of ONOO$^\bullet$ (O$_2^{\bullet-}$ + NO → ONOO$^\bullet$). In view of the fact that the ageing process is associated with increased oxidative stress linking to an increased risk for cardiovascular disease, an increasing accumulation of vitamin A in the liver and the vascular wall with age can be of serious concern.

In recent years, vitamin A toxicity has been suggested to be one of the risk factors for osteoporotic fracture in older population. Thus, there have been numerous animal studies demonstrating that a prolong excessive intake of vitamin A can cause ossification of cartilage, increased bone resorption, extraosseous calcification, hypercalcaemia, and suppressed parathyroid hormone levels [23-25]. More recently, there have been many cross-sectional studies (Table **3**), which have compared the dietary intake of retinol and Bone Mineral Density (BMD). In these studies, the retinol intake was estimated from dietary records and a food-frequency questionnaire. Bone Mineral Density was measured with dual-energy x-ray absorptiometry. Hip fracture was identified by using hospital discharge records and was confirmed by record review. According to multivariate analysis of data, retinol intake was negatively associated with BMD. According to a cox regression analysis the risk of fracture was found to be the highest among men with the highest levels of serum retinol in a population-based (2322 men, 49-51 years) longitudinal cohort study [22]. These results are consistent with the results reported from animal and epidemiologic dietary studies.

Crandall [26] re-examined 20 clinical studies; of these 3 were randomized controlled trials, 14 were observational studies, and 3 were case reports. In these studies, retinol intake from diets or supplements has been found to be negatively associated with lumber, femoral neck and trochanter bone mineral density. There is a graded increase in relative risk of hip fracture with increasing intake of retinol, but not ß-carotene intake. Most studies point to a positive relationship between high intake (>1500 ug/d) or high plasma level (>2.25 umol/L) of vitamin A and reduced BMD or an in-

creased rate of bone fracture. Indeed, a relationship between hypervitaminosis A and skeletal malformation and teratogenesis in fetuses have been recognized for many years [27]. On the basis of such recognition, the American College of Obstretricians and Gynaecologists [28] has recommended a maximum dose of 1500 ug/d prior to and during pregnancy.

Table 3. Studies Reported Fracture Risk[1] with High Preformed Vitamin A (Retinol) Intake

Reference #	No. of Subjects	Length of Study
[17]*	1,120	64 mo
[18]*	72,337	18 yrs
[19]*	958	4 yrs
[20]**	2,332	30 yrs
[21]*	2,799	22 yrs
[22]*	34,703	9.5 yrs

[1]bone mineral density (BMD), hip fracture, relative risk.
*postmenopausal women; **men

It is noteworthy that worldwide the highest incidence of osteoporosis occurs in Northern Europe, a population with a high intake of vitamin A. However, a decreased biosynthesis of vitamin D associated with a minimized levels of sun exposure in this population may also contribute to this finding. This has been the basis for some but preliminary studies (to date) to examine the relationship between vitamins A and D in the context of bone metabolism.

DOES VITAMIN A ANTAGONIZES THE ACTION OF VITAMIN D?

In an experimental study involving weanling male rats, increasing levels of retinyl acetate (0-8621 ug/d for 21 d) in a diet eliminated the ability of vitamin D to elevate the levels of serum calcium while in the absence of the vitamin, these rats maintained a normal serum calcium [29]. These results suggest that vitamin A in excess antagonized the action of vitamin D on intestine and bone. A similar effect has been reported in human subjects [30].

Vitamin D is a prohormone. It must be metabolized in order to be activated. The liver and kidney are the main sites for its metabolic activation. Vitamin D is first hydroxylated in the liver at the 25-carbon atom of its molecule to form 25-OH-D; the latter is then further hydroxylated in the kidney at carbon 1-position, forming the active metabolite, 1,25 (OH)$_2$-D. The classical actions of this active metabolite are to regulate calcium and phosphate homeostasis and to promote the mineralization of bone [31]. Most of the biological effects occur through the direct transcriptional regulation of specific target genes by binding to the vitamin D receptor (VDR), which binds to its response element in target genes as a heterodimer with the retinoid receptors, termed retinoid X receptors, PXR [31]. In respect of PAR to influence the activation or expression of appropriate target genes, the preferred receptor partner for heterodimerisation is PXR. Thus, there is potential for interaction between retinoic acid and vitamin D signalling pathways [32, 33].

CONCLUSIONS

Current evidence points to a possible association between an excessive intake of vitamin A, either alone or in combination with low vitamin D, and the risk of osteoporosis. There is, however, little data available on changes of physiological and biochemical properties in animal caused by the interaction between vitamins A and D. Vitamin A could interfere with vitamin D in terms of its absorption, transport, activation, or vitamin A could stimulate the metabolic degradation of vitamin D. The mechanism of antagonism of vitamin A to vitamin D is far from being clearly understood. Furthermore, it is not yet possible to set a specific level of retinol intake above which bone health is compromised. Based upon the available evidence to date, it is hypothesized that a prolong intake of excess vitamin A affects vitamin D metabolism and that such effect is exacerbated in the presence of a borderline intake of vitamin D.

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