Vitamin A

Vitamin A		Women	Men	Children		
	RE/d			2-5 y	6-9 y	10-13 y
Recommended intake	RI	700	900	350	400	600
Average requirement	AR	500	600			
Lower intake level	LI	400	500			
Upper intake level	UL	3000* 1500#				

*as pre-formed retinol.
#Post-menopausal women

Introduction

Vitamin A is a term reserved to designate any compound possessing the biological activity of retinol (IUBAC-IUB 1982). The term retinoids include both the naturally occurring forms of vitamin A as well as the many synthetic analogues of retinol, with or without biological activity (Sporn et al 1976).

All-trans retinol, the parent retinoid compound, is a primary alcohol. In most animal tissues, the predominant retinoid is retinyl palmitate, but other fatty acid esters, such as retinyl oleate and retinyl stearate, are also found. Most of these metabolites occur in the all-trans configuration. Furthermore, the 11-cis aldehyde form, 11-cis retinal, is present in the retina of the eye, whereas several acid forms such as the all-trans retinoic acid, 13-cis retinoic acid and 9-cis retinoic acid, may be present in many tissues (Blomhoff 1994, Sporn et al 1984).

Vitamin A exists in the plant world only in the form of precursor compounds such as β-carotene. β-Carotene is one of 50-60 members with vitamin A activity of a large class of naturally occurring compounds called carotenoids. In all cases, a requirement for vitamin A activity is that at least one intact molecule of retinol or retinoic acid can be obtained from the carotenoid.

Recommendations on vitamin A include both vitamin A-activity as retinol and some pro-vitamin A carotenoids. The term ‘retinol equivalents’ (RE) is used to convert all sources of preformed retinol and provitamin-A carotenoids in the diet into a single unit. The conversion factors for the relevant carotenoids are based on human studies showing percentage absorption of a single dose of 45 μg to 39 mg β–carotene ranging from 9 to 22 % (IoM 2001). In addition, a number of factors such as protein-energy malnutrition, zinc-deficiency, dietary fat, alcohol, infections and degree of food processing/food matrix affect the bioavailability and bioconversion of retinol and carotenoids (Blomhoff

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1994, Sporn et al 1984, IoM 2001). Based on these and similar studies the U.S. FNB (2001) introduced the concept 'retinol activity equivalents' (RAE). 1 RAE is equal to:

- 1 μg of dietary or supplemental preformed vitamin A (i.e. retinol)
- 2 μg of supplemental β-carotene
- 12 μg of dietary β-carotene
- 24 μg of other dietary provitamin A carotenoids (e.g. α-carotene and β-cryptoxanthin)

In NNR the same factors are used, but the term "retinol equivalents" (RE) is maintained.

Dietary sources and intake

Vitamin A is present in the diet either as preformed vitamin A (i.e. retinol and its fatty acyl esters) in animal sources such as milk, eggs, butter and fish liver oils or as provitamin A carotenoids in dark green leafy vegetables and in red or orange coloured fruits and vegetables such as carrots. In addition, preformed vitamin A is also contained in a number of mono and multivitamin supplements (Blomhoff et al 2003).

Mean intake of preformed retinol in the Nordic countries varies from 740 to 1200 μg/10 MJ. In general, Icelanders have the highest intake followed by Norwegians. The main sources of retinol are liver and liver products, edible fat, milk and milk products, including retinol fortified margarine, spreads and milk. Cod liver oil is an important source of retinol in Iceland and Norway (Blomhoff et al 2003).

The 10 % of the adult population with the highest intake (the 90th percentile) have daily intakes of preformed retinol from foods that are up to 2-3 times higher than the RI for vitamin A. The 90th percentile intakes were the following among men: in Denmark 1600 μg, in Finland 1600, Norway 2800 and in Sweden 1900 μg retinol per day. For women the corresponding intakes were: Denmark 950, Finland 1200, Norway 2500 and in Sweden 1200 μg retinol per day (Blomhoff et al 2003, Männistö et al 2003, Lyhne et al 2005).

Physiology and metabolism

Vitamin A is essential for the life of all vertebrates. The vitamin has numerous important functions including a role in vision, maintenance of epithelial surfaces, immune competence, growth, development and reproduction (Blomhoff 1994, Sporn et al 1984, , Ross et al 2000). When intake of vitamin A is inadequate to meet the body's needs, clinical vitamin A deficiency characterised by several ocular features (xerophthalmia) and a generalised impaired resistance to infection occurs. A series of epidemiological and intervention studies in children living under poor conditions have documented a relationship between poor vitamin A supply and increased rates and severity of infections, as well as mortality related to infectious diseases such as measles (D'Souza and D'Souza 2002). Vitamin A deficiency is a public health problem in over 120 countries (WHO 1995). The problem is probably uncommon in developed countries but may be under-recognised since simple screening tests to measure sub-clinical deficiency is lacking. Vitamin A, may however, be a double-edged sword since it has been suggested that intake marginally above the recommended dietary intake is associated with embryonic malformations (Ross et al 2000, Rothman et al 1996), reduced bone mineral density and increased risk for hip fracture (Melhus et al 1998).

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The major dietary sources of vitamin A are provitamin A carotenoids from vegetables and preformed retinyl esters from animal tissues (Blomhoff 1994, Sporn et al 1984, Blomhoff et al 1990, Blomhoff et al 1982). Carotenoids such as α- and β-carotene and β-cryptoxanthin are absorbed by passive diffusion. After entry into the enterocytes, provitamin A carotenoids are cleaved yielding either one or two molecules of retinol. Absorption of retinyl esters includes enzymatic conversion to retinol in the intestinal lumen prior to entry into enterocytes. Retinol is then esterified to long chain fatty acids before incorporation into chylomicrons. Generally 70-90% of ingested preformed vitamin A (e.g. retinol) is absorbed.

Most of the chylomicron retinyl esters are transported to the liver. In vitamin A sufficient states, most of the retinyl esters taken up by hepatocytes are transferred to perisinusoidal stellate cells in the liver for storage. Normally, 50-80 % of the body's total retinol is stored in the hepatic stellate cells as retinyl esters. The normal reserve of stellate cell retinyl esters is adequate to last for several months (Blomhoff and Wake 1991).

Retinol bound to retinol-binding protein is released from the liver and circulates in plasma, ensuring an ample supply of retinol to target cells. Inside target cells, retinol is oxidized to retinal and retinoic acid which are the active retinol metabolites. These metabolites are usually synthesised in target cells by a complex metabolic system involving numerous enzymes and binding proteins (Blomhoff 1994, Sporn et al 1984, Blomhoff et al 1990, Blomhoff et al 1982). Retinal functions as a chromophore in the visual process while retinoic acid activates specific nuclear retinoic acid receptors and thereby modulates gene transcription (Gudas et al 1994).

Requirement and recommended intake

Earlier recommendations have mainly been based on studies aimed at eliminating symptoms of vitamin A deficiency. In the Sheffield study (Hume and Krebs 1949), symptoms of vitamin A deficiency (reduced plasma retinol, reduced dark adaptation, dryness of the skin, eye discomfort) developed in several of 16 healthy men following intake of a diet essentially free of vitamin A for 8 months. Of the 16 subjects studied, only 3 had changes in dark adaptation of sufficient magnitude to serve as a criterion to investigate the curative ability of varying amounts of retinol and β-carotene. Addition of 390 μg retinol per day to one of the individuals with vitamin A deficiency eventually improved dark adaptation and also improved somewhat the plasma retinol levels. Supplementation with 780 μg retinol per day for 45 days had little further effect on the subject's plasma retinol level. However, retinol supplement of 7200 μg retinol per day increased his plasma retinol above his initial level of 1.2 μmol/L. Furthermore, it was demonstrated in the other vitamin A-deficient individuals that daily intake of 1500 μg β-carotene in oil, but not 768 μg β-carotene in oil, improved dark adaptation and plasma retinol levels. Hume and Krebs (1949) concluded that daily retinol intake of 390 μg represented the minimum protective dose. This figure should be raised to 470 μg to correct for an error in the conversion factor used in the analytical measurements (Leitner et al 1960).

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Similar observations were obtained in the Iowa study (Sauberlich et al 1974) where vitamin A deficiency developed in 8 healthy men after several months on a vitamin A-deficient diet. Abnormal electroretinograms occurred at plasma retinol levels of 0.1-0.4 μmol/L and impaired dark adaptation was observed at plasma retinol levels of 0.1-0.9 μmol/L, whereas follicular hyperkeratosis was found at plasma levels of 0.3-1.3 μmol/L. Plasma levels below 1.1 μmol/L were associated with a mild degree of anaemia that responded to retinol supplementation. The Iowa study also observed that daily intake of 300 μg retinol partially corrected the abnormal electroretinograms, whereas supplements of 600 μg/day were needed to prevent eye changes in adult men. By using isotope-labelled retinol it was calculated that the average rate of utilization of retinol during the state of vitamin A depletion was about 910 μg retinol/day. The study (Leitner et al 1960) concluded that a daily retinol intake of 900 μg per day would maintain a plasma level of 1.1 μM in most adult men. For women, the requirement would be reduced in proportion to body weight.

The US DRIs (IoM 2001) for vitamin A were based on estimated requirements that assure adequate body stores of retinol where no clinical signs of deficiency are observed, adequate plasma retinol levels are maintained and there is protection against vitamin A deficiency for approximately 4 months on a vitamin A-deficient diet. The underlying evaluation assumes that the body turn-over of retinol is 0.5 %, the minimal liver reserve is 20 μg/g, the liver weight : body weight ratio is 1:33, the total body:liver vitamin A reserve is 10:9, and that the efficiency of storage (i.e. retention of absorbed vitamin A in liver) is 40 %. Based on these assumptions (IoM 2001), and using reference weights for US adults, the estimated average requirement of preformed vitamin A required to assure an adequate body reserve in an adult male is 627 μg/day. The corresponding value for women was estimated to 503 μg/day. Using a factor of 1.4 to cover the variation, a recommended daily allowance was set to 900 μg/day for men and 700 μg/day for women above 19 years of age (IoM 2001). These estimations are in general agreement with a large number of recent studies using functional criteria for vitamin A status, such as dark adaptation, papillary response test, conjunctival impression cytology and markers of immune function (see IoM 2001 for a review of these studies).

In a more recent study (Haskell et al 2011), estimated average requirement for vitamin A in adult males was studied using the deuterated retinol dilution (DRD) technique in 16 men in Bangladesh. The results indicated that 254-400 μg/day was sufficient to assure an adequate body reserve (equivalent to 362-571 μg/day for a 70 kg male in the USA), which is lower than the AR in the NNR 2004. Using the factor of 1.4 to cover the variation this would result in a recommended intake of 500-800 μg/day. However, more studies of the variation in the AR are needed before a change in the current recommendations can be discussed.

Using the above factorial method for the Nordic reference subjects, the estimated average requirement for vitamin A would be very similar as for the US reference subjects, i.e. close to 600 and 500 μg/d for men and women respectively. In NNR 2004, the recommended intakes for adults were based on these considerations and thereby set to 900 RE/day for men and 700 RE/day for women. There are limited scientific data to change the reference values from NNR 2004. Therefore, the RI of 900 RE/day for men

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and 700 RE/day for women are maintained. Also, the average requirements of 600 and 500 RE/d for men and women, and the lower intake level of 500 RE/d for men and 400 RE/d for women, respectively, are kept unchanged.

In infants, no functional criteria of vitamin A status have been published that reflect the response to dietary intake. Breast milk from well-nourished mothers in the Nordic countries usually contains sufficient amounts of vitamin A. For non-breastfed infants, vitamin A content of formula is sufficient. No specific recommended intake of vitamin A for infants aged 0-6 months is therefore given. Any contribution by carotenoids was not considered since the bioconversion of carotenoids in infants is not known.

Direct studies on the requirement for vitamin A are not available to estimate an average requirement for infants, children and adolescents ages 1-17 years. Thus, the RIs for children and adolescents are extrapolated from those for adults by using metabolic body weight and growth factors ($BW^{0.75}$, see IoM 2001).

Experimental data to estimate an average requirement during pregnancy are lacking. Using the retinol accumulation in foetal liver as a criterion, about 50 µg vitamin A per day would be needed in addition to the AR for non-pregnant women (IoM 2001). The RI for pregnancy is set to 800 RE/d to cover the individual variation.

The vitamin A content of breast milk varies with the dietary vitamin A intake. Reported values for Western countries are 450-600 RE/L. With an average milk production of 750 mL/d, this corresponds to 350-450 RE/d. An additional intake of 400 RE/d is therefore recommended during lactation.

In elderly subjects, intakes of 800-900 RE/d vitamin A seem more than adequate (Russel and Suter 1993). Some early studies (Garry et al 1987) have found an age-related trend toward higher serum retinol values with advancing age, but recent studies have found trends towards a slight decrease (Haller et al 1996). None of these elderly subjects had retinol values below a cut-off value of 0.35 µmol /L. Using a cut-off value of 0.7 µmol /L as proposed by NHANES data from 18-74-year-old subjects only resulted in very few subjects at risk (21). In a Danish cross-sectional study of 80-year-old men and women, 10 % had a dietary intake of vitamin A below the lower limit, but only one subject had a retinol value below 0.7 µmol /L (Pedersen 2001). Use of the same vitamin A-containing supplements have been linked to higher circulating retinyl ester values in elderly subjects compared to younger (Krasinski et al 1989), due perhaps to delayed plasma clearance in the elderly (Krasinski et al 1990). Anintervention study found an altered postprandial plasma retinol concentration in older subjects compared to younger, while the intestinal absorption and esterification were the same in the elderly compared to the younger subjects (Borel et al 1998).

Serum retinol levels are generally considered to be a relatively poor reflection of vitamin A status, unless liver stores are either very depleted or highly saturated, while plasma β-carotene seem to be a possible biomarker of the β-carotene status (Nielsen 1998). Several studies (Haller et al 1996, Heseker and Schneider 1994, Bates et al 1999) have found a positive relationship between plasma levels and the intake of β-carotene in elderly subjects. Consumption of carotene-containing fruits and vegetables is inversely related to overall mortality and cardiovascular mortality, even in the elderly (Gaziano et

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al 1995, Sahyoun et al 1996). However, the role of β-carotene in the prevention of age-related diseases is still too weak to use as a basis for vitamin A recommendations. The RI for elderly subjects > 60 years of age is the same as for younger adults.

Reasoning behind the recommendation

There are limited scientific data to change the reference values from NNR 2004. Therefore, the RI of 900 RE/day for men and 700 RE/day for women are maintained. Also, the average requirements of 600 and 500 RE/d for men and women, and the lower intake level of 500 RE/d for men and 400 RE/d for women, respectively, are kept unchanged.

Upper intake levels and toxicity

Several studies have shown that doses up to 180 mg β-carotene per day as supplements may be used for many years with no evidence of vitamin A toxicity and without the development of abnormally elevated blood retinol concentrations. Serious adverse effects of β-carotene in the form of supplements have, however, been reported but these are not related to its conversion to retinol (see discussion in Chapter Antioxidants).

Adverse effects of dietary retinol needs to be considered in Nordic populations where the dietary intake of preformed retinol has been relatively high, especially in Iceland.

Vitamin D antagonism

Several studies have provided evidence of an antagonism between retinol and vitamin D both in animals (Grant and O´Hara 1957, Aburto et al 1998, Metz et al 1985, Aburto and Britton 1998 a; b) and humans (Johansson & Melhus 2001). Animal studies have shown that retinol serves as an antagonist to vitamin D action, not only in toxic amounts but also at the physiological level (Rohde et al 1999). In a meta-analysis, which included all cases of retinol intoxication published in scientific literature up to year 2000 (Myhre et al 2003), it was observed that the mean dose of retinol causing hypervitaminosis A was higher when the dose originated from a formula containing vitamin D. This observation may imply increased sensitivity for retinol toxicity among subjects with vitamin D insufficiency.

Risk of acute and chronic hypervitaminosis A

Retinol toxicity related to osteoporosis and teratogenicity is discussed in separate sections below. There have been no reports in the Nordic countries describing either classical chronic or acute hypervitaminosis A due to intake of foods such as liver, except a few cases of early Arctic explorers eating Polar Bear liver (CEC 1993). Although adults in the Nordic countries have a generous intake of retinol, very few if any healthy individuals are likely to ingest amounts that may lead to classical hypervitaminosis A. Thus, the risk of hypervitaminosis A due to retinol-rich foods is very low.

A major issue when evaluating the potential toxicity of retinol is the observation that intake of retinol in various physical forms appears to have different thresholds for toxicity (Blomhoff et al 2003, Myhre et al 2003). Retinol in water-soluble, emulsified or

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solid (i.e. tablets) preparations generally seems to have more acute toxic effects than retinol in foods or oils (Myhre et al 2003). This may be relevant for potential hypervitaminosis A from supplements and foods fortified with retinol. Several foods commonly used in the Nordic countries are fortified with retinol. If the diet consists of large amounts of retinol-fortified foods, the daily intake may approach the upper safe levels. Therefore, oil-based retinol preparations should preferably be used in supplements and fortification of foods. Supplements and fortification with water miscible / emulsified preparations should be kept to a minimum.

A total of 17 suspected cases of supplement-induced chronic hypervitaminosis A, but no acute cases, have been reported in scientific literature in the Nordic countries up to 2003 (Blomhoff et al 2003). Chronic hypervitaminosis A is induced after daily doses of 2 mg/kg/day of retinol in oil-based preparations for many months or years (Myhre et al 2003). In contrast, only a few weeks of intake of doses as low as 0.2 mg/kg/day of retinol in emulsified/water-miscible and solid preparations caused hypervitaminosis A (Blomhoff et al 2003). Thus, emulsified/water-miscible and solid preparations of retinol are about 10 times more toxic than oil-based preparations of retinol. The safe upper single dose of retinol in oil or liver seems to be about 4-6 mg/kg bodyweight (Myhre et al 2003). These thresholds do not vary considerably with age.

Hepatotoxicity is a manifestation of hypervitaminosis A and toxic symptoms seem to depend on both the amount and duration of exposure. Mechanisms of hepatic effects are linked to overload of the storage capacity of the liver for vitamin A which may cause cellular toxicity, production of collagen and eventually fibrosis and cirrhosis. The lowest dose reported to cause cirrhosis was a consumption of 7500 μg RE/day for 6 years, and it can be hypothesized that this value might be the upper threshold of the storage capability of the liver (SCF 2002).

Risk of retinol-induced teratogenicity

Animal studies demonstrate that both retinol deficiency and retinol excess may give rise to embryonic malformations, and that a single high dose of retinol or retinoic acid may be teratogenic if given at a susceptible stage of early embryonic development (see discussion in Blomhoff et al 2003 and references therein). In humans, several cases of teratogenicity have been reported due to retinoic acid medication, but no cases due to preformed retinol in foodstuffs. Epidemiological data suggest that intakes of retinol supplements up to 3 mg vitamin A per day during pregnancy are not associated with an increased risk of giving birth to a malformed child. And since epidemiological data indicate that the threshold for teratogenicity is higher than 3 mg retinol/day it is assumed that this level offers adequate protection against teratogenic effects (SCF 2002). Thus, it is recommended that the intake of retinol supplements during pregnancy should be limited to no more than 3 mg per day unless other medical aspects argue for a higher intake. As the possible adverse effects of excess intake of retinol appear very early during pregnancy, this advice is expanded to all women of childbearing age. Furthermore, it is recommended that pregnant women should avoid eating liver as the main course of a meal.

Risk of retinol-induced osteoporosis

Results from animal experiments, in-vitro studies, pharmacological studies and clinical observations have shown that retinol intoxication is associated with severe detrimental

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effects on the skeleton (see Blomhoff et al 2003). Most human studies published during the last decade have, however, not shown any association between retinol intake and bone density (Maggio et al 2003; Kaptoge et al 2003; Suzuki et al 2003; Macdonald et al 2003; Rejnmark et al 2004; Wolf et al 2005; Barker et al 2005; Penniston et al 2006: Hogstrom et al 2008; Forsmo et al 2008), which is in line with animal data (Johansson et al 2003). In studies on rats bone density was unaffected while bone diameter and strength were diminished. This seems to be related to increased periosteal bone resorption and reduced bone formation (Lind et al 2010, Kneissel et al 2005). Observations on a human fetus have identified that a mutation in the enzyme (CYP26B1) that specifically inactivates the vitamin A-active metabolite retinoic acid has effects resembling those seen when high retinol doses are administered to experimental animals, e.g. a pronounced reduction of the diameter of the long hollow bones (Laue et al 2011).

Retinol and fractures

A few prospective and case-control studies have found an increased risk for fractures in groups with retinol intakes from foods and supplements >1.5 mg/d (e.g. Feskanich et al 2002; Melhus et al 1998 and Michaëlsson et al 2003). Caire-Juvera and coworkers (2009) found no overall association between total retinol intake and the risk of hip or total fractures among 75,747 postmenopausal women from the Women's Health Initiative Observational Study. However, an increased risk for fracture was seen in the group with the highest quintile of total retinol intake ($\geq$ 1.426 µg/d) among women with a vitamin D intake below the mean ($\leq$11 µg/d), but the overall trend was not significant. In other studies no association between retinol intake from foods (Rejnmark et al 2004) or from foods or total intake (Lim et al. 2004) and fractures have been found. There are also a few studies indicating associations between use of dietary supplements containing vitamin A and fractures (Lim et al 2004; White et al 2006). Mean retinol intakes varied between studies and some only measured retinol from foods (Melhus et al. 1998; Rejnmark et al. 2004), while other reports associations for both total and food retinol intake (Feskanich et al 2002; Michaëlsson et al. 2003; Lim et al. 2004; Caire-Juvera et al. 2009). There are also some studies showing an association between serum retinol levels and fractures (Michaelsson et al. 2003; Barker et al 2005, Opotowsky et al 2004). However, in the studies by Barker et al (2005) and Opotowsky et al (2004), no intake data were available.

Setting an upper intake level for retinol or retinyl esters

Toxic effects have primarily been linked to preformed vitamin A, i.e. retinol or retinyl esters. It is clear that the hazards and their associated doses are different for different groups of the population and the severity of the adverse effect varies from minor to irreversible.

Taking into account the low margin between the recommended intake value and doses that might pose a risk to different groups of the population setting an upper level of intake is not easy. In NNR 2004 the recommended maximum intake of 3 mg/ day of retinol supplements for women of childbearing age was chosen as the upper level for the whole population. This level is 2.5 times below the level which may cause hepatotoxicity. This UL is kept unchanged in NNR 2012.

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In NNR 2004, an UL of 1500 µg/d was set for postmenopausal women in order to reduce the possible risk of osteoporosis. The results from the studies published after NNR 2004 are contradictory and don´t give any clear indication for at what levels of intakes the risk for fractures increase. Still, it cannot be ruled out that long-term intakes above 1500 µg/d may increase the risk for fractures. Thus, the previous recommendation that postmenopausal women who are at greater risk for osteoporosis and bone fractures should restrict their intake to 1500 µg/d, is therefore maintained.

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